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Hot Topics in Infection and Immunity in Children VII

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Preface

Each of the chapters in this book is based on a lecture given at the seventh 'Infection and Immunity in Children' (IIC) course held at the end of June 2009 at Keble College, Oxford. Thus, it is the seventh book in a series, which collectively provide succinct and readable updates on just about every aspect of the discipline of Paediatric Infectious Diseases.

The eighth course (28–30 June 2010) has another exciting programme delivered by renowned top-class speakers, and an eighth edition of this book will duly follow.

The clinical discipline of Paediatric Infectious Diseases continues to grow and flourish in Europe. The University of Oxford Diploma Course in Paediatric Infectious Diseases, started in 2008, is now well established with a large number of trainees enrolled from all parts of Europe. The Oxford IIC course, as well as other European Society for Paediatric Infectious Diseases (ESPID)-sponsored educational activities, is an integral part of this course.

We hope this book will provide a further useful contribution to the materials available to trainees and practitioners in this important and rapidly developing field.

Melbourne, Australia — Nigel Curtis
Bristol, UK — Adam Finn
Oxford, UK — Andrew J. Pollard

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Sue Sheaf has administered and run the course for several years now. Her quiet efficiency and effectiveness are vital to its success. As course organisers we are indebted to Sue and we are enormously appreciative of her efforts. We give our heartfelt thanks to Sue on our behalf and also on behalf of all the speakers and delegates who have benefited from her behind the scenes administrative, organisational and diplomatic skills.

Pamela Morison administered the production of this book. In addition to carefully correcting and formatting the chapters and liaising with the publishers, she quickly learnt the subtle art of persuading authors (and editors) to meet deadlines, read formatting instructions and answer e-mails. We thank Pam for her patient and cheerful approach to this difficult task, and we gratefully share with her the credit for this book's production.

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The Value of Vaccination

David E. Bloom

Abstract Vaccination is most often studied from a scientific, clinical, or epidemiological perspective, and rightly so, for vaccines are meant to improve health outcomes. But these are not the only lenses through which the effects of vaccination programs can be understood. This chapter provides an economic perspective on vaccination programs, detailing in particular a new line of inquiry that makes a case for the importance of vaccination to achieving national economic aims. Research has shown that national spending on childhood vaccination programs does more than just reduce morbidity and mortality in a country: it also promotes national economic growth and poverty reduction. The chapter begins with a look at recent research that demonstrates powerful links that run from population health to economic well-being. Second, it discusses how knowledge of the economic benefits of health fundamentally transforms how we understand the value of vaccination. And third, it provides evidence for the scale of the returns that countries receive when they invest in immunization programs – returns that have not been fully captured by traditional economic analyses.

1 Population Health and Economic Well-Being

Since 1950, many parts of the world have seen remarkable health gains. Life expectancy has increased by more than two decades, and the global infant mortality rate has been reduced by two-thirds over the same time period. Smallpox has been eradicated, and polio nearly so. These health improvements are examples of what one might consider truly extraordinary achievements. By defining what might be possible, they can – and should – make us even more ambitious about what can be achieved in the future.

But these have also been accompanied by a colossal set of failures in the health arena, failures that indicate the extent and severity of human misery and insecurity

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on the planet. In particular, there has been a demonstrated reversal of health gains in some countries. Largely as a consequence of the HIV and AIDS epidemics, life expectancy in several sub-Saharan African countries has fallen, in some cases by roughly 15 years, beginning in the 1990s [1]. As another example, two-thirds of the roughly 9 million deaths of children under the age of 5 worldwide that will occur this year will be due to causes that could be easily prevented or cured with existing knowledge [2].

Even more troubling than these health deficits are the gross health disparities between rich and poor nations. In 2005, 86% of the world's health expenditure took place in the OECD countries – which are home to but 15% of the world's population [3]. At least 20% of the world's children are still not immunized with DTP3, with research in *The Lancet* suggesting that this number is likely to be closer to 26% [4]. Child mortality is currently an order of magnitude higher in developing countries than it is in the wealthy industrial countries. Significant disparities also prevail in infant mortality and life expectancy (see Table 1). Large disparities also exist not only between but also within countries, typically between urban and rural populations, racial and ethnic groups, and income classes.

Most of the financial resources for improving population health – for addressing these failures and disparities – will have to come from the public sector. There are four classic arguments in support of devoting public resources to the promotion and protection of health. The first set of arguments has moral, ethical, and humanitarian roots – i.e., devoting resources to health is fair and just. The second argument is that health is a "fundamental human right," a legal claim to which all human beings are entitled. The third argument is that health is essential to building strong societies. In this view, improved health is a key ingredient in the formation of social capital and societies that are cohesive, peaceful, equitable, and secure. A fourth argument has to do with the character of health and of health services, from an economic standpoint. For a number of reasons, unregulated markets do a poor job of achieving socially

Table 1 Health disparities between developed and developing countries [5]

	1950–1955			2005–2010		
	World	Developed countries	Developing countries	World	Developed countries	Developing countries
Infant mortality rate (deaths per 1,000 live births)	152	59	174	47	6	52
Child mortality rate[a] (deaths per 1,000 live births)	109	18	122	71	8	78
Life expectancy (years)	47	66	41	68	77	66

[a]Child mortality rate is for 1980–1985 and 2005–2010

desirable levels of health provision. This means that governments have a natural (and essential) role to play in the health sector.

These four arguments on behalf of devoting public resources to health are each logical and coherent. However, neither individually nor collectively have governments or other institutions been able to use them to mobilize the resources necessary to make a significant dent in the world's health deficits and disparities. It is evident that more is needed to make a persuasive case.

Another powerful justification for devoting public resources to health has recently come to the fore, one that will perhaps add to the collective power of the above justifications for health spending. This argument has to do with the relationship between health and the macroeconomy. Essentially, it argues that a healthy population is an important engine of economic growth [6].

Figure 1 shows one of the best established patterns in the field of global health – the positive association between health and wealth. Each point is a country, with the location of the point reflecting the country's income per capita and the life expectancy of its people. The basic pattern shown on the chart is that countries with higher incomes tend to have healthier populations. This pattern holds for different income and health measures and at different points in time [7].

Another key feature of the chart is the arrangement of the variables on the horizontal and vertical axes. Income is placed on the horizontal or *X* axis, which means it is the independent variable. This is a clear suggestion that the variable income affects the dependent variable on the vertical or *Y* axis, in this case, health. In other words, the centerpiece of this very famous scatterplot is a causal link that runs from income to health.

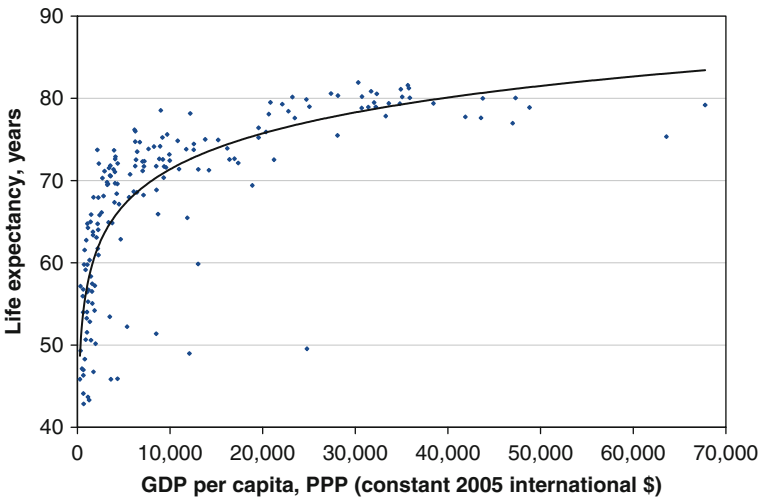

Fig. 1 Life expectancy and income [8]

This is not a startling idea. When people have more money, they tend to have better nutrition, better access to safe water and sanitation, access to more and better health care, and better psychosocial resources like community recreation facilities. These mechanisms allow one to conceptualize population health as a consequence of economic growth, which has been the dominant view on the health–income nexus going back to the birth of modern economics over two centuries ago.

However, the reverse causal link – from health to income – may be equally plausible, for several reasons [9]:

First, a healthier workforce tends to be a more productive workforce, with more energy, better mental health, and less absenteeism.

Second, economic outcomes can be improved through better education, which is in turn improved by health. Healthy children tend to stay in school longer and have better cognitive development. Thus, educational investments in healthy children yield high returns, which will naturally lead to an expansion of those investments. Education is virtually undisputed among economists as being one of the most powerful instruments of economic growth and poverty alleviation.

Third, health and longevity affect savings and investment. Healthy populations have higher savings rates as people save more in anticipation of longer periods of retirement. Savings lead to investment, which results in the accumulation of physical and human capital, and technological progress. These are, of course, the classic drivers of economic growth. It is also worth noting that healthy populations are better able to attract foreign direct investment [10], which often carries with it new technology, job creation, and increased trade.

Finally, demographic change provides yet another casual link from health to economic improvement, a link that was vitally important to the so-called economic miracles experienced in a number of countries in East Asia and Ireland. Essentially, the idea is that health improvements trigger a process of demographic change, beginning with lower fertility rates, that promotes an age distribution that is increasingly favorable to economic growth. This demographically induced boost to economic growth has come to be known as the demographic dividend.

The reverse link from health to income has been the subject of much statistical and econometric analysis in the past few years. There are different ways of looking at the link and at data pertaining to the link – varying time periods, control variables, data sets, statistical tools, theoretical frameworks, etc. For the purposes of this chapter, it is enough to say simply that population health is an exceedingly robust and powerful predictor of economic growth.

This premise can be illustrated through a thought experiment: Imagine two countries that are identical in all key dimensions pertinent to economic growth, except that the people in one are healthier than those in the other. The new finding tells us that the healthier country will increase its average income and reduce its poverty rate faster than the less healthy country. It also tells us that a 5-year advantage in life expectancy translates into between 0.3 and 0.5 additional percentage points of annual growth of income per capita [11].

A 1% point advantage may not sound like much, but in a world economy in which per capita income typically grows at 2–3% per year, it is quite meaningful.

A 1% point gain is also meaningful because a 10-year gain in life expectancy is well within the grasp of a very large number of countries. It corresponds roughly to the life expectancy improvement that developing countries – where average life expectancy is currently 66 years – would enjoy if they achieved the same life expectancy as today's developed countries – where it is currently 77 years. It also corresponds to the life expectancy improvement that many demographers project for the wealthy industrial countries during this century.

2 A New Paradigm for the Value of Vaccination

The new perspective outlined above has important implications for assessing the value of immunization programs. There are two standard approaches to conducting an economic evaluation of the desirability of a health intervention: cost-effectiveness analysis and benefit–cost analysis. Today, benefit–cost analysis is the economic tool of choice with respect to assessing the value of vaccination [12].

In carrying out a benefit–cost analysis, decisions must be made regarding what constitutes a cost and what constitutes a benefit. With respect to vaccination, there is nothing particularly tricky about measuring costs. These include the cost of the immunizing agent, the cost of administering that agent, and the value of time associated with getting a child to a medical practitioner, along with any associated transportation costs.

The calculation of benefits is less straightforward. Economists traditionally focus on a narrow range of implications of vaccination programs. They assume that with vaccinated children not getting sick, medical costs are avoided. In addition, they assume that parents may benefit by not having to miss work to look after sick children or take them to the doctor. These two benefits are correctly treated as benefits of a vaccination program. However, they are just two components of the much wider set of overall benefits that vaccination potentially confers on children, their parents, and their communities.

For example, healthy children have, as mentioned above, better records of school attendance. They also attend school for more years and learn more each year they are enrolled. Vaccinated children also tend to avoid the long-term sequelae associated with certain childhood diseases, such as neurological impairments, hearing loss, and a variety of other physical disabilities. Better educated and healthier than their peers, vaccinated kids will therefore tend to be more productive workers when they grow up.

Such benefits do not only accrue to children. With respect to parents and grandparents, they tend to be healthier themselves if their children and grandchildren are healthy. They also have lower rates of absenteeism, and they avoid the anxiety associated with having children and grandchildren who are ill.

Society also derives benefits from vaccinated, healthy children. These benefits relate first to herd immunity, where even individuals who are not immunized gain protection from disease when other members of the community are immunized.

Immunologists and clinicians express this herd immunity bonus in terms of additional numbers of effectively immunized people; economists focus on the monetary aspects, where those people who avoid illness because of herd immunity will tend to be more productive and require less resources for medical care. Societal benefits also include decreased antibiotic resistance. Because immunization means less need to treat diseases with antibiotics, it decreases the development of antibiotic resistance and the need to resort to what are often far more expensive second-line drugs. Finally, the expectation that children will grow up healthy leads naturally to families having fewer children, a benefit that helps trigger the demographic dividend described above.

The central premise, then, of the new paradigm for the economic evaluation of vaccination is a broad view of the benefits of vaccination, one that incorporates impacts on the many factors listed here, in addition to averted medical care costs and the cost of parental work loss [13]. In other words, if one accepts the argument that "healthier means wealthier," it stands to reason that a proper accounting of the benefits of vaccination must, at a minimum, include the future productivity gains of children who grow up healthier, smarter, and better educated, as well as the economic gains enjoyed by others in their families and communities.

3 Applications of the New Approach

A review of some recent research will demonstrate the kind of results that are produced via the new paradigm for conceptualizing, measuring, and accounting for the full benefits of childhood vaccination. Two studies serve to illustrate the change: one focuses on a Global Alliance for Vaccines and Immunisation (GAVI) program and the other analyzes some data from the Philippines [14].

The GAVI proposal aims to extend the use of a variety of vaccines to 75 low-income countries during 2005–2020, at a cost of US $13 billion. GAVI seeks to expand the traditional basic childhood vaccination package; to increase coverage of the under-used Hib, hepatitis B, and yellow fever vaccines; and to help finance the introduction of vaccines covering meningococcus, pneumococcus, and rotavirus. In principle, this ambitious program will save lives, save medical care costs, and encourage higher labor productivity by supporting the physical and mental development of children. GAVI's epidemiologists estimate that this program will reduce the child mortality rate in the 75 GAVI countries by 4 deaths per 1,000 live births initially (by 2005), and by 12 deaths per 1,000 live births (by the year 2020), a sizable decline.

In an initial, albeit somewhat crude, attempt to estimate the rate of return on this investment, a group of researchers calculated the likely effect of the program on worker productivity at the individual level. The headline result was striking, in which a conservative approach estimated the rate of return on investment in the GAVI immunization program to be 12% by 2005, rising to 18% by 2020. These rates of return compare favorably with rates of return on other highly regarded investments in economic growth and development.

A second study that took a relatively broad view of the benefits of vaccination examined data from the Cebu Longitudinal Health and Nutrition Survey on efforts to immunize children in the Philippines against DTP, TB, polio, and measles. The analysis focused on children's cognitive development and directly links vaccination experience in the first 2 years of life to cognitive function at age 10, as measured by test scores on language, math, and IQ tests.

Using a range of propensity score methods to deal with the problem of non-random assignment, the study found a significant positive effect of childhood vaccination on all three test scores. When international evidence was used to translate those test-score benefits into earnings gains as adults, and to compare those earnings gains to the $20 cost of the vaccine package, another striking result appeared: a 21% rate of return on the vaccine spending.

4 Two Calls to Action

The results of the GAVI study and the Philippines study are at best suggestive. But they both point toward the eminent economic sensibility of immunization programs by virtue of the handsome rate of return they deliver – a return that is higher than previously recognized and that is comparable to estimated rates of return on investments in education, the most exalted instrument of development.

Education economists have long understood that one compelling argument to justify incurring the out-of-pocket and foregone earnings costs of schooling today is the enhancements that schooling yields to productivity and earnings tomorrow.

What the above research suggests is that we acknowledge that the same reasoning applies to spending on vaccination research and coverage. Spending on immunization programs today promotes increased productivity and increased earnings tomorrow – and these increases need to be meaningfully, consistently, and comprehensively measured in the interest of better public and private policy decisions about resource allocation.

Thus, this research potentially provides more than just an incremental contribution to knowledge. It actually has transformative potential: it has the capacity to transform vaccination policy debates from discussions of vaccination programs as burdensome costs into discussions of vaccination programs as income-generating investments. Such a transformation is guaranteed to get the attention of economic policymakers because they are accountable for income growth and poverty reduction. And this can be very fortuitous, since economic policymakers also have the "power of the purse." The first call to action is thus that policymakers, in allocating resources to national vaccination budgets, acknowledge that the rate of return offered by vaccination is likely higher, perhaps considerably, than has been previously thought.

One other certain implication of this research is that the literature on the economic evaluation of vaccination needs to be reconsidered. Most books and articles on the benefits and costs of vaccination discuss only the reduction in healthcare costs

that stem from vaccination, while a few sources also make a passing nod to the benefits of reducing time away from work. As a result, there is much research to be done. The second call to action is directed toward economists and other researchers, who must conduct a new set of benefit–cost analyses, vaccine by vaccine and country by country, to widen and deepen the evidence base regarding the full benefits of vaccination programs.

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Recent Trends in Global Immunisation

Gustav J.V. Nossal

1 Introduction

In the midst of the global financial crisis, it may be difficult to argue that increased aid to the poorest countries, particularly those in Africa, is of high priority. Nevertheless, a dissection of recent trends in global immunisation should be embedded in an analysis of the global health scene. It could be argued that some global inequities are just intolerable and that therefore inertia and indifference are no longer possible. That being said, it is encouraging that global immunisation programmes are on the improve and that despite everything progress in global health is possible.

2 Official Development Assistance at the Global Level

After the Gleneagles meeting of the group of eight richest nations in 2005, a decision was taken to increase aid substantially, particularly to Africa. In the event, while not every country has lived up to its pledges, total Official Development Assistance in 2008 rose by 10.2% from the 2007 base, reaching US $119.8 billion or 0.3% of global Gross National Income. Of that, aid to sub-Saharan Africa was US $22.5 billion. Bearing in mind that the United Nations many years ago set 0.7% of Gross National Income as the desirable benchmark, it is interesting to note that only five countries actually managed to reach that goal, namely Denmark, Luxemburg, The Netherlands, Norway and Sweden. In terms of actual monies contributed, the volume leaders are USA ($26 billion but only 0.18% of Gross National Income), Germany, UK, France and Japan in that order. Following a strong commitment by the Rudd Government, Australia's aid rose 13.8% in 2008 to 0.33% of Gross National Income. It is planned to go to 0.5% of GNI by 2015.

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One frequently hears an argument against overseas aid which suggests that aid is not worthwhile because of rampant corruption and what is really needed is increased trade with developing countries. Actually, saying that aid is all wasted on corruption is a bad excuse for doing nothing. It is true that there is much corruption in developing countries, and it is important to "corruption proof" particular grants as much as possible, but for the poorest countries frankly there may be nothing to trade without aid. Unquestionably, reduction of trade barriers would be very helpful to developing countries and therefore the real answer is that we clearly need both trade and aid. It is essential to realise that aid works only if there is a true partnership, and the methods of giving aid need careful examination. For example, micro-credit has a proud history in the short time that it has been promoted.

An important point to make is that the world, even in a global financial crisis, can increase aid if it truly wants to. Indeed, the global financial crisis shows just what huge funds governments can mobilise if the will is there. For example, the US Wall Street Bailout cost US $700 billion and the G20 stimulus packages agreed at the recent G20 Summit meeting totalled well over US $1 trillion. The wars in Iraq and Afghanistan cost the USA alone US $150 billion/year. Putting this into perspective, it has been estimated that most of the Millennium Development Goals could be achieved if an extra US $120 billion/year in Official Development Assistance were available, i.e. a doubling of the present level and still short of the 0.7% GNI benchmark.

Increasing aid does not depend upon the decisions and pronouncements of politicians alone. It becomes feasible when ordinary people become committed realising that it is in the long-term interest of social stability and peace. The matter is well summarised by the following two quotes:

> Each of the great social achievements of recent decades has come about not because of government proclamations, but because people organised, made demands, and made it good politics for governments to respond. It is the political will of the people that makes and sustains the political will of governments. (The late James Grant, former Executive Director of UNICEF)

> Every day, 50,000 people die needlessly as a result of extreme poverty. Poverty can be eradicated only if governments of both developed and developing countries live up to their promises. (Ban Ki-moon, Secretary General UN, 2008)

3 Health Progress is Possible

Nothing illustrates more starkly the degree of global inequities in health than life expectancy and mortality statistics. Some illustrative examples for 2007 are given in Table 1. How can we continue to live in a world where life expectancy is twice as long in the "best" countries than in the "worst", let alone where deaths under 5/1,000 live births are nearly 100-fold different between "best" and "worst"?

Despite these alarming statistics, it is clear that health progress is possible. For example, when we look at the under 5 mortality, this was a record low of 9.2 million deaths in 2007 vs. 13 million in 1990 despite an increased population. It comes as no

Table 1 Life expectancy and mortality statistics 2007

	Males	Females
	Life expectancy	
Japan	81	86
Australia	79	84
USA	76	81
Afghanistan	41	42
Sierra Leone	39	43
	Deaths under 5 per 1,000 live births	
Sierra Leone	262	
Afghanistan	257	
USA	8	
Australia	6	
Japan	4	
Sweden	3	

surprise that the bulk of these deaths were in sub-Saharan Africa (4.5 million) and in South Asia (3.0 million). About two-thirds of these deaths were preventable, among these were pneumonia (1.8 million), diarrhoea (1.6 million), malaria (780,000), measles (390,000) and AIDS (290,000).

In part, this health progress has been secured through some massive new programmes since the year 2000, of which the largest are the Global Fund to Fight AIDS, TB and Malaria initiated in 2002; the President's Emergency Plan for AIDS Relief (PEPFAR) initiated in 2004; and the many programmes of the Bill and Melinda Gates Foundation starting from the year 2000. These new and very large programmes should not obscure the fact that other and more traditional programmes are getting traction, including polio eradication, the Stop TB Partnership, various malaria control programmes, and ambitious plans to contain filarial diseases including river blindness and lymphatic filariasis.

4 The GAVI Alliance, Formerly the Global Alliance for Vaccines and Immunisation

A major example of a Gates Foundation-initiated programme is the GAVI Alliance [1]. Launched in 2000, this has three main aims, namely increased coverage in the poorer countries with the standard childhood vaccines; introduction into immunisation programmes of newer vaccines; and increased research and development of new and improved vaccines for third world use.

As a result in its relatively short history the GAVI Alliance has ensured that a cumulative 51 million extra children got their three doses of the diphtheria–pertussis–tetanus vaccine, a surrogate for the six common childhood vaccines. A

cumulative 192 million children have been immunised with hepatitis B, and coverage with this important vaccine is now 60% worldwide. A cumulative 42 million children have been immunised against *Haemophilus influenzae B*, and given that this conjugate vaccine was so successful, the Gates Foundation has helped to introduce conjugate pneumococcal vaccines as well. Planning is well advanced for introduction of rotavirus vaccines and vaccines against typhoid, rubella, Japanese encephalitis and cervical cancer.

As a result of the above initiatives, it is estimated that a cumulative number of 3.4 million deaths have been averted.

One fine example of what can be achieved with developmental research and technology transfer is the plan to control the shocking outbreaks of meningococcal meningitis that sweeps across the so-called meningitis belt of sub-Saharan Africa. In a partnership between the Gates Foundation, the World Health Organization (WHO) and the non-governmental organisation PATH (Program for Appropriate Technology in Health), the Serum Institute of India was contracted to develop a meningitis A conjugate vaccine and helped with significant technology transfer. They have pledged to make the vaccine available at US 40¢ per dose. They have already succeeded in showing that the vaccine is 20 times more immunogenic in 12- to 23-month-old children than the carbohydrate vaccine, through trials in Mali, The Gambia and in Ghana. The Dell Foundation has pledged to fund a demonstration study in which all 1- to 29-year-olds in Burkina Faso will be given a single dose of the vaccine in 2009–2010. In parallel further large phase III trials in Mali and India are planned for 2009–2010. If successful, and there is little reason to doubt that the trials will be successful, 250 million 1- to 29-year-olds and 23 million infants in 24 other "meningitis belt" countries will be immunised between 2010 and 2015. The result would be to protect 430 million people in 25 countries from Senegal to Ethiopia from this horrible disease with its 10% case fatality rate and 20% of serious sequelae, including mental retardation.

5 Polio Eradication Still Somewhat Problematic

Within the field of immunisation some areas are still problematic. For example, the polio eradication campaign is way behind where its planners hoped it would be at this stage. There are still four countries (Nigeria, India, Pakistan and Afghanistan) in which transmission has never been interrupted. Furthermore, 14 countries in which poliomyelitis had been eradicated have reported re-introduction, admittedly small numbers of cases, but showing that the threat is still quite real.

Dr. Margaret Chan, the Director-General of WHO, has termed polio eradication as WHO's top operational priority. With respect to the fact that it is proving so difficult to immunise children in some of the poorest areas, such as Northern India, the question has been raised as to whether the injectable polio (Salk) vaccine may need to be used in such areas. Furthermore, given the occurrence of intercurrent diarrhoea, it has been postulated that zinc supplementation may have a role to play.

6 Recent Developments in Malaria

There has recently been considerable public health progress in the field of malaria [2]. Of course malaria remains a very serious public health problem. There are at least 300 million attacks per year, at least 1 million deaths, mainly in children under 5, and resistance of the parasite to first-line, cheap drugs and also resistance of mosquitoes to insecticides remain big problems. However, progress has been on three fronts. Insecticide-impregnated bednets pre-sprayed with pyrethroids have proven a singularly effective and relatively cheap weapon. At less than $5 per bednet, malaria mortality has been decreased by more than 50%, resulting in the fact that all-cause mortality has been reduced by 20%. This has been a real boon in areas of high malaria endemicity. Frequently it has been accompanied by residual spraying of dwellings by pyrethroids as well. Second, after a rather fallow period, new drugs for malaria are at last coming forward. For example, the "Medicines for Malaria" venture represents a public–private partnership between the WHO and 39 research and development partners. Initiated in 1999 it already has 11 drugs in clinical trials. Many of these are derivatives of artemisinin. In fact, artemisinin-based combination therapy (ACT) is now best practice for attacks of malaria. Some combinations include chlorproguanil–dapsone–artesunate, pyronaridine artesunate and also artemisinin together with drugs like amodiaquine or piperaquine. A related step forward is intermittent preventive treatment (IPT) for malaria in infants. This involves a full course (for example of sulfadoxine–pyrimethamine) given to asymptomatic infants in areas of high risk. Similarly, IPT is also effective in pregnancy, frequently with two courses given during the pregnancy. Third, there has been progress on the malaria vaccine front. A vaccine prepared by GlaxoSmithKline called RTS,S based on the circumsporozoite protein showed a 66% efficacy in 554 African infants when given as three doses at 10, 14 and 18 weeks of age. In view of these encouraging results, phase 3 trials have been started in nine countries and should finish by October 2011. It is planned to enrol 16,000 infants aged 5–17 months in ten different sites in seven countries.

RTS,S is not the only progress in malaria vaccines. For example, the Gates Foundation is backing a whole portfolio of alternative approaches. The firm Sanaria is embarking on clinical trials of live X-irradiated mosquito salivary gland-derived sporozoites following trials in human volunteer challenge studies that showed 90% efficacy. A wide variety of blood stage antigens are in late pre-clinical or early clinical development. Liver cell-specific antigens are being progressed, particularly by the International Centre for Genetic Engineering and Biotechnology in New Delhi, India. Various vaccines depending on viral vectors, leading to T-cell immunity, are under development, frequently with protocols favouring a "prime–boost" approach, i.e. a different vaccine for the priming and the boosting protocol.

In view of all this activity, in September 2008 the United Nations called a special summit meeting and launched a $3 billion plan to "end all malaria deaths by 2015". While this might be unduly optimistic, it is an indication of how seriously the malaria control field is moving.

7 HIV/AIDS Vaccine – A Long Way to Go

The news is not as good with respect to an HIV/AIDS vaccine [3]. The failure of Merck's adenovirus 5-vectored vaccine efficacy trial in 2007 was a big disappointment. At the time of writing, the Sanofi-Pasteur ALVAC-HIV prime VaxGen gp120 boost vaccine in adult Thai men is the only efficacy trial ongoing. Other T-cell-based strategies include novel vectors (non-human adenoviruses, CMV, NDV, measles, fowlpox, BCG) sometimes encoding interleukins, dendritic cell targeting ligands or TLR ligands. These strategies usually involve prime–boost protocols.

Antibody-based strategies for an HIV/AIDS vaccine fall into two groups. First there are attempts to define the epitopes which bind broadly neutralising monoclonal antibodies, which are then synthesised or mimotopes of them constructed. Then there are attempts to target the conserved, briefly revealed co-receptor binding site on the envelope protein. These could be conformationally constrained gp120-CD4 constructs, computer-generated mimotopes or peptide-scaffold molecules.

One problem which constrains all HIV/AIDS vaccine research is the lengthy and difficult process of clinical trials, particularly given that ethical concerns mandate that strict safe sex education must be given at all trial sites, with documented evidence that this alone reduces rates of acquisition of seropositivity.

8 Measles Remains a Threat

With all this activity in research on vaccines which do not yet exist, it is easy to forget that in the developing countries measles still remains a real threat. For reasons that are not entirely clear, the case fatality rate of measles in a developing country setting is up to 2%, very high for a disease which essentially every non-vaccinated child gets at some time. A serious problem with respect to measles vaccination is that the live attenuated vaccine can usually not be given before 9 months of age. As maternal immunity wanes at about 4 months of age, there is a substantial gap during which infants remain highly vulnerable.

This induced a group led by Dr. Peter Aaby in Guinea-Bissau [4] to go against the conventional wisdom and to trial measles immunisation at 4.5 months of age. A group of 441 children in Guinea-Bissau received such immunisation vs. 892 children that remained in the control group before both groups were given the regular 9-month dose of the live attenuated measles vaccine. Monthly measles incidence was charted and turned out to be 0.7% in the immunised group vs. 3.1% in the control group. Cumulatively, by 9 months of age, 14.4% of unvaccinated but only 3.1% of vaccinated infants had contracted measles. Even more startling was vaccine efficacy against admission to hospital for measles, which was 100%. Equally, deaths from measles were 7 in the unvaccinated but 0 in the vaccinated, again 100% efficacy. The treatment group had no more non-measles deaths than controls.

It is clear that this was a relatively small and preliminary trial. Nevertheless the results are sufficiently intriguing as to warrant serious follow-up.

9 Anti-Vaccine Activists are a Real Danger

Unfortunately, anti-vaccine activists constitute a real danger in both the industrialised and the developing countries. In the richer countries, these activists are emboldened by the fact that most mothers have little or no experience of how fierce epidemic disease can be. Two recent examples illustrate the point. In the United Kingdom, claims that the measles–mumps–rubella vaccine caused autism saw a disastrous drop in immunisation coverage, at a time when measles transmission had practically come under control. This necessitated extensive and expensive studies to disprove the claim but the UK immunisation programme has still not fully recovered. In France, false claims that the hepatitis B vaccine could cause multiple sclerosis seriously set back the use of this important tool.

The worst example in a developing country comes from Kano State in Nigeria. Here, a rumour spread that the oral polio vaccine was really a Western plot to render female Muslim babies sterile. This entirely fanciful notion caught hold, derailed the polio eradication effort for more than a year and resulted in the fact that polio became resurgent in Nigeria and, just as disastrously, polio spread from Nigeria to numerous neighbouring African countries. Through belated government action, the polio immunisation programme in Nigeria is now more or less back on track, but harm such as this takes a long time to undo.

The fact of the matter is that serious adverse events after immunisation do occur, but are vanishingly rare. For example, the oral Sabin polio vaccine can occasionally revert to neurovirulence, but this occurs approximately once per 2 million doses! The measles vaccine can very rarely cause thrombocytopenic purpura, but at an incidence that is at least 1,000-fold less than that at which measles itself causes this complication. Other claims, such as encephalitis after the pertussis vaccine, have also not been proven. The risk–benefit equation is enormously on the side of vaccine benefit.

10 Conclusion

There is room for cautious optimism in the global public health scene. It is clear that the Gates Foundation has unleashed some powerful and dynamic forces. Equally, it is evident that some governments are taking their responsibilities towards developing countries more seriously. The emergence of talented and idealistic health leaders in many developing countries is also to be welcomed. The statistics are there for everyone to see and it will take some time for them to become less scandalous. What is needed is the continuance of scientific progress and political will.

This chapter has been as much about politics as it has been about science. As it is based on a lecture given at Oxford University, it may be apt to end with a quote from one of Oxford's greatest sons. Sir Peter Medawar said: "If politics is the art of the possible, research is surely the art of the soluble. Both are immensely practical-minded affairs".

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New Advances in Typhoid Fever Vaccination Strategies

Zulfiqar A. Bhutta, M. Imran Khan, Sajid Bashir Soofi, and R. Leon Ochiai

1 Introduction

Salmonella belong to the group of Enterobacteriaceae that are aerobic, gram-negative rods and approximately 1–3 μm × 0.5 μm in size [1, 2]. Currently there are approximately 2,400 pathogenic species of *salmonella*. *Salmonella* was first identified in 1880 by Eberth from the mesenteric nodes and spleen of a patient dying from typhoid fever [3, 4]. Later in 1884 Gaffky was able to isolate the bacillus. A year later Salmon and Smith described a bacillus that is now known to be *S.* Choleraesuis, the first bacteria that affects both human and animals [5]. *Salmonella* possess a flagellar antigen (H), somatic (O), and a surface antigen Vi. *Salmonella* are divided into two subspecies of *S. enterica* and *S. bongori*. *S. bongori* contains 8 serovars and *S. enterica* contains the other approximately 2,300 serovars that are divided into 6 subspecies based on flagellar H antigen. *Salmonella* nomenclature has undergone many changes [6]. Serotypes of *Salmonella* are recognized using the technique recommended in the Kauffman–White scheme.

Only few *Salmonella* serovars have been identified to cause disease in animals [7]. *Salmonella* subspecies *enterica* serovar Typhi is the most common cause of infection in humans and serologically is placed in *Salmonella* group D due to O antigens 9 and 12 [8]. The genetic makeup of the organism has not shown variation geographically and is stable with a few exceptions of isolates from Indonesia that have slightly different flagellar antigens. *S.* Typhi expresses a polysaccharide capsule Vi (virulence antigen) on its surface and is highly stable serologically compared to other *Salmonella* serotypes [9]. Presence of Vi prevents the binding of O antigen to the O antibody and thus enables the pathogenesis of the organism. Clinical severity of typhoid fever is a result of the Vi antigen that increases the infectivity [10]. However, Vi-negative strains have also been identified; therefore, Vi presence is not essential for *S.* Typhi-related typhoid fever. In vitro studies have shown that the Vi antigen of *S.* Typhi has anti-opsonic and antiphagocytic characteristic that reduces

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the level of secretion of *Salmonella* serovar Typhi-induced tumor necrosis factor alpha (a marker of activation) by human macrophages and increases the level of resistance of the organism to oxidative killing [8].

2 Typhoid Fever Epidemiology

A recent analysis estimated that there are 21 million typhoid fever cases per year and 216,000 deaths [11]. An earlier WHO estimate of the global typhoid disease burden based on a study from 1984 indicated around 17 million cases and approximately 500,000–600,000 deaths per year [11, 12]. Recent analysis assumes an average case fatality rate (CFR) of only 1%, which is at the low end of most estimates in the literature. Typhoid fever is considered endemic in most of the developing world. An estimated 90% of typhoid-related deaths occur in Asia [11, 13].

The recent burden of disease analysis was based on data derived from selected studies in a total of only 10 developing countries that included only one from sub-Saharan Africa (South Africa). High incidence rates of typhoid have been documented for south and Southeast Asia, but arbitrary estimates were made for many regions of the developing world that lacked any data, especially Africa. The paucity of reliable incidence data from most developing countries reflects the fact that laboratories capable of bacteriologic confirmation are lacking in much of the developing world [13]. As well as typhoid fever being endemic, the disease has also appeared as epidemic forms in central Asia, Africa, and south Asia [14].

The incidence of typhoid fever may vary considerably not only between, but also within, countries [15] (Fig. 1). In some countries, evidence suggests that residents of

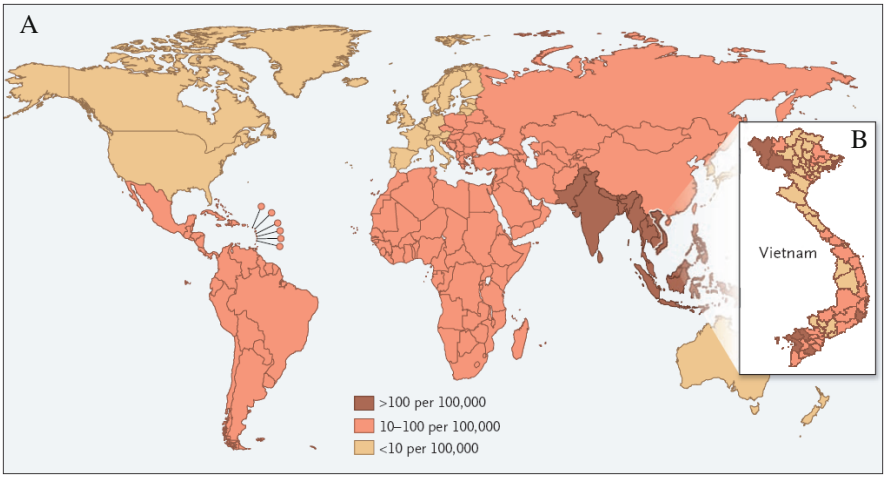

Fig. 1 Estimated distribution of typhoid fever burden in 2000 (a) and the geographic differences of typhoid fever incidence in Vietnam (b) [15]

poor urban areas are at considerably higher risk than rural dwellers. Until recently typhoid was considered as a disease of school-aged children. More recent systematic population-based studies from India, Bangladesh, and Pakistan have confirmed that the incidence is higher in young children [16–19].

Typhoid fever is a waterborne disease transmitted by the ingestion of food or water contaminated with excreta of patients and asymptomatic carriers and is therefore most common in areas with poor water and sanitation systems and practices. Common sources include polluted water and contaminated food (e.g., milk products), often eaten outside of the home and handled by infected persons. Other risk factors for increased transmission include recent typhoid fever in the household, a lack of toilet in the household, drinking unboiled water, not using soap for hand washing, and sharing food from the same plates as others [20, 21].

3 Typhoid Fever: Clinical Presentation and Outcome

Typhoid fever caused by *Salmonella* Typhi is an acute generalized infection of the reticuloendothelial system, intestinal lymphoid tissue, and gall bladder. The clinical presentation of the disease varies from high-grade fever to more systemic involvement of nervous system [22]. Typhoid is often confused with other acute febrile illnesses until it persists for more than 3 days and does not respond to symptomatic treatment or first-line antimicrobial therapy [23]. Typhoid was recognized as a distinct disease in the earlier quarter of nineteenth century and soon gained significance as a serious health problem due to its ability to spread quickly in populations, especially those living collectively such as army soldiers and in dormitories [2, 20, 24].

Presentation of typhoid fever varies ranging from mild fever to more severe forms such as toxic shock. Symptoms include sustained high-grade fever (~104°F), profuse sweating, altered bowel habits from constipation in adults to diarrhea in children, malaise, myalgia, a dry cough resembling bronchitis, anorexia, nausea, and in some cases non-bloody diarrhea. If fever lasts for more than 5 days, a rash of flat, rose-colored spot may appear. The incubation period for a non-complicated case of typhoid fever is 10–14 days. Malaise and lethargy can continue for a couple of months even when the disease may have resolved. If left untreated, typhoid fever progresses through the four stages, each lasting approximately 1 week. In the first week, there is a slowly rising temperature with relative bradycardia, malaise, headache, and cough. In some cases bleeding from nose (epistaxis) and abdominal pain may also occur. The number of circulating white blood cells decreases with eosinopenia and relative lymphocytosis; blood cultures are positive for *Salmonella* Typhi, while Widal is negative in the first week [25, 26].

In the second week, fever has a plateau of around 104°F and heart rate is slow with a thready pulse. Delirium is frequent and calm, but sometimes agitated. Rose spots appear on the lower chest and abdomen in around 30% of patients. Abdominal symptoms become more obvious with pain in the right lower quadrant. Diarrhea with a frequency of six to eight stools per day may occur during this time; however,

constipation is also frequent. The spleen and liver become palpable and tender at this time. Elevation of transaminases can be seen on liver enzyme tests. Anti O and Anti H on Widal are strongly positive; blood culture may also be positive depending on the quantity of blood taken from the patient. In the third week of fever, complications appear including intestinal hemorrhage, encephalitis, metastatic abscess, cholecystitis, endocarditis, and osteitis. Overall 10–15% of typhoid fever cases develop complications. Intestinal perforation may occur in 1–3% of cases leading to peritonitis and ultimately to death if proper surgical intervention is not undertaken. In the fourth week fever is still high and oscillates very little. The patient has delirium due to dehydration. Other complications include disseminated intravascular coagulation that may lead to early death. Pneumonia is more common in children than in adults. Some of the rare outcomes reported are hepatic, splenic, and bone marrow granulomas; splenic and liver abscesses; pleural effusion; phagocytic syndrome; pseudotumor cerebri; hemolytic endocarditis and pericarditis. Arrhythmias or cardiogenic shock are manifestations of toxic myocarditis with fatty infiltration of the heart [1, 2, 24].

Hospitalization rates of typhoid fever cases vary from 10 to 40%, while the rest either self-medicate or are treated on an outpatient basis [16]. Population-based studies have reported variation in hospitalization rates. In settings where early treatment was provided due to extensive and systematic surveillance, it was possible to treat typhoid early. On the other hand patients who followed the regular health system mechanism had higher rates of hospitalization and complication. The average length of hospital stay ranges from 10 to 15 days.

Following recovery, convalescing patients may continue to excrete *S*. Typhi in the feces for almost 3 months. One to four percent of cases become long-term carriers, excreting the organism for more than 1 year. Most carriers are asymptomatic. The average case fatality rate is less than 1%, but this is variable among the endemic countries, with Pakistan and Vietnam having a case fatality rate of less than 2% and Indonesia and Papua New Guinea as high as 30–50%. Young children have been found to be at a higher risk of severe typhoid. Case fatality rates have been found to be 10 times higher in children younger than 4 years compared to older children. The most significant contributor to a poor outcome is a delay in the initiation of an effective antibiotic treatment. In untreated cases, fatality can go as high as 10–20%. The gall bladder carriage rate is 1–5% of the survivors of typhoid infection. Carrier status also increases the chances of hepatobiliary cancers [24, 27–30].

3.1 Diagnosis

Following ingestion of the *Salmonella* pathogen, there is an asymptomatic period. The incubation period for typhoid fever is 7–14 days and is influenced by the dose of the inoculum. Secondary bacteremia follows infection and coincides with the onset of symptoms such as high-grade fever and malaise. Other symptoms and signs that may help in the clinical diagnosis are loss of appetite, abdominal discomfort, headache, and severe myalgia. A coated tongue, tender abdomen, hepatomegaly,

and splenomegaly are also common. Delirium, confusion, and convulsions may also occur in children less than 5 years. As a result of bacterial dissemination throughout the body, the patient may present with systemic involvement such as respiratory, neurological, and abdominal illnesses.

The diagnosis of typhoid fever in endemic settings is mostly clinical and relates to the clinical experience of the attending physician. There have been repeated and regular attempts to establish diagnostic criteria that combine clinical presentation and laboratory investigations. Such attempts have not resulted so far in the development of a diagnostic technique that will help overcome current diagnostic challenge. Despite reservations about the sensitivity, specificity, and predictive value of Widal, it is the most common laboratory method used for diagnosis of typhoid. Widal detects antibodies that are also cross-reactive with other Enterobacteriaceae. In typhoid patients, antibodies only appear in the second week; therefore, usefulness of the test is limited in the initial stages of the disease [31]. Other serological tests such as Tubex and Typhidot have not shown promising results. The gold standard for the diagnosis of typhoid is isolation of the bacteria from blood and/or bone marrow. Bone marrow cultures have higher sensitivity compared to blood culture. The bone marrow culture is positive for 80–95%. In cases where patients have been treated with antimicrobials, the bone marrow culture may still lead to *S. typhi* isolation. Blood culture is positive 60–80% of the time but the yield varies with the quantity of blood taken [23, 32–35].

3.2 Management

Lack of simple, accessible cost-effective tools for accurate diagnosis of typhoid fever results in delayed diagnosis and failure to adequately treat the disease. These factors in turn contribute to the high emergence of severe form of the disease in endemic settings. In initial stages, the disease is either treated at home or by informal health sector. Improper diagnosis leads to inappropriate management and resultant increase in severity of the disease ultimately leading to hospitalization and fatal outcomes. Careful assessment of fever cases is recommended. In cases where fever is more than 5 days, laboratory investigation such as blood culture is advised. However, clinical symptoms and signs should be correlated with laboratory findings. In cases where either the provisional diagnosis is typhoid fever or there is serological or bacteriological evidence of disease, first-line antimicrobial therapy should be initiated. Third-generation cephalosporins are the most effective treatment for typhoid fever with cure rates of 90–98% [27].

4 Control Strategies

Similar to other diseases spread by the fecal–oral route, typhoid fever predominates in areas with inadequate water and sanitation systems and/or poor hygienic practices. Typhoid was effectively eliminated in developed countries mainly through large-scale development of water treatment (e.g., chlorination), construction of deep

wells, and piped water and sewerage systems. Impact of safe drinking water and adequate sanitation on diarrheal diseases has also been demonstrated in northeast Brazil where a 22% reduction in diarrheal diseases was evident after an expenditure of nearly 900 million dollars on infrastructure development. Infrastructure development for provision of safe water and proper sanitation is costly to build for many developing country government budgets. Considering most of the typhoid fever cases occur in urban slums of Asian cities, diversion of development budgets seems unrealistic in near future [36–38].

In lieu of the existing situations, alternative short-term interventions are recommended for the reduction of disease burden in these areas. These interventions include intensive hygiene education for hand washing using soap, discouraging open defecation especially by children, and the proper disposal of garbage and feces. There is evidence that such interventions have been effective in the control of enteropathogens at small scale. The practicality of such interventions at large scale has still not been answered systematically [39–42].

In the existing circumstances, a typhoid fever vaccination program may provide a short-term alternative strategy coupled with a continuous advocacy for development of infrastructure for safe water provision and clean and hygienic sanitation. There is evidence that immunization can virtually eliminate typhoid fever in a relatively short period of time, especially when targeted toward high-risk age groups and geographic areas. Due to the reduction in the price of the vaccine, it is now becoming more affordable to countries with high burden of typhoid fever. In order to make a typhoid fever vaccination program more effective, it must be introduced as a typhoid fever control program that should have other components such as hygiene education messages, sanitation improvements (e.g., latrines), and improved water supply and quality measures [19, 37, 43–47].

4.1 Antimicrobial Resistance

Increasing resistance to available antimicrobials is another challenge for typhoid fever control. Outbreaks of *S.* Typhi strains resistant to chloramphenicol first appeared in the 1970s in several parts of the world. As new drugs such as ampicillin and co-trimoxazole became available, resistance against these drugs also emerged. Outbreaks of multi-drug resistance (MDR), defined as resistance to first-line antibiotics, were first reported in the late 1980s in south Asia and the Middle East that later spread to east Asia and Africa. In Vietnam, 86% of all isolates were found to be multi-drug resistant. MDR typhoid has been associated with more severe illness and higher rates of complications and deaths, especially in children under 2 years of age. The emergence of multi-drug resistance *S.* Typhi strains has led to the widespread use of fluoroquinolones, such as ciprofloxacin and ofloxacin. However, outbreaks of nalidixic acid-resistant typhoid (called NARST) started to occur in Vietnam and Tajikistan in the early 1990s and then spread to Pakistan and India [32]. Nalidixic acid-resistant typhoid cases respond less well to fluoroquinolones, exhibiting more prolonged fever than sensitive cases, and, in one study, a 10-fold higher rate of

post-treatment stool carriage was observed compared to sensitive cases (20% vs. 1.8%), increasing their potential to infect others. Cases of full-blown resistance to ciprofloxacin have also reported from Pakistan and India [33, 48–53].

More recent data from population-based studies confirm that multi-drug and nalidixic acid resistance is a serious problem in south and southeast Asia [14]. Sixty-seven percent of isolates tested in Karachi, 22% in Hue, and 7% in Kolkata were multi-drug resistant, and high rates of nalidixic acid resistance were found in all three sites – 59% in Karachi, 58% in Kolkata, and 44% in Hue. Two isolates in the India site (1.6%) were found to be ciprofloxacin resistant. On the other hand, no drug resistance was found in the Indonesian and Chinese sites (Table 1). The increase in the resistance to available antibiotics may result to increase in the fever duration, decrease in management options, and an economic burden on the families. The often non-specific symptoms of typhoid fever can make the clinical diagnosis difficult and it can be confused with malaria, dengue fever, influenza, and other febrile illnesses. Confirmed diagnosis requires isolating *S.* Typhi in the laboratory through blood cultures, bile-stained duodenal fluid culture, or occasionally through bone marrow culture. Unfortunately, such invasive tests are not conducted for the majority of patients in developing countries, especially those treated in non-hospital settings [14, 24, 54].

Table 1 Antibiotic resistance among *Salmonella typhi* isolates from five Asian study sites in the DOMI program

	Hechi, China	Kolkata, India	N. Jakarta, Indonesia	Karachi, Pakistan	Hue, Vietnam
Total number of isolates tested	15	122	131	127	18
Antibiotic resistance (%)					
Chloramphenicol	0 (0%)	9 (7%)	0 (0%)	85 (67%)	6 (33%)
Ampicillin	0 (0%)	9 (7%)	0 (0%)	84 (66%)	6 (33%)
TMP–SMX[a]	0 (0%)	11 (9%)	0 (0%)	84 (66%)	4 (22%)
MDR[b]	0 (0%)	9 (7%)	0 (0%)	83 (65%)	4 (22%)
Ciprofloxacin	0 (0%)	2 (2%)	0 (0%)	0 (0%)	0 (0%)
Ceftriaxone	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Nalidixic acid	0 (0%)	69 (55%)	0 (0%)	75 (59%)	8 (44%)

Source: DOMI program; data from [14]
[a]TMP–SMX, trimethoprim–sulfamethoxazole
[b]MDR, multi-drug resistant (i.e., resistant to chloramphenicol, ampicillin, and trimethoprim–sulfamethoxazole)

4.2 Vaccination

The use of new-generation antimicrobials to manage increasing resistance has increased the cost of treatment. The cost of illness, due to antimicrobial-resistant typhoid, is on average nearly four times greater than those who responded well to

Table 2 Total cost of blood culture-confirmed typhoid fever illness by the type of patient and study site (US $2005) in the DOMI program

Type of patients and costs	Hechi, China	Delhi, India	Kolkata, India[a]	N. Jakarta, Indonesia	Karachi, Pakistan[b]	Hue, Vietnam
Sample size	58	98	79	107	66	16
Ages (years) included in surveillance	5–60	All ages	All ages	All ages	2–15	5–18
Total costs for hospitalized patients	215	820	129	432	210	157
Costs for outpatients	67	95	13	57	38	38
Hospitalization rate (%)	40	12	2	20	10[a]	28
Weighted average costs (hospitalized and outpatient)						
Private costs	126	79	11	106	53	38
Direct	101	43	6	61	45	33
Indirect	26	36	5	45	9	5
Public costs	0	101	4	26	2	33
Total weighted average costs	$126	$182	$15	$132	$55	$71
Average household monthly income in sample (US $2005)	121	N/A	N/A	207	158	84
Percent of families that borrowed money for typhoid treatment (%)	14	N/A	49	24	N/A	18

Sources: Poulos C et al. Cost of illness due to typhoid fever in study sites in five Asian countries *Unpublished* (*Manuscript submitted*)

[a]Bahl et al. [55]

[b]Results from Karachi are based on local expert opinion and reflect the costs of disease in children 2- to 15-year-old

the first-line antimicrobial treatment (Table 2). Typhoid fever can have a devastating financial impact on families in several of these largely poor communities as the majority of costs of illness are private costs equivalent of 1.6 and 1.2 months of an average household income. These findings of disease burden from various parts of the world have important implications for typhoid fever immunization strategies at the country level, as they suggest that in many countries, vaccination in geographically targeted, high-risk populations, rather than universal immunization, will be potentially the most cost-effective means of controlling the disease [55].

Typhoid vaccine use for prevention of disease dates back to 1896 with the inoculation of heat-inactivated vaccine. This was the first bacterial vaccine to be widely used in humans. The vaccine was obtained by inactivating the virulent microorganisms with heat or chemicals. The associated adverse effects after administration of killed whole-cell vaccine restricted its wider public health use. The adverse events included fever (6–30%), malaise, local reaction (35%), and headache (10%). During the World Health Organization-sponsored trials, the efficacy of the vaccine was 51–66%, but it was highly reactogenic. Among vaccinees 25% had systemic and local reactions post-vaccination. It is believed that during the process of vaccine

production, destruction of some heat-labile antigens resulted in the low efficacy and associated adverse effects. Irrespective of being highly reactogenic, the vaccine was widely used in the military in the early twentieth century due to high reporting of typhoid fever in the sick reports of the English and American armies. The vaccine was shown to reduce typhoid incidence by more than 90% from the time before vaccine introduction. Similarly the Belgian government conducted mass vaccination of the civilian population during the First World War in 1915. More recent examples of use of the killed WC vaccine are in schools and high-risk population in Thailand in the 1960s and early 1970s. A drop in the incidence of typhoid fever was noticed after the introduction of the vaccine. Similarly Cambodia used the vaccine during an outbreak; however, adverse events resulted in the dropping of the fourth dose [7, 56, 57].

In early twentieth century inactivated oral vaccines (acetone-inactivated vaccine and formalin-inactivated vaccine) were used to assess local immunity. These oral inactivated vaccines were evaluated in volunteers and field studies in the 1960s and 1970s. These vaccines could not make it to efficacy assessment and are no longer under consideration for production. The two new-generation typhoid vaccines that are currently internationally licensed and available are the injectable Vi polysaccharide vaccine and the oral, live-attenuated Ty21a vaccine [58–61].

4.2.1 Ty21a

Ty21a is an orally administered, live-attenuated Ty2 strain of *S.* Typhi in which multiple genes have been chemically mutated, including those responsible for the production of Vi. The vaccine was developed in the 1970s and first licensed in 1989, but initially used only in developed countries (Table 3). This lyophilized vaccine is currently available in two formulations. The enteric-coated capsules given in three to four doses and a liquid suspension consisting of the vaccine in one sachet and a buffer in another are combined with water before administration. The liquid formulation is given in three doses. For both formulations, the doses are administered every other day (e.g., over a 5-day period). The vaccine is licensed for use in persons 6 years and older. While the capsules are often used for travelers to developing countries, the liquid formulation is the one most likely to be used by public health programs in developing countries. The vaccine requires a cold chain (at 2–8°C) and survives for approximately 14 days at 25°C. Ty21a vaccine has been shown to be well tolerated and to have low rates of adverse events. In three double-blinded, randomized controlled efficacy trials in Chile and Indonesia involving approximately 550,000 school children, reactogenicity of the Ty21a vaccine was assessed through active surveillance. The rates of side effects (diarrhea, vomiting, fever, and rash) in the vaccinated groups were not found to be significantly greater than those in the control groups for both the enteric-coated capsule and liquid formulations. In large-scale field trials in children in Egypt, Chile, and Indonesia, Ty21a was found to have protective efficacy rates against blood culture-confirmed typhoid fever of 33–67% for the enteric-coated capsules and 53–96% for the liquid formulation (53–78% for the currently licensed liquid formulation) after 3 years of follow-up, when each was

Table 3 Description of efficacy and effectiveness trials of Ty21a oral typhoid vaccine

Study (Year)	Reference	Formulation	Number of study subjects	Ages (years)	Follow-up period	PE for blood culture-confirmed typhoid (95% CIs)	Incidence rate in control group (per 100,000)
Alexandria, Egypt (1978–1980)	Wahdan et al. [83]	Liquid given with tablet of $NaHCO_3$	32,388	6–7	36 months	96% (77–99%)	50
Area Occidente, Santiago, Chile (1983–1986)	Levine et al. [84]	Three doses of enteric-coated capsules given (1–2 days between doses)	140,000	6–19	36 months 7 years	67% (47–79%) 62%	110
Area Sur Oriente, Santiago, Chile (1986)	Black et al. [85]	Three doses of enteric-coated capsules (1–2 days between doses)	81,321	6–19	3 years	33% (0–57%)	100
		Three doses liquid suspension (1–2 days between doses)			3 years 5 years	77% 78%	
Sumatra, Indonesia (1986–1989)	Simanjuntak et al. [63]	Three doses of enteric-coated capsules (7 days between doses)	20,543	3–44	30 months	42% (23–57%)	810
		Three doses liquid suspension (7 days between doses)				53% (36–66%)	

given in three doses every other day (except in Indonesia, where dosing occurred every 7 days). The vaccine appeared to be more efficacious in areas with lower incidence of typhoid (Egypt, Chile) than in hyper-endemic areas, such as Indonesia. Ty21a is therefore considered to provide protection for at least 5–7 years. Large-scale vaccination with Ty21a also appeared to confer herd protection in Chile. These data suggest that the systematic application of live oral typhoid vaccine can notably reduce the incidence of the disease in endemic areas [12, 62–65].

4.2.2 Vi Capsular Polysaccharide

Vi is a subunit vaccine consisting of the purified Vi ("virulent") polysaccharide outer capsule of the Ty2 strain of *S.* Typhi. The vaccine is administered subcutaneously or intramuscularly as a single dose of 25 μg. It was first developed in the 1970s and further developed for large-scale manufacture at the US NIH, in collaboration with Pasteur-Merieux-Connaught. First licensed in the USA in 1994, the vaccine is in the public domain and is now being produced by several multi-national and developing country manufacturers. Like other T-independent purified polysaccharide vaccines, Vi does not elicit adequate immune responses in children less than 2 years of age, and thus is licensed for use in persons 2 years and older. The vaccine is highly heat stable and is able to retain its physicochemical characteristics for 6 months at 37°C and for 2 years at 22°C (room temperature). Vi vaccines have been extensively tested in humans and demonstrate a strong safety profile (Table 4). No serious adverse events and minimum side effects were associated with Vi vaccination in large field trials. In a recent multi-center study of Vi effectiveness, the vaccine showed safe and with minimal side effects. There is no booster effect of Vi vaccine [8, 10, 66–72].

4.2.3 New Vaccines in Pipeline

The low-efficacy estimates, inability to confer lifelong immunity, and difficulties in administration through regular and routine public health programs have limited the use of available typhoid vaccines. Therefore, a search for new improved vaccine is on the agenda in the vaccine field. There have been attempts to produce conjugate typhoid vaccines in both oral and parenteral forms. The aim of a conjugate vaccine is the production of T-cell-dependent immunity where the serum antibody response can be boosted and results in long-term immunity. Tetanus and diphtheria toxoid, cholera toxin, cholera toxin B subunit of recombinant exotoxin A of *Pseudomonas aeruginosa* are being tested for conjugation to Vi. An earlier Vi conjugate vaccine did not produce significant results due to the high Vi volume. Recent advances in the conjugation of Vi to a carrier protein have led to significant antibody responses in adults and children in endemic settings. A similar approach has been adopted for oral vaccines using recombinant techniques. The aim is to have a vaccine that will be single dose and will induce sufficient immunity to protect the population for life. However, to have such a vaccine seems overambitious at this moment. Both Vi conjugate vaccines, designed to be effective in infants, and new oral live vaccines, designed to be highly immunogenic in a single dose, are currently in

Table 4 Description of Vi Polysaccharide vaccine efficacy trials

Study (year)	Reference	Formulation	Number of study subjects	Ages (years)	Follow-up period	PE for blood culture-confirmed typhoid (95% CIs)	Incidence rate in control group (per 100,000)
Kathmandu Valley, Nepal (1986–1988)	Acharya et al. [68]	One dose of Vi (25 μg)	6,907	5–44	17 months	72% (42–86%)	926
E. Transvaal, South Africa (1985–1988)	Klugman et al. [69]	One dose of Vi (25 μg)	11,384	6–14	21 months 36 months	64% (36–79%) 55%	773
Quan County, Guangxi Province, China (1995–1997)	Yang et al. [86]	One dose of locally produced Vi (30 μg)	131,271	3–50 (92% school age)	19 months	69% (28–87%) (72% in school children)	63–78

development. A prototype Vi conjugate vaccine was found to be highly efficacious (91%) in Vietnamese toddlers for at least 4 years and serum antibody responses suggest that it can protect for at least 10 years in persons 5 years and older. Several groups are now developing Vi-diphtheria toxoid (DT) conjugate vaccines, with the goal of transferring technology to appropriate developing country producers, so that low-cost typhoid conjugate vaccines can ultimately be incorporated into the infant EPI schedule for high-risk populations. A number of improved live oral vaccines are currently in clinical trials. However, all of these newer generation typhoid vaccines are still several years away from being licensed and available on the market. The future promise of these vaccines should not preclude the more immediate use of currently available new-generation vaccines in endemic populations.

4.3 Perceived Risk of Disease and Vaccination Acceptance

Research suggests that vaccine acceptance or demand can be influenced by the perceived prevalence of the disease in the community, as well as by beliefs regarding the severity of the disease, the risk of its striking one's household, attitudes toward vaccination in general and perceived benefits and risks of specific vaccines. Among other factors, knowledge of and experience with the disease are also important factors. Communities also exhibit a strong understanding of how common the disease is in their communities. There is a strong correlation between actual incidence and perceptions of typhoid being a "common" or "very common" disease in their community. High-risk communities also tend to have good knowledge of how to prevent typhoid fever. There has been interest from high-risk population and demand for new-generation typhoid vaccines (Table 5). The findings from socio-behavioral studies also highlight the demand for typhoid vaccine in areas where incidence of typhoid fever was not high [47, 73, 74].

4.4 The Market (Vaccine Demand and Supply)

According to preliminary estimates, the potential demand for a typhoid vaccine was calculated for 30 countries in regions considered to have high typhoid incidence (>100/100,000/year). The estimated number of doses required each year was approximately 136 million. Given that there are several high-quality producers of Vi the issue of supply of Vi vaccine does not appear to be a problem with manufacturers being able to meet an increased demand for new-generation typhoid vaccines created by their introduction into public health program in endemic countries.

In the years since the WHO recommendation, several developing country manufacturers have acquired the technology to produce Vi. This proliferation of Vi producers has been facilitated by technology transfer from the US National Institutes of Health (NIH) to several companies, the lack of patent protection,

Table 5 Results of the DOMI socio-behavioral studies on population knowledge, perceptions, and beliefs in five Asian sites

Data	Hechi, China	Kolkata, India	N. Jakarta, Indonesia	Karachi, Pakistan	Hue, Vietnam
Annual typhoid incidence in 5- to 15-year-olds (per 100,000)	29	494	180	413	24
Percent of respondents who have heard of or are familiar with typhoid fever (%)	73	93	N/A	86	77
Households who report past experience with typhoid fever in the household (%)	14	37	48	31	2
Percent who believe that typhoid fever is "common" or "very common" in community (%)	2.5	66	25	47	4
Percent who think the chances of household members getting typhoid fever are	(regarding children)				
• Very likely (%)	0.4	12	66	52	0
• Likely or somewhat likely (%)	9	64	24	6	48
Percent who consider typhoid fever in infants or children to be	N/A				
• Very serious (%)		56	22	58	57
• Serious (%)		38	67	37	39
• Total (%)		94	89	95	96
Percent who think that typhoid vaccines should be used in community (%)	50	93	97	95	N/A

as well as the relatively simple, low-cost production process involved. Two additional developing country producers are in the process of developing Vi vaccines, in collaboration with the International Vaccine Institute and the US NIH.

4.4.1 Vaccination Strategies

The typhoid fever burden estimates are available only from few countries globally. For countries where estimates are available, data come from small-scale population-based studies or conducted as part of surveillance for vaccine trials. Therefore, the introduction of typhoid vaccines for mass immunization is questioned. A more practical approach recommended by the WHO is to consider targeted introduction of the vaccine in national vaccination programs. The policy decision for typhoid vaccine uptake is largely dependent on the perception of typhoid endemicity in the country.

The estimates of clinical protection for typhoid fever have been consistent around 70% for at least 3 years across field trials. However, there has been little evidence on the effectiveness of the vaccine until recently. Results of the indirect protection in Kolkata suggest the actual impact of the vaccine is much higher than expected.

4.4.2 Determining Endemicity

The widespread use of antimicrobials has reduced complication rates of typhoid fever. However, population studies directed by hospital estimates have shown that high rates of typhoid incidence are captured once systemic surveillance is undertaken. Population-based studies are expensive and time-consuming. Therefore, in settings where typhoid fever is expected to be found, alternative methods can be adopted to assess disease burden. A rapid assessment of outpatient hospital visits, admissions, and outcome of fever episodes can provide approximate estimates about the most affected age group, geographic location, and socio-economic classes affected. Such data can then be used for typhoid fever advocacy, guiding control strategies and in determining the target population for vaccination. In endemic settings, focusing on the high-risk groups can be a cost-effective strategy. A vaccination campaign targeting high-risk populations such as school age children, food handlers will affect transmission of the pathogen and hence circulation in the environment. Such effects can reduce the burden of disease beyond controlled efficacy results for the vaccine.

A common source of typhoid spread in a high endemicity setting is food handlers. Unhygienic food is sold without control by street vendors. Considering the prevalence of typhoid fever, the chances that these food handlers will be carriers of typhoid qualify them as a priority group for vaccination. Typhoid incidence estimates from south Asia have shown that children of school age are at highest risk. Considering that 5% of cases become carriers after being infected, school-age children will have the highest rates of transmission and close interaction of children in school and sharing of food increases the risk of spread of *S*. Typhi infection from an infected child to other typhoid-susceptible children. Vaccinating school-aged children will also have a greater impact in disease reduction. School-based immunization in Thailand with the killed whole-cell vaccine in the 1980s provides lesson for countries with endemic typhoid [75].

5 Population Impact

Among the two vaccines available in the market, only Vi polysaccharide vaccine has been used at large scale in countries with a high burden of typhoid fever. The introduction of the vaccine resulted in a significant reduction of typhoid fever presenting to health clinics. However, a more scientific evaluation of the effect of the vaccine has not been done that could single out Vi vaccine use as the important factor in disease reduction.

5.1 Guangxi Province, China

Typhoid fever has been endemic in many southern provinces of China. An annual incidence rate of 113/100,000 in the general population was reported in Jiangsu

Province in 1988, and an average annual incidence of 53/100,000 between 1995 and 1999 in Hechi City in Guangxi Province. An immunization program using locally manufactured Vi vaccine was undertaken in these typhoid endemic areas in the 1980s. Initially the old- generation killed whole-cell vaccine and the new-generation oral live Ty21a vaccines were used. However, due to adverse events association with killed vaccine added with high cost and a difficult schedule of administration of Ty21a, the Ministry of Health switched to Vi polysaccharide vaccine in the program. Local production was a result of technology transfer to six institutes of biological products by the National Institute of Health United States.

Vi polysaccharide vaccine was introduced in the province of Guangxi in 1995; however, there are other provinces in China (provinces of Jiangsu, Hunan, Hubei, Yunnan, Guizhou, and Sichuan, and the cities of Beijing and Lanzhou) that have used Vi polysaccharide vaccine in a targeted program to reduce the burden of typhoid fever. Approximately 26 million doses of vaccine were given to school children and other high-risk groups such as food handlers. The most robust data on the impact of Vi polysaccharide vaccine on the incidence are available from the city of Guilin in Guangxi Province in southwest China from 1995 to 2006. Between 1995 and 2006, more than 1.3 million doses were administered to all target groups, peaking in 2000 and 2001. In all, 77% of the vaccine was given to students and 23% went to food handlers and residents of outbreak areas. Coverage rates have varied broadly from year to year, but have averaged 60–70% for students over the 11-year period and 80–85% for the other target groups [76].

The annual incidence of typhoid reported in the city averaged 57/100,000 in the student population and 42/100,000 in the non-student population from 1991 to 1994. Annual incidence rates of typhoid fever in Guilin from the National Notifiable Infectious Disease Reporting (NIDR) system showed the incidence declined to very low levels (0.2–4.5/100,000) in both the student and non-student population from 1995 to 2006 after vaccination [29]. Vaccine coverage ranged between 3 and 13% among the general population; between 15 and 74% among students. Approximately 3.5 million vaccines were provided to the target region in the specified period of time.

Typhoid vaccine is also recommended for use in outbreak settings in China. The recommendation is based on an effectiveness study of *S.* Typhi outbreak in China in 1999.

5.2 National Immunization Program, Vietnam

In 1997, the National Immunization Program (NIP), Vietnam, took the initiative of typhoid fever vaccination as a regular program. This decision was driven by the increase in the reporting of clinical typhoid fever and the rise in incidence of antibiotic resistance. Typhoid vaccination was limited to half of the 61 high incidence provinces. The vaccines were provided by the National Institute of Vaccines and Biological Substances (IVAC) to the NIP at price of approximately US $0.52 a dose. The typhoid vaccination program involved annual campaigns in which children

3–10 years of age were vaccinated with Vi polysaccharide in selected districts. Children as well as adults were vaccinated in districts with reported typhoid fever outbreaks. More than half a million doses of typhoid vaccine were given to 3- to 10-year-olds in the selected 30 provinces.

Review of the data from the NIP on the use of Vi polysaccharide vaccine in the northwestern region showed a clear decline in the incidence of typhoid fever from 97/100,000 persons per year in 1999 to less than 20/100,000 from 2006 after the introduction of Vi polysaccharide vaccine. Vaccine coverage in the general population ranged between 0.1 and 4%, but it was much higher among the targeted age group. A similar decline in the incidence of typhoid fever was seen not only from the southern Mekong delta region but also from other regions with medium typhoid incidences where Vi polysaccharide vaccine was introduced.

A meta-analysis of typhoid incidence data using prospective surveillance study results and the government's routine disease reports suggests that a targeted immunization strategy is appropriate to reduce the number of cases. An impact and financial analysis further suggests that Vi polysaccharide vaccination in these provinces would need to be more intensive (e.g., covering all districts in a given province) and systematic than the current program in order to have a significant impact on disease incidence in the country as a whole.

5.3 Delhi State, India

The State Government of Delhi, India, funded a typhoid vaccination program for 2- to 5-year-old children with Vi polysaccharide vaccine. The program represented the first public sector typhoid vaccination program in India since 1987 when the old whole-cell vaccine was discontinued due to its reactogenicity and due to the perception that typhoid fever was not a major cause of mortality. The impetus for Vi polysaccharide vaccine introduction was the emergence of multi-drug-resistant typhoid fever among children coming to the city's hospitals. The program targeted 2- to 5-year-old group children that are reported to be at a higher risk. The State Directorate of Family Welfare and the Delhi Municipal Corporation, which provides around 85% of the state's government health services, ran the program. The vaccines are purchased for US $0.53 from a local producer. Since the start of the program, approximately 1 million children have been vaccinated at a rate of 300,000–325,000 children per year. A systematic evaluation of the program is not available, and it is therefore not possible to assess the impact of vaccination on the incidence of culture confirmed or clinical typhoid in the age group and on the general population [77].

5.4 Disease of Most Impoverished (DOMI) Studies in South and SouthEast Asia

Through the DOMI Program, the Vi polysaccharide vaccine was used for a series of effectiveness trials in Asia. Project sites were established in five Asian countries:

Hechi, China; Kolkata, India; North Jakarta, Indonesia; Karachi, Pakistan; and Hue, Vietnam. Study sites were chosen in discussion with the local public health specialists on the basis of a high perceived burden of typhoid fever, absence of control programs against the disease, and willingness of the community to participate. The age groups selected were thought to be the likely targets for typhoid vaccination under a public health program. The projects were designed as a cluster randomized controlled effectiveness trial in all sites except for North Jakarta, which conducted a demonstration project to assess mass vaccination feasibility and safety. The project mimicked the way Vi polysaccharide vaccine might be delivered under public health conditions. In Indonesia and Vietnam, it was deemed most appropriate to target the school children at schools. In other sites, community-based vaccination was considered most appropriate. These decisions were made by the local public health experts and implemented for the projects.

Mass vaccinations were conducted in 2003 and 2004 in five sites, having more than 190,000 people vaccinated with Vi or a control agent. The program proved that very large mass vaccination campaigns are feasible and safe. The vaccination coverage in the target population was between 58 and 91%. The highest coverage rate (91%) was achieved in a school-based program in North Jakarta, Indonesia. The lowest coverage rate was observed in another school-based program in Hue, Vietnam. The community-based mass vaccination campaigns in China, India, and Pakistan had participation rates that ranged between 68 and 78%. Variations in the vaccination coverage might have been related to the different study designs [78–81].

A cluster randomized trial assessed the effectiveness of Vi polysaccharide vaccine through a cluster randomized effectiveness trial in Kolkata, India. 37,673 individuals of more than 2 years of age either received the Vi polysaccharide vaccine or the active control hepatitis A vaccine (Table 6). Protective effectiveness (PE) of Vi polysaccharide vaccine against typhoid fever was calculated to be 61% (95% CI: 41–75) 2 years after vaccination. The trial reported for the first time the Vi polysaccharide protection in children aged 2–5 years with a PE of 80% (95% CI: 53–91). The study reported no serious adverse event associated with the vaccine [82].

Table 6 Vi Polysaccharide effectiveness estimates from Kolkata, India [81]

Age group	Vaccine group		Total protection Vaccine protective effectiveness (95%CI)
	Vi	Hepatitis A	
2–4 years (cases/population)	5/1,097	27/1,095	82% (95%CI: 58%, 92%)
Incidence per 1,000 population	2.3	12.9	
5–14 years (cases/population)	21/4,282	54/4,584	59% (95%CI: 18%, 79%)
Incidence per 1000 population	2.5	6.1	
≥15 years (cases/population)	8/13,490	15/13,125	48% (95%CI: –44%, 81%)
Incidence per 1,000 population	0.3	0.6	

6 Conclusion

Typhoid fever in childhood differs significantly from clinical presentation from adults and case fatality rates are higher in children under 5 although complication rates are almost similar. There are few community-based studies that have looked specifically for typhoid fever. The global estimates of typhoid fever grossly under-report rates of complications and have no data on severity of disease and outcome. There are regional differences in presentation which may reflect differences in care-seeking patterns, health systems, and co-morbidities. Case fatality rates from sub-Saharan and North Africa were higher than Asia and those from central Asia. This may have resulted due to reporting during an outbreak period. There is no evidence that MDR typhoid is associated with consistently higher rates of complications and mortality. Recent emergence of nalidixic acid-resistant strains poses enormous challenges for developing countries with few affordable options for treating typhoid in public health settings. There is an urgent need for expanding the antibiotic pipeline for typhoid and innovative approaches including combination therapies, antibiotic cycling, and reverting to first-line therapy in sensitive cases.

Vi-PS vaccine, unless used at scale for mass vaccination, may not provide protection against typhoid among young children (under 5) in endemic areas. The last Vi-conjugate vaccine efficacy trial (with 89% protection) was over 10 years ago. There is need for alternative strategy of fast tracking Vi-conjugate vaccines in endemic areas, potentially in combination with other antigens (e.g., paratyphoid A).

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Prevention of Vertical Transmission of HIV in Resource-Limited Countries

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1 Introduction – Global Status of Efforts to Prevent Vertical Transmission of HIV

Prevention of vertical (i.e., mother-to-child) transmission of HIV is essential to reduce significant HIV-related child morbidity and mortality in developing countries. Globally, pediatric infections comprise about 15% of all new HIV infections each year and virtually all pediatric infections can be prevented by eliminating vertical transmission [1]. The World Health Organization (WHO) recommendations (revised in 2006) for prevention of mother-to-child transmission (PMTCT)[1] include a four-pronged comprehensive strategy [2]. Although we acknowledge the critical role that all approaches play in reducing pediatric HIV infection, the focus of this chapter is on strategies that address the third prong: preventing HIV transmission from infected mothers to their infants. Considerable achievements have been made on this front, including many clinical trials demonstrating good efficacy. Yet after more than 10 years of global efforts to prevent vertical HIV transmission, only an estimated 18% of pregnant women in 2007 had access to services designed to interrupt vertical transmission [3].

Ministries of health and supporting partners in resource-limited settings have successfully demonstrated the ability to deliver these services and have learned important lessons about how the implementation of services can be improved. Most of the countries that have been hardest hit by HIV have developed guidelines and strategies to achieve national coverage of appropriate HIV prevention services. WHO publishes global guidance on the provision of services, which often serves as the foundation for these programs and national strategies [2]. WHO, the United Nations Children's Fund (UNICEF), and the Joint United Nations Program on HIV/AIDS (UNAIDS) estimate that in developing countries only 33% of pregnant

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[1] Since submission, the WHO has released revised PMTCT guidelines available at: http://www.who.int/hiv/pub/mtct/advice/en/index.html.

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women with HIV and only 20% of HIV-exposed infants are receiving antiretrovirals (ARVs) for prevention of vertical transmission of HIV [3]. This estimate demonstrates the generally poor coverage of services to prevent vertical transmission for pregnant women with HIV. It is also estimated that only 12% of HIV-positive pregnant women themselves eligible for antiretroviral therapy (ART) receive it [3].

2 Program Experience

The Thailand and Botswana national programs and those of the Elizabeth Glaser Pediatric AIDS Foundation (EGPAF) in several countries exemplify what can be achieved. The specific methods employed in the Foundation's programs have been described elsewhere [4, 5].

2.1 Thailand and Botswana National Programs

National programs to prevent vertical HIV transmission in Thailand and Botswana are largely organized and supported by local governments and demonstrate the potential effectiveness and feasibility of interrupting vertical transmission on a national scale with adequate resources [6, 7]. These countries are leading middle- and lower-income countries in successfully implementing national strategies that have been documented to decrease vertical transmission.

2.1.1 Thailand

Successful approaches to reduce vertical transmission have been documented in Thailand [6]. The country reports that 95% of pregnant women attend antenatal care (ANC) and 97% have access to PMTCT services. The vast majority (85%) of deliveries take place in public hospitals and 94% of pregnant women are tested for HIV. The HIV seroprevalence among pregnant women in Thailand is 1.5%, and 70% of HIV-positive women receive ARV prophylaxis to prevent vertical transmission.

The impressive results of Thailand's program from 2001 to 2003 report a total of 2,200 HIV-exposed infants registered in six provinces. There were known outcomes for 1,667: 1,509 (90.5%) were uninfected and 158 (9.5%) were infected [6]. The cohort which is non-breastfeeding was followed for a minimum of 2 years [6]. The observed vertical transmission rates by birth year were 10.3% in 2001, 9.4% in 2002, and 8.6% in 2003 [6]. These rates are reportedly 46–58% lower than before the PMTCT program was initiated, with dramatic decreases in transmission achieved through the provision of 4 weeks of zidovudine (AZT) to mothers during the antepartum period only. The program's successful service coverage and uptake resulted in a final observed transmission rate of less than 9% [6].

2.1.2 Botswana

PMTCT services have been available in every public antenatal care (ANC) clinic in Botswana since 2002. Botswana's national program provides AZT to pregnant women with HIV from 28 weeks' gestation, with single-dose nevirapine (sdNVP) given at the onset of labor for women with CD4 counts >200 cells/mm^3. Women with lower CD4 counts receive ART when eligibility is determined. Botswana also provides free replacement formula feed for HIV-exposed infants. Data from Botswana's national program were presented at the International AIDS Conference in Mexico City in 2008. Among 10,516 HIV-exposed infants who received polymerase chain reaction (PCR) testing to determine their HIV status between October 2006 and November 2007, the vertical transmission rate in mothers who received no prophylaxis was 12% whereas for mothers receiving sdNVP only, transmission was 7%, a 43% reduction [7]. Transmission was lower (0.7%) among mothers who initiated ART prior to pregnancy [7]. Those with low CD4 counts (<200 cells/mm^3) who initiated ART during pregnancy transmitted HIV to their infants at a rate (2.3%) comparable to that (3.3%) of mothers with higher CD4 counts receiving at least 4 weeks of AZT plus sdNVP [7]. Among mothers receiving less than 4 weeks of AZT, the transmission rate was approximately 5% [7].

2.2 Elizabeth Glaser Pediatric AIDS Foundation Program and Experience

The Elizabeth Glaser Pediatric AIDS Foundation (EGPAF) has supported programs to prevent vertical transmission and/or to provide care and treatment in 22 countries since 2000.

Most of the countries supported are located in southern and eastern Africa, the regions of the continent with the highest HIV disease burden. Seven countries have continuing programs which no longer require EGPAF support (see Fig. 1; shown in yellow). The programs were originally focused on PMTCT but as they have rapidly grown in size and number, they have also gradually expanded to include a mix of adult and pediatric care and treatment services as well.

Over time, experience and shifts in procedure and policy have improved the uptake of services. At the same time the program has expanded, reaching almost 2 million women during 2003–2005, compared to 250,000 in 2000–2002, and in the years 2006–2008, more than 4.5 million women accessed ANC services in 18 countries. Pregnant women routinely receive HIV counseling, 85% are tested for HIV and at least 80% of HIV-positive pregnant women receive ARV prophylaxis (see Fig. 2). The greatest limitation to preventing even more infections is the level of service coverage achieved to date.

Infant uptake of ARV prophylaxis is consistently lower than maternal ARV uptake. This needs to be understood in order to improve programs further. Frequency of delivery in a facility (or by a skilled birth attendant) varies by country and region but is generally lower than ANC attendance. Infants born at home usually do not

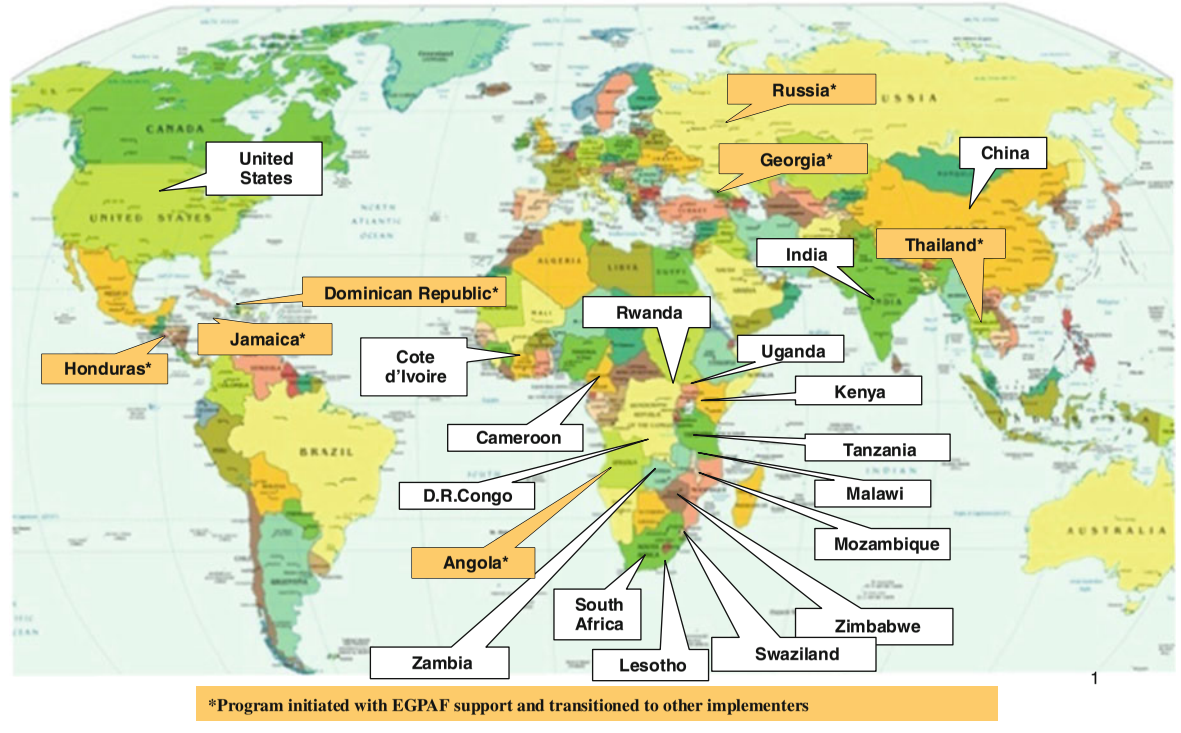

Fig. 1 The Elizabeth Glaser Pediatric AIDS Foundation Global Program Map

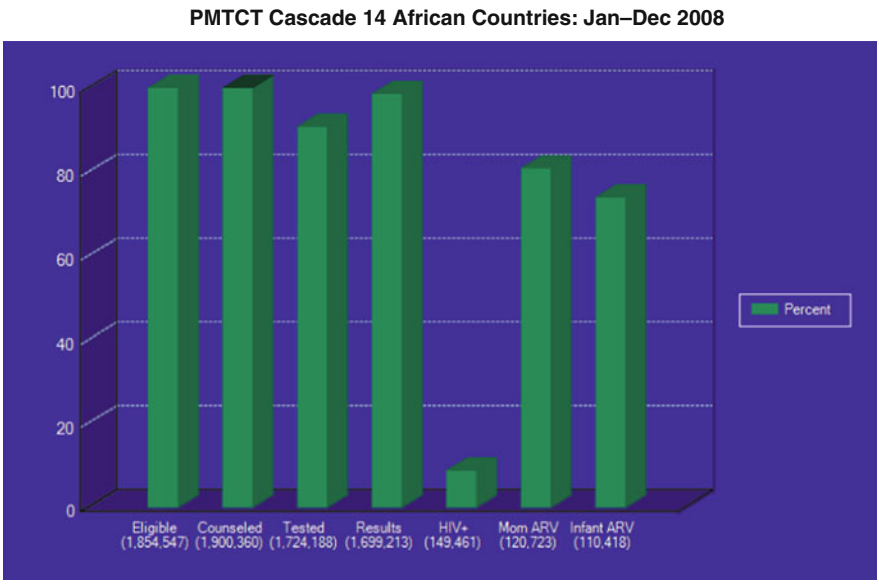

Fig. 2 The Elizabeth Glaser Pediatric AIDS Foundation Africa Program Cascade. ARV, antiretroviral; PMTCT, prevention of mother-to-child transmission. *Source*: EGPAF program data. *Calculation Footnote*: *Mother ARV = sdNVP in ANC + sdNVP in L&D + ANC ART. Infant ARV = sdNVP in ANC + sdNVP in L&D + combination regimen in L&D*

receive ARV prophylaxis. The reported percentage of deliveries taking place in EGPAF-supported health facilities has remained approximately the same over time despite the addition of PMTCT services to these facilities. One must assume that the provision of ARVs for infants is not communicated or is not perceived as sufficient incentive to deliver in a facility or that the general barriers (financial, geographic, logistic, or gender-related) which women face in accessing facilities for delivery have not yet been adequately addressed.

As of December 2008, health facility delivery rates in 17 countries in the program ranged from 28% in Rwanda to 88% in China [8] and were less than 60% in the majority of the African countries [8]. Despite effort to improve the health system infrastructure and quality of maternal and child health (MCH) services for women with HIV, the reality is that almost half of HIV-positive women will not return to deliver their infants in a health facility. Consequently, in many countries, infant ARV prophylaxis is provided in an oral syringe for the mother to take home after her initial ANC visit. Improved uptake is seen using this intervention [4] but increasingly complex combinations of drugs for both mother and infant require programs to evaluate and adapt these approaches.

In Uganda, where roughly 40% of infants are delivered in a health facility, there is roughly 45% uptake of the infant ARV dose. By contrast, in Swaziland, where

75% of infants are delivered in a health facility and where infant ARV prophylaxis is dispensed during the antenatal period, there is close to 100% uptake of the infant dose.

3 Lessons Learned

3.1 Counseling

Counseling for pregnant women initiating antenatal care has evolved from one-on-one pretest counseling toward an approach in which the majority of HIV messages are integrated into a group counseling session. This also covers other important health topics and is intended to be useful to all pregnant women, not just those living with HIV. Posttest counseling continues to be provided one-on-one and is a key opportunity to reinforce HIV prevention messages for all women regardless of their HIV serostatus. Information on exclusive breastfeeding is given to all women as well. For those who are HIV positive, counseling is tailored to provide specific information on future infant feeding choices and should be the point of initiation into longitudinal HIV care, beginning with staging and screening for treatment and enrolment into care.

3.2 Testing

An important early program change was the introduction into antenatal care of rapid HIV tests and their inclusion in national policies [4]. Providing same-day results dramatically improved the percentage of women who received their results and had the opportunity to access ARV prophylaxis and/or continued care and treatment.

Initially pregnant women were asked if they would like to receive special counseling and testing for HIV. In other words, they had to "opt in." As counseling and testing coverage remained at steady but suboptimal rates, HIV counseling and testing were integrated into routine ANC services and are provided unless women specifically refuse, or "opt out," This "provider-initiated" approach has resulted in substantial increases in the proportion of women receiving counseling and testing in ANC [5].

3.3 ARV Prophylaxis

Provision of ARV prophylaxis in the form of sdNVP to women with HIV and their infants has been the catalyst of most PMTCT services in the countries where EGPAF works. The simplicity of this regimen (one dose for the mother in labor and one dose for the infant within 72 h of delivery), and a donation program that provides NVP for free to PMTCT programs in selected resource-limited countries, has permitted

the initiation of PMTCT services in facilities with no prior ARV experience, including primary care and rural health facilities. In 2006, WHO revised its guidelines to emphasize the use of combination ARV regimens (starting AZT antepartum at 28 weeks' gestation, sdNVP plus AZT/lamivudine (3TC) intrapartum, and AZT/3TC for 7 days postpartum) for pregnant women with HIV who do not yet require ART themselves. These combination regimens have been shown to be more efficacious in preventing vertical transmission and potentially to limit development of viral resistance in the mother to non-nucleoside reverse transcriptase inhibitors (NNRTI) following sdNVP [2].

While most countries have revised their national policies to favor the use of combination regimens, many countries have been slow to roll them out owing to the need for additional training of MCH staff and logistical considerations. EGPAF is supporting ministries of health and other in-country partners to introduce the use of combination regimens at the lowest level including the most remote health centers. They can also be introduced during the initial staff training for new sites. Nevertheless, sdNVP remains an important option as PMTCT services continue to expand at a rate that exceeds the roll-out of the training and systems improvements required for complete coverage of combination regimens.

3.4 Uptake of Maternal and Infant Prophylaxis

Optimally, all pregnant women with HIV not eligible for treatment should be started on AZT during pregnancy (at 28 weeks gestation) and should receive sdNVP during labor when they come to the health facility to deliver. Improvement of maternal uptake of ARV prophylaxis has been achieved by dispensing ARVs at the time of HIV diagnosis rather than waiting for delivery. Optimally, pregnant women should receive at least four prenatal examinations. However, in most of the countries where the Foundation works less than 60% of pregnant women achieve this and the rates can be very low as, for example, in Rwanda where it is only 13% even though 94% come for one ANC visit [8]. Logically, therefore, expectant mothers should receive ARVs when they first come to the ANC clinic. While it is not guaranteed that a woman will actually take the ARVs dispensed, she must have the medication on hand in order to have access to the intervention. Dispensing ARVs at the time of diagnosis has been shown to increase the proportion of women receiving ARVs to about 90% in studies conducted in Tanzania, Cameroon, and Kenya [4]. Despite this, dispensing of ARVs at the first ANC visit is not permitted in many countries. Foundation-supported programs have seen significant improvement in ARV uptake where such policies have been changed [5]. Dispensing is more complicated for combination regimens, as women must return several times to receive longitudinal ARVs (usually AZT) for the remainder of gestation, while NVP and the initial month of AZT can be dispensed at the time of diagnosis. AZT can safely be given from 14 weeks of gestation without increasing viral resistance rates. Despite this some national policies still prohibit giving AZT prior to 28 weeks, thus denying

access to the intervention to women who do not return 12 weeks or less prior to delivery.

3.5 HIV Testing in Labor and Delivery

Ideally, the HIV serostatus of all pregnant women arriving in labor and delivery should be known. However, for a variety of reasons (e.g., not attending ANC, attending ANC that has no PMTCT services, disruption of services), the HIV serostatus of a significant number of women arriving in labor and delivery is unknown. Most, but not all, countries have changed national policies to allow routine HIV counseling and testing in labor and delivery.

Diagnosing a pregnant woman with HIV infection in labor and delivery is far from optimal, especially if combination regimens and/or ART were available to her in the ANC setting, but it still affords an important opportunity to provide ARV prophylaxis to both mother and infant to reduce the risk of vertical transmission. From when the Foundation first began collecting information on HIV testing in labor and delivery in 2005 until December 2008, of the countries that are able to report on HIV status in maternity, 8.6% of more than 4 million women in 18 countries were reported as "HIV status unknown" in labor and delivery. Of these 88.7% were then tested for HIV and 5.4% were found to be HIV positive. Such testing permits access to ARV prophylaxis to this significant group [9].

4 Modeling Service Coverage

There is a need to enhance and expand geographic coverage (i.e., access to PMTCT services by pregnant women), coverage by the provided services (enhancement of each part of the cascade of services), and coverage with therapy (the proportion of immunocompromised HIV-positive women eligible for therapy who receive it). Achieving significant progress in service coverage, which could avert the majority of the pediatric HIV infections worldwide, will require the full support of national governments as well as adequate and sustained financial resources from donors.

Table 1 illustrates the theoretical success of PMTCT programs in averting HIV infections along the steps of the basic PMTCT cascade. Using a theoretical sample of 100 HIV-positive pregnant women, the table shows the proportions utilizing ANC services, counseled, tested, and receiving ARV prophylaxis based on average uptake percentages in actual EGPAF programs for the stated time intervals. We assume for the purposes of this model that HIV-positive women who do not know their status attend ANC at the same rate as other women. Vertical transmission rates in this model have been calculated for three different scenarios of ARV prophylaxis provision and all are based on the assumption that all women with CD4 counts of <200 cells/mm^3 receive ART. Calculation #1 assumes that all HIV-positive

Table 1 Modeling of effectiveness of interventions to prevent vertical transmission: using a theoretical sample of 100 HIV-positive pregnant women

	2000–2002	Number included in program	Number missed	2003–2005	Number included in program	Number missed	2006–2008	Number included in program	Number missed
Attend ANC 1×percent	90%	90	10	90%	90	10	90%	90	10
Counseled percent	84%	75	25	93%	84	16	100%	90	10
Tested percent	80%	60	40	82%	69	31	89%	80	20
Mom ARV percent	60%	36	64	76%	52	48	82%	66	34
	Expected TR	Number infected	Overall TR		Number infected	Overall TR	Number infected		Overall TR
In absence of ARV	25%	16			12		8.5		
Calculation #1	8%	2.9	18.9%		4.1	16.1%	5.3		13.8%
Calculation #2	3%	1.1	17.1%		1.6	13.6%	2.0		10.5%
Calculation #3	2%	0.7	16.7%		1.0	13%	1.3		9.8%

Source: Barker [10].

ANC, antenatal care; ARV, antiretroviral; TR, transmission rate or total number infected (number infected with no ARV + number infected by calculation 1, 2 or 3 per 100 patients).

women with CD4 counts >200 cells/mm^3 receive sdNVP. Calculation #2 assumes that all HIV-positive women with CD4 counts >200 cells/mm^3 receive 12 weeks of AZT + sdNVP. Calculation #3 assumes that all HIV-positive women, regardless of CD4 count, receive ART. The proportion of women visiting an antenatal clinic at least once during their pregnancy is currently estimated at approximately 90% in Foundation-supported countries, with this proportion varying greatly by country [8]. According to the model, out of a theoretical sample of 100 pregnant women with HIV, 10 will not access any antenatal services.

4.1 2000–2002

Looking at the years 2000–2002 (Table 1), with counseling accessed at a rate of 84%, 75 women with HIV would have been counseled and offered testing, and 60 women (80% of 75 counseled) would have been tested, leaving 40 HIV-infected women (30 who did access antenatal care) with unknown HIV status. If ARVs were given to 60% of women known to be HIV positive, then 36 women out of the original 100 would go on to receive the prophylactic intervention and 64 would not. If there were a vertical transmission rate of 25% for all those who failed to receive ARVs, then of the 64 who missed services, 16 would transmit infection. Depending upon the ARV regimen available, vertical transmission varies from 2 to 8% among the women who receive it. An estimated overall rate of 8% of HIV-positive women receiving sdNVP or ART, depending upon their CD4 count, will transmit the virus to their infants. A combination regimen of AZT plus sdNVP lowers the transmission rate to 3%, and if ART were given to all HIV-positive pregnant women a transmission rate of 2% could be achieved [10]. Adding the instances of transmission among those not accessing the intervention together with transmission instances among those who received the intervention results in overall transmission rates of 16.7–18.9%. Note that this model does not take into account late postpartum transmission from breastfeeding. This model demonstrates that the ARV regimen used has less of an impact on transmission than do increases in the number of women attending ANC and in improvements in uptake of counseling, testing, and delivery of ARVs to all those who come in for services.

4.2 2003–2005

In 2003–2005, ARV prophylaxis was dispensed to approximately half the women eligible to receive it. Of the 48 women who would have missed services according to the model, an estimated 12 mothers would have transmitted HIV to their infants. From 1.0 to 4.1 infants are estimated to become HIV infected among mothers receiving ARV for prophylaxis or therapy, depending upon the ARV regimen. The overall effectiveness of the ARV intervention is calculated with a transmission rate of 13.0–16.1% (see Table 1).

4.3 2006–2008

In 2006–2008 (Table 1), significant improvements were seen in the uptake of services. However, with 10% of all HIV-positive pregnant women not receiving antenatal care, 10% of those counseled not being tested, and 18% of those known to be HIV positive not receiving ARVs, there were 34 out of 100 HIV-positive women who missed the benefit of services. The overall vertical transmission rates were lower for this time period, calculated at 9.8–13.8%, depending upon the ARV regimen used. Again it is noted that successful delivery of services is more influential than the specific regimen used to prevent transmission. Combination regimens are more efficacious, but even with ART administered to every pregnant woman identified as HIV positive (Scenario #3), the model shows that overall transmission rates of less than 9.8% cannot be achieved due to current gaps in service delivery.

5 Effectiveness of Prevention of Vertical Transmission Programs: The PEARL Study

Global goals to reduce vertical transmission are ambitious and appropriate in their scope, yet a lack of clarity and consensus regarding how to monitor the effectiveness of PMTCT programs makes it difficult for policymakers to mount a coordinated response [11]. Some have advocated for the use of population-level "HIV-free child survival" as a gold standard metric to measure the effectiveness of PMTCT programs [11]. A recent CDC-supported study, "PMTCT Effectiveness in Africa: Research and Linkages to Care and Treatment," or "PEARL," measured PMTCT program effectiveness in nonclinical trial settings in Cameroon, Côte d'Ivoire, South Africa, and Zambia [12]. All 43 health centers included in the study offered ongoing PMTCT services that provided a minimum of sdNVP and, in some cases, combination prophylaxis regimens or ART. The study design and assessment of program effectiveness are partially described in a published report preceding the study results [11]. A novel community-survey-based approach was adopted in the hope that it could be implemented widely in developing countries, with only minor modifications required to the existing demographic and health surveys [11].

The study also obtained umbilical cord blood specimens from 28,061 women in delivery to determine their HIV status and to measure the presence of NVP [12, 13]. This method provided a means to document whether dispensed prophylaxis was actually taken [12].

The study documented pervasive gaps in service delivery in representative urban and rural clinics in four countries [12]. Failures were observed at each step of the PMTCT cascade: health facilities failed to test women, provide test results, and dispense NVP and mothers failed to ingest the prophylaxis they were given (see Fig. 3). Service coverage (defined as including dispensing of maternal and infant prophylaxis) across the facilities in four countries was found to be only 50% [12].

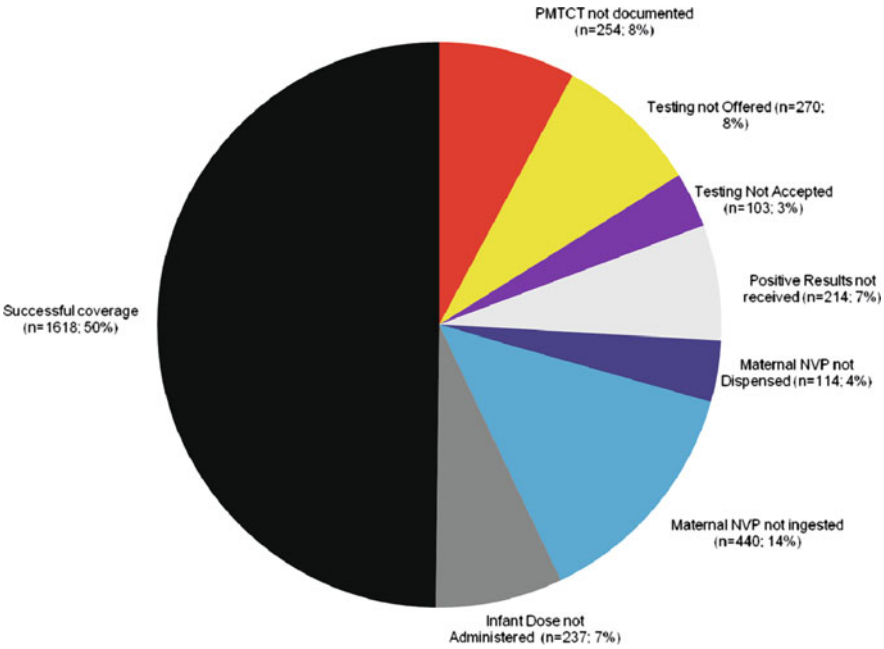

Fig. 3 Results of the PEARL study. PMTCT, prevention of mother-to-child transmission; NVP, nevirapine. *Source*: Stringer [12]

In addition to the functional problems described, women not actually ingesting the ARVs represents an additional barrier that needs to be addressed.

6 Importance of Identifying Pregnant Women Eligible for ART

A crucial element in the effort to enhance the overall impact of PMTCT services is to identify pregnant women with HIV who are eligible to receive ART. It is estimated that over 80% of cases of vertical transmission and the same proportion of maternal deaths occur among women with CD4 counts <350 cells/mm^3 [14]. It is likely that as many as 30–50% of women diagnosed with HIV infection in ANC have CD4 counts <350 cells/mm^3 [15]. Treatment of these women could therefore dramatically decrease vertical transmission rates while maintaining maternal health. However, identifying women most in need of treatment remains a challenge, as most ANC sites in resource-limited settings do not have the tools (e.g., CD4 testing, viral load testing) to monitor disease stage.

Ideally, ART eligibility is ascertained at the time of HIV diagnosis through staging performed in ANC and/or by obtaining a CD4 count. Clinical staging is difficult and particularly insensitive during pregnancy, and point-of-service CD4 testing is often unavailable at lower-level clinics. If CD4 testing cannot be performed at the facilities, blood samples must be sent to a central testing facility, and it can take

several days to several weeks for results to be returned to the original clinic. This requires one or more additional ANC visits by the pregnant woman, depending on how long it takes to obtain the test results. Additionally, if the ANC clinic cannot initiate ART, the mother must be referred, and these additional visits and actions required by the woman are critical barriers to her accessing appropriate care. Such logistical barriers are partially responsible for the low proportion of women who are actually screened and staged and eventually enrolled into care or initiated on ART.

Because ANC and other HIV-related services are often provided by different units or facilities that lack common patient information systems to track individual women, following women with HIV identified in ANC and reporting the numbers subsequently enrolled into care and those started on treatment has proven difficult (see Fig. 4). When sites do report these data, it is evident that too few women are receiving ART. At Foundation-supported sites, a very small percentage of HIV-positive women are reported to receive combination therapy (see Fig. 5). This is consistent with UNICEF reports and highlights the need for care providers to focus on improving the identification and treatment of pregnant women eligible for ART.

To improve the identification and initiation of treatment of eligible women on ART, many countries are now moving to integrate ART services into the MCH units [16–18]. For example, in Swaziland, a pregnant woman initiates treatment in MCH and is followed there postpartum until her child is 2 years old, at which point she is then referred to the regular ART clinic. This requires adequate staff in the busy MCH settings who are trained to undertake WHO clinical staging and/or have a process for drawing, tracking, and sending blood samples for CD4 count and are able to interpret results and initiate treatment. Experience has shown that training is not enough, and MCH staff need continued mentoring to provide good quality services [19].

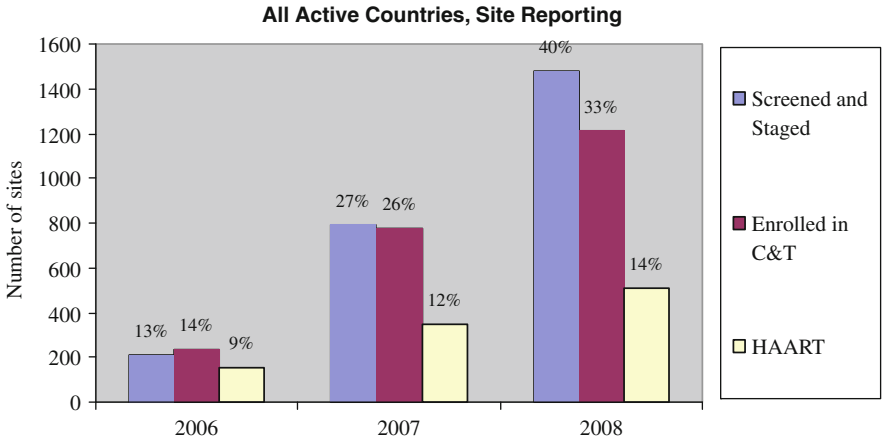

Fig. 4 All active EGPAF country programs, site reporting. *Note*: % refers to proportion of total PMTCT sites. C&T, counseling and testing; HAART, highly active antiretroviral therapy; PMTCT, prevention of mother-to-child transmission. *Source*: EGPAF program data

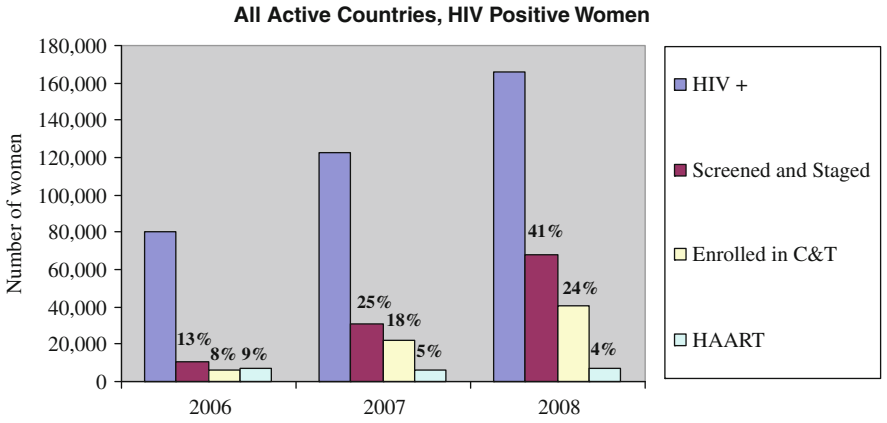

Fig. 5 All active EGPAF country programs, HIV-positive women. *Note*: % refers to proportion of HIV-positive women. C&T, counseling and testing; HAART, highly active antiretroviral therapy. *Source*: EGPAF program data

7 Prevention of Vertical Transmission During Breastfeeding

The finding that HIV can be transmitted from a mother to her infant during breastfeeding has created major dilemmas in resource-limited settings, with an estimated 40% of vertical transmission attributed to breastfeeding in these settings [20]. Improving *HIV-free survival* of infants and children is the ultimate goal of all programs aimed at preventing vertical HIV transmission, yet it has been repeatedly shown that replacement feeding in these settings carries a significant risk of morbidity and mortality for HIV-exposed infants [21, 22]. Improving HIV-free survival of infants must be accomplished while achieving optimal breastfeeding practices. Exclusive breastfeeding for the first 6 months and continued breastfeeding up to 11 months is the single most effective preventive child-survival intervention for all infants [23]. Therefore, HIV-exposed infants should be afforded the same health benefits of exclusive breastfeeding for the first 6 months of life as all other infants.

In the context of HIV, it is imperative that optimal infant and young child feeding practices be ensured. The possibility of reducing postnatal HIV transmission by providing ARVs to the lactating mother or the breastfeeding child has been studied extensively in recent years. It has also been shown that early weaning is not safe in most resource-limited settings. In the PEPI study in Malawi, prolonged ARV prophylaxis administered to the infant was found to reduce postnatal HIV infection significantly [24]. However, early weaning was encouraged, and two-thirds of mothers had stopped breastfeeding after only 9 months postdelivery. Observational data suggest that ART administered to mothers during lactation can reduce transmission of HIV to the infant; additionally, women who require ART for their own health should receive therapy regardless of the vertical transmission prevention benefits. There is a current debate regarding whether mothers with higher CD4 counts should also receive ART during lactation. The recently concluded BAN study in Malawi

demonstrated that both maternal ART and infant NVP administered for 28 weeks were safe and effective in reducing postnatal vertical transmission [25]. The study was not large enough to compare the efficacy of the two regimens. Despite these findings, programs have not yet begun to administer postpartum NVP to infants or ART to mothers routinely regardless of maternal CD4 count, as the feasibility of these interventions still needs to be established.

Based on recent findings, WHO recommends exclusive breastfeeding for the first 6 months of an infant's life and weaning at about 1 year of age [26]. In settings and individual instances in which mothers can safely manage replacement feeding according to the AFASS (acceptable, feasible, affordable, sustainable, and safe) criteria, replacement feeding or early weaning is suggested.

8 Conclusion

Prevention of vertical HIV transmission is of critical importance, yet developing functional programs to deliver badly needed services is a complex undertaking. Low- and middle-income countries, especially those hardest hit by HIV, need to prioritize these services while dedicating appropriate resources to initiate and build sustainable programs. Eliminating pediatric HIV infection is possible, but careful attention must be paid to research establishing the efficacy of various interventions and barriers to their implementation while outlining solutions to guide program implementation.

Access to prevention programs must be expanded for all pregnant women to improve service coverage for women with HIV. Service providers must work hard to optimize each step of the PMTCT cascade in order to improve coverage and thereby achieve optimal outcomes. Focusing on identification and enrollment of women with HIV who are eligible for ART is essential for maternal health and to decrease vertical transmission maximally among this higher-risk group. To achieve significant increases in the HIV-free survival of infants, breastfeeding practices must be optimized, along with ARV regimens shown to be effective in decreasing transmission during the breastfeeding period.

The evolution of the Foundation's PMTCT programs has taught us some important lessons about how these programs can be optimized, but the numerous challenges described indicate that much work remains. However, experience from national programs in Thailand and Botswana shows that it is possible to achieve low transmission rates.

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Pneumonia in Children in Developing Countries

Frank Shann

1 Introduction

Pneumonia is the commonest cause of death in children [1, 2]. The World Health Organization (WHO) and UNICEF estimate that pneumonia is the primary cause of 19% of all deaths in children less than 5 years of age. In addition, most of the 10% of deaths caused by neonatal sepsis are associated with pneumonia and bacterial pneumonia is an important factor in many of the deaths caused by measles, pertussis and HIV [2]. Pneumonia therefore causes approximately one-third of all child deaths. Far too little effort has gone into research on pneumonia and its prevention and treatment.

Many factors are known to increase the risk of death from pneumonia. These include malnutrition, low birth weight, failure to breastfeed exclusively during the first four months of life, lack of measles immunisation, indoor air pollution and crowding [1].

2 Aetiology

Until the early 1980s, the conventional view was that "no specific causative agent is found in most patients" dying from respiratory tract infection and therefore that little could be done to reduce mortality from pneumonia [3, 4]. It was felt that attention should instead be focussed on diseases that could be treated, such as diarrhoea with dehydration and malaria. This view was based on studies that searched carefully for evidence of viral infection in children with pneumonia but did not involve bacterial cultures of percutaneous lung aspirates from children who had not received antibiotics [5–7].

Several lines of evidence suggest that most fatal pneumonia in children is caused by infection with *Streptococcus pneumoniae* or *Haemophilus influenzae* [8]. Firstly are findings from studies conducted in the United States and Europe during the

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period before antimicrobial therapy was available. Bacteria were grown from percutaneous needle aspirates of the lung in 57% of children with pneumonia. Mortality was 65% in 99 children with a positive blood culture, compared to 17% of 826 children with a negative blood culture. Secondly, in controlled trials, the mortality rate from pneumonia was 2.3% in 824 children treated with sulphonamides, compared to 5.4% in 1491 control children. Thirdly, percutaneous needle aspirates of the lung yielded positive cultures in 71% of 321 children with pneumonia in developing countries in the pre-antibiotic era and in 58% of 705 children in the antibiotic era [8].

A detailed study of the bacteriology and virology of severe pneumonia was performed in 83 children in Papua New Guinea in the early 1980s [9]. Bacteria were grown from lung aspirate or blood in 68% of 71 children who had not received antibiotics, and viruses from 29% of 62 children; isolation of bacteria was strongly associated with mortality, but isolation of viruses was not. *Streptococcus pneumoniae* or *H. influenzae* or both were isolated from 52% of the children and from seven of the eight children who died. Among the *H. influenzae* isolated from lung or blood, 56% were non-serotypable strains, 25% were non-b serotypes (a, c, d, e or f), and only 19% were type b. Studies in several other developing countries have confirmed that strains of *H. influenzae* other than type b are an important cause of pneumonia in children [10]. In the early 1980s, this evidence convinced WHO to set up a programme to reduce mortality from acute respiratory infections in children [11–14].

The aetiology of pneumonia in children can only be determined reliably by doing needle aspirations of the lung in children who have no antibiotic activity present in blood or urine [15]. Studies that use blood culture and antigen detection without lung aspiration yield misleading results and studies that include children who have received antibiotics will grossly underestimate the proportion of cases caused by bacteria. The history is not a reliable indication of antibiotic use: the child's blood or urine must be tested for antibiotic activity [15].

3 Standard Management

Mortality from pneumonia caused by *S. pneumoniae* or *H. influenzae* can be substantially reduced by early treatment with antibiotics [8]. However, mortality is highest in the remote and disadvantaged regions of low-income countries [1] and early treatment will be possible only if we can train and support primary health-care workers to give antibiotic therapy in these regions. The antibiotics need to be effective, inexpensive, and suitable for use in primary health care. Since very few antibiotics meet all these criteria, their use needs to be restricted to limit the development of resistance.

In high-mortality areas, the guidelines for primary health-care workers should use the *minimum* number of criteria needed to decide on treatment reliably, and the *minimum* treatment needed to reduce *mortality* [16]. Unfortunately, these principles

have not always been observed and the WHO guidelines have become more complicated than necessary, and have sometimes been based on potentially misleading information from medium- or low-mortality areas [17, 18]. A clear distinction needs to be made between guidelines written for very high-mortality areas with minimal health-care resources and guidelines written for medium–low-mortality areas where more resources are available for health care and viruses cause a higher proportion of severe respiratory tract infection.

4 Which Children Need an Antibiotic?

Children with pneumonia have cough or difficulty in breathing. However, most children with cough or difficulty in breathing do not have pneumonia: they have only a mild respiratory infection and do not need an antibiotic. The best indication that children need antibiotics is their respiratory rate; in general, children without tachypnoea do not need an antibiotic and children with tachypnoea are more likely to benefit from antibiotic therapy [12, 19–21]. Fever is not a useful discriminator: children with viral infections often have a fever when they do not need antibiotics. Listening with a stethoscope is far less reliable than counting the respiratory rate and looking for chest indrawing and absence of prolonged expiration.

In high-mortality areas, it is important to decide which criteria for the use of antibiotics will save the most lives, while minimising the use of antibiotics (to delay the development of resistance). This is *not* the same as deciding which children have pneumonia; for example, young infants have a higher case fatality rate and are more likely to benefit from antibiotics than are older children with the same clinical findings. WHO suggests that antibiotics should be given to children aged 2–12 months if they have a respiratory rate $\geq$ 50 breaths/min and to children aged 12–59 months if they have a respiratory rate $\geq$ 40 breaths/min [18]. This age-dependent definition has a higher sensitivity for the diagnosis of pneumonia than using a rate $\geq$ 50 breaths/min for all children aged 2–59 months. However, children aged 15–59 months who present with a respiratory rate of 40–49 breaths/min have a low incidence of pneumonia and a low case fatality rate, so treating these children greatly increases the use of antibiotics with very little effect on total mortality [22]. For this reason, when deciding which children should receive antibiotics (rather than which children have pneumonia), tachypnoea should be defined as $\geq$ 50 breaths/min in all children aged 2–59 months.

There are important causes of false-positive and false-negative findings with tachypnoea. False-positive findings occur in children with bronchiolitis or asthma who often have tachypnoea when they do not need antibiotics. In very high-mortality areas, it is probably sensible to give antibiotics to infants with bronchiolitis and asthma is usually uncommon in these areas, so this is not an important problem. However, in medium–low-mortality areas, a high proportion of infants with tachypnoea have bronchiolitis or asthma, so tachypnoea (and chest indrawing) are unreliable indications of the need for antibiotics unless small airway obstruction

has been excluded. This is best done by observing the chest and looking for a prolonged active expiratory phase and *not* by listening for wheeze with a stethoscope. Separate guidelines are needed for medium–low-mortality areas when bronchiolitis and asthma are common.

False-negative findings with tachypnoea may occur in children with very severe pneumonia: such children sometimes have slow, laboured respiration. However, all these children will have chest indrawing (see below). Children without chest indrawing should be given antibiotics only if they have tachypnoea.

5 Which Children Need Admission?

Among children who do not have small airway obstruction, tachypnoea is a sign that the lungs are stiff (have reduced compliance) from inflammation and accumulation of pus in the alveoli. As lung compliance falls even further, chest retraction (or chest indrawing) develops; this is the best sign that a child has severe pneumonia and requires admission for intensive antibiotic treatment [16, 20, 21]. The WHO definition of the term chest indrawing was initially vague; it should be used to mean the presence of subcostal retraction plus either intercostal or supracostal retraction [16, 21]. False-positive chest indrawing may occur in the absence of pneumonia in children with airway obstruction (those with small airway obstruction will have prolonged expiration or audible wheeze) and in preterm babies (who all have a degree of chest indrawing).

Two controlled trials performed at tertiary-care facilities have suggested that children with chest indrawing could safely be treated at home with oral amoxicillin [23, 24]. However, it is likely that few of the children had bacterial pneumonia: lung aspirates were not cultured, only 14 of the 3,739 children died, and 63% had wheeze, so these results should not be extrapolated to areas with a high mortality from pneumonia.

6 Which Children Have Very Severe Pneumonia?

WHO defines very severe pneumonia as the presence of chest indrawing plus either central cyanosis or severe respiratory distress (for example, head nodding) or the inability to drink – providing small airway obstruction has been excluded [17, 18]. There is considerable evidence to support these criteria [21, 25]. Unfortunately, the clinical diagnosis of cyanosis is not reliable. When pulse oximetry is not available, it is helpful to compare the colour of the child's tongue to that of the mother's tongue. The lips look "cyanosed" in pigmented children, and all people look "cyanosed" in ordinary fluorescent lighting.

There is confusion about the measurement of hypoxaemia. In places fortunate enough to have pulse oximetry available, a clear distinction should be made between the use of saturation as an indication of the severity of pneumonia (less than 90% is associated with a much higher mortality) [21] and as a guide to when to give

oxygen. With normal cardiac output, haemoglobin concentration and pH, arterial oxygen saturations of 68% or more are not dangerous [26], which suggests that it might be sensible to give supplemental oxygen only when the saturation is less than 80% if oxygen supplies are limited. When comparing the effectiveness of different methods of giving oxygen, arterial oxygen tension is much more sensitive than oxygen saturation because of the sigmoid shape of the haemoglobin–oxygen dissociation curve [27].

7 Which Antibiotic for Outpatients With Non-severe Pneumonia?

Most children with bacterial pneumonia recover without antibiotic therapy [8]. Consequently, antibiotics and vaccines may have a substantial effect on mortality even if they tip the balance only slightly in favour of the host. For example, if 90% of children recover from pneumonia without antibiotics, an increase in survival of only 5% (to 95%) means that mortality has halved from 10 to 5%.

At the time that the WHO protocols were developed in the late 1970s and early 1980s, most strains of *S. pneumoniae* and *H. influenzae* were sensitive to benzyl penicillin (but not phenoxymethylpenicillin), amoxicillin, and cotrimoxazole. Pharmacokinetic studies performed in children in Papua New Guinea suggested that mean serum penicillin levels were greater than 1.0 μg/mL for about 11 h after 48 mg/kg (48,000 U/kg) of procaine penicillin given by intramuscular injection [28]. The early WHO protocols therefore suggested that children should be treated with intramuscular injections of 50 mg/kg of procaine penicillin given daily, or 15 mg/kg of amoxicillin orally three times a day, or cotrimoxazole (4 mg/kg of trimethoprim and 20 mg/kg of sulphamethoxazole) orally twice a day.

Cotrimoxazole is inferior to amoxicillin for the treatment of proven infections with *H. influenzae* or *S. pneumoniae* in bacterial pneumonia [29] and in otitis media [30, 31]. Otitis media provides useful information about the treatment of pneumonia because it is caused by the same organisms, and bacterial cultures can be performed using tympanocentesis before, during and after treatment. Two studies that were not supported by microbiological evidence concluded that cotrimoxazole is as effective as amoxicillin or procaine penicillin for pneumonia, but only 66 children received cotrimoxazole in the first study [32] and the other study involved only children with non-severe pneumonia where a very large sample size would have been needed to detect a difference in efficacy [33]. Cotrimoxazole should no longer be used as a first-line treatment for pneumonia in high-mortality areas [29–31] except as an adjunct to amoxicillin in HIV-affected areas when pneumocystis pneumonia is prevalent.

Streptococcus pneumoniae resistance to penicillin and other antibiotics is an increasing problem throughout the world and it is therefore surprising that WHO has recently recommended that amoxicillin be given fewer times a day and for fewer days to children with pneumonia. Amoxicillin remains an effective treatment for

pneumonia caused by strains of *S. pneumoniae* with an intermediate level of resistance (MIC 2–4 μg/mL), provided that amoxicillin levels remain above the MIC for at least 50% of the time between doses [34, 35]. To increase the proportion of time that the levels of amoxicillin are above the MIC, it is much more effective to give the same dose more often, rather than a higher dose at the same frequency [34]. Because of problems with compliance, the dose of amoxicillin recommended by WHO has been changed from 15 mg/kg three times a day to 25 mg/kg twice a day [36]. Unfortunately, the serum level remains above a given MIC for a smaller percentage of the time with the new regimen [37], so it will be less effective for the treatment of partially resistant strains of *S. pneumoniae*. In the management of otitis media caused by *H. influenzae* or partially resistant *S. pneumoniae*, 45 mg/kg amoxicillin twice a day is clearly superior to 20–25 mg/kg twice a day [38, 39]. In high-mortality areas, the best policy may be to recommend giving 45 mg/kg amoxicillin twice a day orally or (preferably) 30 mg/kg three times a day for greater efficacy.

A controlled trial performed in four tertiary-care hospitals in Pakistan compared 45 mg/kg/day amoxicillin with 90 mg/kg/day in children with non-severe pneumonia [40]. The study failed to detect any benefit from using a higher dose of amoxicillin. However, it is likely that few of the children had bacterial pneumonia: lung aspirates were not performed, none of the 876 children died and 42% had wheeze, so these findings should not be extrapolated to regions with a high mortality from pneumonia.

WHO originally recommended that children with tachypnoea and no chest indrawing should be treated with antibiotics for 5 days, and this is still the case in areas where HIV infection is common [36]. In areas with low HIV prevalence, WHO now suggests that amoxicillin be given for only 3 days. This recommendation is based on the findings of three studies (one unpublished) in 5,763 children, where the relative risk of failure was 1.07 (95% CI 0.92–1.25) with 3 days compared to 5 days of treatment [41]. However, it is likely that few of the children had bacterial pneumonia: only nasopharyngeal bacteria were cultured, and there was only one death among the 4,188 children in the two published studies, so these results should not be extrapolated to areas with a high mortality from pneumonia.

8 Which Antibiotics for Severe Pneumonia?

Pharmacokinetic studies performed in children in Papua New Guinea found that the mean serum level of penicillin was more than 5 μg/mL for 3 h after a dose of 35 mg/kg of benzyl penicillin given by intramuscular injection. WHO recommends that children with chest indrawing who do not have signs of very severe pneumonia should be treated with 50 mg/kg of benzyl penicillin given by intramuscular injection every 6 h for at least 3 days and then 25 mg/kg of oral amoxicillin twice a day (which perhaps should be 30 mg/kg three times a day – see above). This high dose of benzyl penicillin is likely to provide effective treatment for most strains of

H. influenzae and *S. pneumoniae*, including strains with intermediate resistance to penicillin (MIC 2–4 μg/mL) [35].

When a child with severe pneumonia cannot be referred for treatment with intramuscular benzyl penicillin, WHO suggests that treatment can be safely given at home with 45 mg/kg amoxicillin orally twice a day for 5 days [36]. This recommendation is based on two controlled trials performed in urban tertiary centres [23, 24]. However, it is likely that few of the children had bacterial pneumonia: lung aspirates were not cultured, only 14 of the 3,739 children died and 63% had wheeze, so these findings should not be extrapolated to regions with a high mortality from pneumonia. As discussed above, 30 mg/kg of amoxicillin given three times a day is likely to be more effective than 45 mg/kg given twice a day [34].

9 Which Antibiotics for Very Severe Pneumonia?

Children are said to have very severe pneumonia if they have chest indrawing plus either central cyanosis or severe respiratory distress or an inability to drink. WHO recommends that these children be treated with ampicillin plus gentamicin, or with chloramphenicol. *Streptococcus pneumoniae* is increasingly resistant to chloramphenicol and two controlled trials suggest that penicillin (or ampicillin) plus gentamicin is more effective than chloramphenicol (the relative failure rate was 1.26, 95% CI 1.03–1.54). Gentamicin has a synergistic effect with β-lactams against many strains of *S. pneumoniae* that have reduced sensitivity to penicillin [42–47] and it has excellent activity against *H. influenzae*. There are advantages in giving 8 mg/kg of gentamicin intramuscularly on the first day followed by 6 mg/kg on subsequent days, rather than 7.5 mg/kg daily [48].

Penicillin plus gentamicin remains an effective treatment for pneumonia caused by strains of *S. pneumoniae* with an intermediate level of resistance, provided penicillin levels remain above the MIC for at least 50% of the time between doses [34, 35]. Penicillin resistance is *not* an indication for the use of third-generation cephalosporins to treat pneumonia, as they are no more effective than penicillin alone [35], let alone penicillin plus gentamicin. Third-generation cephalosporins and fluoroquinolones are much more expensive than penicillin and gentamicin, and they are more likely to induce antibiotic resistance [49].

WHO should recommend penicillin plus gentamicin, rather than ampicillin plus gentamicin, for the treatment of very severe pneumonia. Ampicillin has a much broader spectrum than oral phenoxymethylpenicillin (penicillin V), but there are fewer differences between ampicillin and parenteral benzyl penicillin (penicillin G); in particular, ampicillin and benzyl penicillin have similar activity against *H. influenzae* [28]. Some gram-negative bacilli are sensitive to ampicillin and resistant to benzyl penicillin, but these organisms are almost all sensitive to gentamicin so that organisms that are sensitive to ampicillin plus gentamicin can also be expected to be sensitive to penicillin plus gentamicin. Hospitals have to stock benzyl penicillin to treat severe pneumonia and it would be better just to add gentamicin for

very severe pneumonia, rather than requiring hospitals to stock ampicillin as well. Ampicillin is more expensive than penicillin and it has more side effects.

WHO recommends that treatment be changed if a child with pneumonia "does not improve within 48 h". It is unrealistic to expect that improvement will reliably occur as early as this in bacterial pneumonia; in adults with pneumococcal pneumonia, Robert Austrian noted no difference in mortality between treated and untreated patients in the first 5 days [50] and the controlled trials of both penicillin (or ampicillin) plus gentamicin vs. chloramphenicol for very severe pneumonia assessed the response to treatment after 5 days [51, 52]. It would be more logical to recommend that treatment be changed if the child is getting worse at any time from 48 h onwards or if there is no improvement after 5 days [21, 53].

10 Oxygen Therapy

Hypoxaemia is a major cause of mortality in pneumonia [54]. Studies in guinea pigs and adult humans in the 1920s and in children in Papua New Guinea in the 1970s all suggest that oxygen therapy approximately halves mortality from pneumonia [55]. A recent study found that an improved system for delivering oxygen reduced mortality from pneumonia by 35% among children in Papua New Guinea [56].

However, it is very difficult to deliver oxygen therapy to children in the remote parts of many low-income countries and the cost is considerable. In Papua New Guinea, the improved oxygen delivery system cost US $51 per patient treated and $1,673 per life saved. The world's 49 low-income countries with a population of 1.3 billion had a total health expenditure of only US $22 per person in 2006 [57]. As discussed above, with normal cardiac output, haemoglobin concentration and pH, arterial oxygen saturations of 68% or more are not dangerous [26] and it might be sensible to give supplemental oxygen only when the saturation is less than 80% (rather than 90%) if oxygen supplies are limited.

11 Fluid Therapy

Some children with severe pneumonia present with sepsis and dehydration. If parenteral fluid therapy is available, hypovolaemia should be corrected rapidly with 10 mL/kg boluses of 0.9% saline. However, many patients with pneumonia have low maintenance water requirements because they have high levels of antidiuretic hormone [58]. Once hypovolaemia and hypoxaemia have been corrected, maintenance fluids should be restricted to 1–2 mL/kg/h [59, 60]. There is little evidence to support the widely offered advice that children with acute respiratory infections should be given extra fluids [61].

12 Fever

WHO recommends that paracetamol be administered to children with a temperature greater than 39°C if it "appears to be causing distress" [17]. This recommendation

may be harmful. In randomised trials in mammals with severe infection, antipyretic therapy *doubles* mortality, increases viral shedding and impairs the antibody response [62]. Antipyretics have been shown to prolong the illness in influenza, chickenpox and malaria [63–65]. It is potentially dangerous to recommend the administration of antipyretics to children with pneumonia in high-mortality areas.

13 Neonates, Malnutrition and HIV

Pneumonia, often with systemic sepsis, is common in neonates and infants with malnutrition, and it has a very high mortality rate in these children [66, 67]. Despite their high risk of dying, these children may not appear to be very ill – and the mortality rate is very high indeed in malnourished children with pneumonia if they are afebrile [25]. Gram-negative bacteria are a common cause of sepsis in neonates and malnourished children; so these children should be treated with gentamicin as well as penicillin (or ampicillin).

Children with HIV often present with pneumonia caused by *S. pneumoniae*, *H. influenzae* or *Staphylococcus aureus*. Other common causes of pneumonia in these children are *Pneumocystis jirovecii*, gram-negative bacteria, cytomegalovirus and tuberculosis [68, 69].

14 Overall Effect of Case Management

When case management can be properly delivered, it results in substantial reductions in child mortality. A meta-analysis of the effect of the case management of pneumonia in children in low-income countries found a reduction in total mortality of 27% (95% CI 18–35%) in neonates, 20% (11–28%) in infants and 24% (14–33%) in children 0–4 years of age [70]. There have been three recent reviews of the case management of pneumonia [21, 68, 71].

15 Immunisation

The WHO programme for the management of acute respiratory infections has led to substantial reductions in child mortality [70]. However, pneumonia remains the commonest cause of death in children [1, 2] despite the fact that the WHO programme has been going for almost 30 years [11]. This is largely because it has not been possible to deliver antibiotics reliably to children in the remote regions of many low-income countries, where most of the deaths from pneumonia occur. It is much easier to deliver vaccines at intermittent intervals to children in remote areas than it is to have antibiotics available for administration at all times. Unfortunately, none of the current Expanded Program on Immunization (EPI) vaccines provides specific protection against *S. pneumoniae* or *H. influenzae*, the main causes of fatal pneumonia.

Very substantial additional reductions in mortality would result from immunisation against *S. pneumoniae* and *H. influenzae* [72] and better utilisation of the non-specific effects of vaccines [73]. There is an urgent need for studies of the

effect of immunising mothers with the 23-valent polysaccharide pneumococcal vaccine before pregnancy, during pregnancy or immediately after delivery, in order to increase antibody transfer to the baby across the placenta or via breast milk [74–77]. Unlike the polysaccharide vaccine, the conjugated pneumococcal vaccines are immunogenic in the first few months of life, when most fatal pneumonia occurs, but they cover only a limited number of serotypes, and they are very expensive. Unfortunately, the three trials of conjugated pneumococcal vaccines in children in developing countries all used vaccines that are no longer available [78–80], only two studied the effects on clinical pneumonia [79, 80] and only one studied the effect on all-cause mortality [79]. The vaccines reduced radiological pneumonia by 17% (95% CI 4–28%) in South Africa, 37% (27–45%) in the Gambia, and 23% (–1 to 41%) in the Philippines.

The *H. influenzae* type b conjugate vaccine reduced radiological pneumonia by approximately 20% among children in trials in the Gambia and Chile [81, 82]. However, type b strains cause less than half the cases of *H. influenzae* pneumonia in children in low-income countries; approximately 40% of cases are caused by non-serotypable (unencapsulated) strains and 20% by types a, c, d, e and f [10]. This suggests that a vaccine that protected against all strains of *H. influenzae* would prevent approximately 40% of radiologically proven pneumonia. As with pneumococcal vaccine, there is a strong case for investigating the immunisation of mothers against *H. influenzae* in order to protect their infants through transfer of antibody across the placenta and via breast milk.

A new 10-valent pneumococcal conjugate vaccine has recently been licenced that provides some protection against non-serotypable strains of *H. influenzae* [83, 84]: eight pneumococcal serotypes are conjugated to *H. influenzae protein* D, one to tetanus toxoid and one to diphtheria toxoid. In a controlled trial of the 11-valent prototype, the per-protocol vaccine efficacy was 52% (95% CI 37–63%) against *S. pneumoniae* otitis media, and 36% (4–57%) against *H. influenzae* otitis media [85]. There is an urgent need to test the efficacy of this vaccine against radiological pneumonia in children in high-mortality areas; studies in Argentina, Columbia and Panama are expected to deliver results in the near future.

There is increasing evidence that vaccines have very substantial non-specific (heterologous) effects on mortality among children in low-income countries [73, 86]. For example, BCG and measles vaccines may reduce mortality from diseases other than tuberculosis and measles, and most of the benefits seem to be on mortality from pneumonia [87]. On the other hand, there are studies suggesting that, in some communities, diphtheria–tetanus–pertussis (DTP) vaccine may reduce mortality from diphtheria, tetanus and pertussis but *increase* mortality from pneumonia and diarrhoea [73, 86]. There is an urgent need for further research on the non-specific effects of vaccines in children in developing countries.

16 Conclusion

Although great progress has been made in reducing mortality from pneumonia, it remains the commonest cause of death among children in developing countries.

Further substantial reductions in mortality will require effective antibiotic therapy and immunisation against *S. pneumoniae* and *H. influenzae* to be made available to a high proportion of the children who live in the remote areas of low-income countries.

In recent years, several controlled trials have been performed that have suggested that non-severe pneumonia can be treated with 25 mg/kg amoxicillin twice a day for 3 days [40, 41], and that children with severe pneumonia who cannot be referred can be safely treated at home with 45–90 mg/kg/day amoxicillin [23, 24]. However, it is probable that a very low proportion of the children in these trials had bacterial pneumonia: the trials were performed in urban tertiary centres, no attempt was made to culture lung aspirates, very few children died and wheezing was common in many of the trials. Management based on the findings of these studies may considerably improve the management of children in communities with moderate–low mortality rates, but their findings should *not* be extrapolated to communities where there is a high mortality from pneumonia.

Table 1 outlines the optimal management of acute respiratory infection in children in high-mortality areas, and notes where this differs from the current WHO recommendations. Table 2 summarises the important considerations about pneumonia in high-mortality areas.

Table 1 Suggested management of children with cough or respiratory distress in high mortality areas (who do not have prolonged expiration – or audible wheeze)

Cough or cold
No chest indrawing, respiratory rate <50 breaths/min, age 2–59 months (WHO: <50/min, age 2–12 months; <40/min, 12–59 months)
No antibiotic
Pneumonia
No chest indrawing, respiratory rate ≥50 breaths/min, age 2–59 months (WHO: ≥50/min, age 2–12 months; ≥40/min, age 12–59 months)
Amoxicillin 30 mg/kg oral three times a day (or perhaps 45 mg/kg twice a day) for 5 days (WHO: 25 mg/kg twice a day for 3 days)
Cotrimoxazole should no longer be recommended (except for pneumocystis cover)
Severe pneumonia
Chest indrawing (without cyanosis or severe respiratory distress, and able to drink)
Admit, benzyl penicillin 50 mg/kg IM every 6 h for at least 3 days
When improving, change to amoxicillin 30 mg/kg oral three times a day (WHO: 25 mg/kg bd)
Give antibiotics for a total of 7 days (WHO: 5 days)
Very severe pneumonia
Chest indrawing (not required by WHO) plus either central cyanosis, or severe respiratory distress, or not able to drink
Admit, benzyl penicillin (WHO: ampicillin) 50 mg/kg IM every 6 h plus gentamicin 8 mg/kg IM day 1, then 6 mg/kg daily (WHO: 7.5 mg/kg daily) for 10 days
If getting worse at any time from 48 h onwards or not improving at 5 days (WHO: not improving at 48 h), change to cloxacillin 50 mg/kg IM every 6 h plus gentamicin IM daily for 3 weeks

Table 2 Important considerations about pneumonia in children in high-mortality areas

Epidemiology and aetiology
- Pneumonia is the commonest cause of death in children less than 5 years of age; it is a major factor in approximately 30% of deaths
- Most fatal pneumonia is caused by *S. pneumoniae* and *H. influenzae*

Case management
- Separate protocols are needed for children in medium–low-mortality areas, including children with a prolonged expiratory phase (caused by bronchiolitis or asthma)
- Guidelines for children in high-mortality areas should not be based on data from medium–low-mortality areas (so data from many urban areas should not be used even if they are in low-income countries)
- Guidelines for high-mortality areas should use the *minimum* necessary criteria to decide the *minimum* treatment needed to reduce *mortality*
- The current WHO guidelines are too complicated and influenced by data from medium–low mortality areas
- In children aged 2–59 months who do not have chest indrawing, it would be better to recommend antibiotics only if the respiratory rate is $\geq$50 breaths/min (WHO recommends that antibiotics be given to children aged 12–59 months taking $\geq$40 breaths/min)
- Non-severe pneumonia should be treated with amoxicillin 30 mg/kg oral three times a day for 5 days, or perhaps 45 mg/kg twice a day (WHO recommends 25 mg/kg twice a day for 3 days)
- Cotrimoxazole should no longer be used, except for pneumocystis (WHO policy is unclear)
- Antibiotics should probably be given for 5 days (WHO recommends 3 days) to outpatients in high-mortality areas (regardless of HIV status)
- Chest indrawing should be defined as subcostal plus either intercostal or supracostal retraction (WHO defines indrawing as retraction of the lower chest wall)
- If severe pneumonia cannot be treated with benzyl penicillin given intramuscularly, amoxicillin 30 mg/kg should be given orally three times a day for 7 days (WHO recommends 25 mg/kg twice a day for 5 days)
- Chest indrawing should be a requirement for the diagnosis of very severe pneumonia (WHO does not require this)
- Penicillin (WHO recommends ampicillin) plus gentamicin should be used to treat very severe pneumonia
- Third-generation cephalosporins should *not* be recommended for the treatment of pneumonia; they are no more effective than penicillin plus gentamicin (WHO agrees)
- Chloramphenicol should no longer be used to treat very severe pneumonia (WHO policy is unclear)
- Treatment failure should be defined as progression of disease at 48 h or later, or failure to improve at 5 days (WHO recommends failure to improve at 48 h)
- Fever should *not* be treated with an antipyretic in high-mortality areas (paracetamol is recommended by WHO)
- After correction of hypovolaemia, fluid intake should usually be 1–2 mL/kg/h
- Oxygen therapy for children with an oxygen saturation less than 80% is likely to halve mortality, but it is expensive (WHO recommends giving oxygen if saturation < 90%)

Immunisation
- Treatment with antibiotics reduces mortality from pneumonia, but it is difficult to deliver in remote areas in low-income countries, where most of the deaths occur
- Effective immunisation of mothers and infants against *S. pneumoniae* and *all* strains of *H. influenzae* would be likely to reduce mortality from pneumonia by at least 50% if it were available
- There should be urgent investigation of the evidence that BCG and measles vaccines reduce all-cause mortality (including mortality from pneumonia) in high-mortality areas, but that DTP vaccine may increase mortality from diseases other than diphtheria, tetanus and pertussis in some high-mortality areas

Consideration should be given to immunising mothers with polysaccharide pneumococcal vaccine and ensuring that all infants receive conjugated pneumococcal vaccine. *Haemophilus influenzae* type b causes less than half the cases of *H. influenzae* pneumonia, but the new protein D-conjugated pneumococcal vaccine may protect against pneumonia caused by non-serotypable *H. influenzae* as well as *S. pneumoniae*. Research is urgently needed on the evidence that BCG and measles vaccines may substantially reduce child mortality from diseases other than tuberculosis and measles (including mortality from pneumonia), and the weaker evidence that DTP vaccine may increase mortality from diseases other than diphtheria, tetanus and pertussis in some high-mortality communities.

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Darwin, Microbes and Evolution by Natural Selection

E. Richard Moxon

Abstract Born 200 years ago, Darwin's revolutionary ideas were derived largely from his observations on life forms that evolved relatively recently, including various flowering plants, worms, birds and domesticated animals. Yet, life appeared on planet earth close to 4 billion years ago in the form of unicellular organisms collectively called bacteria. It was only shortly after "*On the Origin of Species*" was published (1859) that the "germ theory" of infectious diseases was formulated. Microbes (viruses, bacteria, fungi and microparasites) received scant mention in Darwin's writings, although pioneers of the Golden Age of Bacteriology, such as Louis Pasteur (1822–1895), were contemporaries. Today, microbes offer extraordinary testimony and powerful model systems of direct relevance to the essentials of Darwinian selection, such as understanding microbial–host interactions, the evolution of pathogens and the emergence of drug- or vaccine-related resistance.

1 Darwin, Microbes and Evolution by Natural Selection

Darwin revolutionised our ideas on how life evolved by arguing that spontaneous variations were a common feature of all life forms. Crucially, he realised that these variations happened independently of external (environmental) changes. Thus, the diversity that is the sine qua non of evolution by natural selection originates independently of the selection itself. Spontaneous variants that are better adapted (more fit) increase in prevalence through natural selection because their progeny inherit these fitness traits and are more likely to survive. Darwin's proposals on the mechanisms of heredity were wide of the mark and although Mendel published his pioneering findings in 1861, there is no evidence that Darwin read the paper or was aware of its importance. Genetics largely unfolded in the twentieth century through the extraordinary contributions of scientists such as Hugo De Vries, Thomas Morgan and Oswald Avery, culminating in the discovery by Jim Watson and Francis Crick

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of the structure of DNA that provided a crucial molecular insight into the mechanism of replication. The amalgam of genetics with Darwin's prescience in recognising the importance of natural selection is the basis of the current theory (Modern Synthesis) of evolution.

In fact, Darwin's arguably most influential (certainly his best known) book, "*On the Origin of Species by Means of Natural Selection*" does not directly address the issue of the origin of species although to him we owe the thought that life might have originated in "*. . . some warm little pond, with all sorts of ammonia and phosphoric salts, light, heat, electricity etc. . .*," an idea that was widely held many years after Darwin's death and led to the testing of his theory by Miller and Urey in the 1950s [1]. No matter how and where life started – in hydrothermal vents in deep oceans, in some sort of primordial soup or soil associated with clay particles – the earliest forms of life, based on the fossil record, were unicellular organisms known generically as bacteria. Indeed, bacteria were the only forms of life on earth for much of its history (Fig. 1). The famous sketch by Darwin depicting the "tree of life" has been overtaken by a wealth of molecular detail (Fig. 2) that emphasises the dominance of bacteria over time. By the time of Charles Darwin's death (1882), a scientific revolution was occurring which established the germ theory of disease, formally proposed through the postulates of Robert Koch. For the first time in the history of civilisation it was recognised that there was a causal link between microbes and many common, but devastating diseases, such as tuberculosis, cholera, anthrax and typhoid.

The available evidence from Darwin's books, essays, letters and diaries is consistent with the fact that he died before microbiology was sufficiently advanced for him to have taken advantage of the "Golden Era of Bacteriology" and the profound changes it exerted. But Darwin was clearly aware of Pasteur's seminal experiments on microscopic life forms that put paid to the theory of spontaneous generation. Further, Darwin lost two children, Charles at the age of 18 months from scarlet fever and his beloved Annie, aged 10, from tuberculosis. Indeed, in "*The Variation*

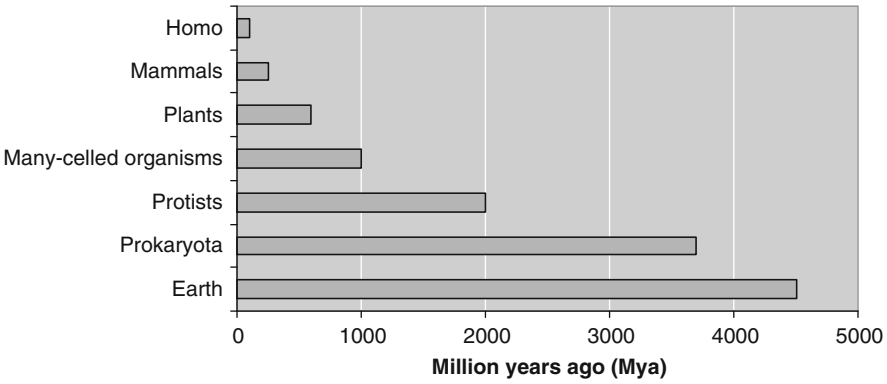

Fig. 1 The first life forms were probably cyanobacteria some 3–4 billion years ago. Amphibians date to about 360 million years and *Homo sapiens* to about 200,000 years ago

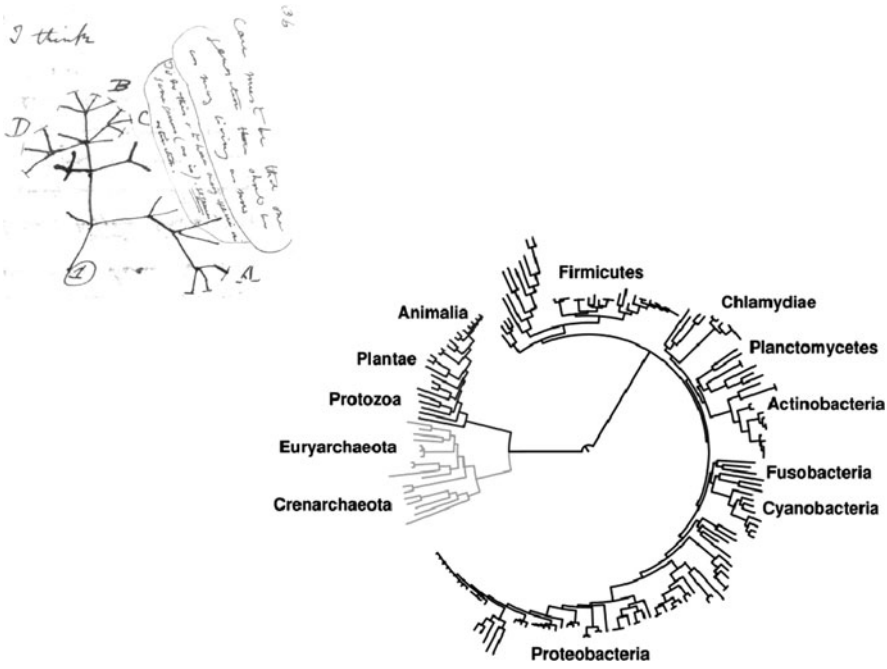

Fig. 2 *Top left*: The original sketch from one of Charles Darwin's notebooks suggesting the concept of a tree of life. *Bottom right*: A recent depiction of an evolutionary tree based on molecular studies showing the common ancestry of all three domains of life

of Animals and Plant under Domestication" (first published in 1868) Darwin seems to anticipate Koch.

> A particle of small-pox matter, so minute as to be borne by the wind, must multiply itself many thousand fold in a person thus inoculated; and so with the contagious matter of scarlet fever. It has recently been ascertained that a minute portion of the mucous discharge from an animal affected with rinderpest, if placed in the blood of a healthy ox, increases so fast that in a short space of time the whole mass of blood, weighing many pounds, is infected, and every small particle of that blood contains enough poison to give, within less than forty-eight hours, the disease to another animal.

But the time was not yet ripe for Darwin to have used microbes to test his theory. Had he done so, bacteria would have provided a powerful experimental system with which to do so. Bacteria can divide in time frames of minutes, not the weeks to years required by the multicellular organisms upon which Darwin largely based his ideas. Further, Darwin would have had access to organisms in which replication occurs by binary fission, uncoupled from the complex events (such as meiosis, gamete production and cell–cell fusion) involved in sexual reproduction. One of the major problems that troubled Darwin was his poor understanding of the mechanism(s) of inheritance in multicellular organisms. If evolution occurs through selection of small, heritable variations in the individuals that make up a species and the members

of a particular species inter-breed, how is natural variation in populations sustained in the face of a reproductive mechanism that would seem to result in blending inheritance? If Darwin had studied bacteria, he would have had a more facile model system at his disposal. Bacteria obtain novel genetic information through unidirectional mechanisms of DNA transfer (transformation, conjugation or transduction) or horizontal gene transfer but, crucially, these mechanisms of genetic acquisition are uncoupled from replication. The promiscuous fashion in which populations of bacteria can access and incorporate DNA into their genomes from other life forms, as compared to the inter-breeding of multicellular organisms, is an important determinant of their evolution (Fig. 3). Microbes as a model system illustrate brilliantly the essentials of Darwin's theory in which the natural phenotypic variation within bacterial populations, based not only on mutation but also on "horizontal" genetic acquisitions, is critical to the generation of diversity and evolution by natural selection.

Following Darwin's seminal contributions to evolutionary theory, it was 80 years before Max Delbrüch and Salvador Luria exploited bacteria as a model system [2]. These scientists turned their attention to investigate the occurrence of resistance of the bacterium *Escherichia coli* to a virus, the bacteriophage T1. They argued as follows: some *E. coli* bacteria are or become resistant to the T1 bacteriophage when the bacteria are exposed to this virus. If so, can one say unambiguously that

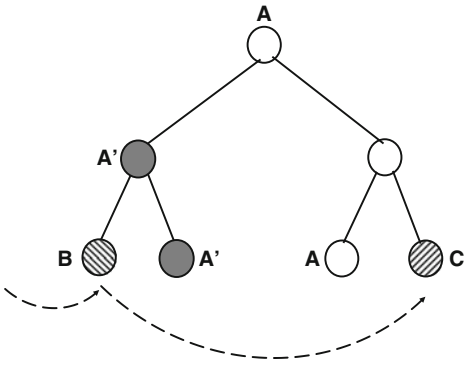

Fig. 3 A cartoon to capture the critical factors that determine bacterial population structure. Starting from an ancestral bacterium, a mutation (e.g. transition, transversion) occurs leading to a variant A′. This diversity occurs through "vertical descent" in the progeny of the ancestor. Most of the progeny of A′ will not be mutants and divergence is very slow. If lateral transfers (inter-genomic recombination events) do not occur then diversity is created only through accumulation of intra-genomic mutations and vertical descent of DNA to progeny. If so, the natural population retains a clonal structure over time. The evolution of diversity is greatly accelerated by lateral transfer (interrupted lines and arrows) from a different species of organism, resulting in variant B, in subsequent generations. This might be acquisition of a DNA element consisting of many genes (an antibiotic resistance element or "pathogenicity island" for example). These elements may then spread to other members of the natural population of the species (e.g. C). The occurrence of recombination events and assorted mechanisms of inter-genomic DNA transfer complicate our ability to trace the history of natural populations of bacteria

the resistant bacteria arose independently of the virus? Two alternatives were proposed: if exposure of *E. coli* to virus drives resistance, then the number of mutants will be a function of the population size; however, if resistance arises by mutation, independently of the virus, then the number of times that the bacteria replicate (number of generations) will be critical. They observed that when several independent cultures of bacteria were exposed to virus, there were enormous variations in the number of resistant bacterial survivors between each of the different cultures. The only explanation that fitted these data was that the mutants had arisen in the bacterial population prior to exposure to virus (Fig. 4). The Luria–Delbrüch fluctuation experiment, as it became known, vindicated Darwin. Selection, not prescription, accounted for the emergence of resistant bacteria.

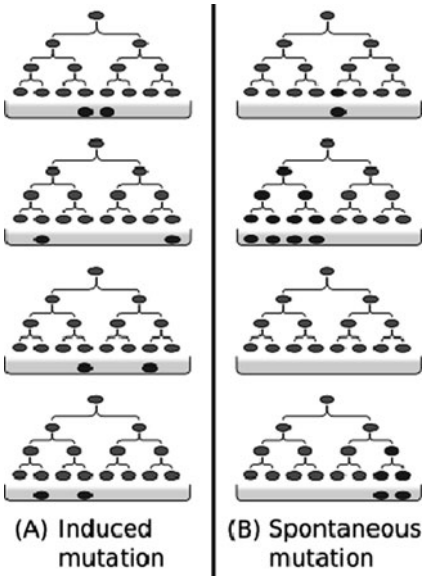

Fig. 4 The two possibilities tested by the Luria–Delbrück experiment. (**a**) If mutations are induced, roughly the same number of mutants is expected to appear on each plate. (**b**) If mutations arise spontaneously during cell divisions prior to plating, each plate will have a highly variable number of mutants

The timing of this elegant laboratory-based experiment coincided with the beginning of a natural occurring experiment in the real world that has proved to be of monumental importance: the emergence of antimicrobial resistance. Antibiotics rank as one of the great discoveries of twentieth century medicine. The inhibitory properties of sulphonamides, first published in 1935, were credited with saving the lives of Franklin Roosevelt and Winston Churchill amongst others. In the 1940s came penicillin, a drug for which Oxford enjoys some prestige although Florey, Chain and Fleming's Nobel Prize arose from a discovery at St Mary's Hospital in London. As dramatic as was the impact of antibiotics on treating infections, the rapid evolution of resistant bacteria was also extraordinary and provides one of the most powerful examples of Darwinian selection. The canonical example was penicillin resistance to the bacterium *Staphylococcus aureus,* an ironic twist in that it was inhibition of *S. aureus* by the penicillium mould that prompted Fleming's famous

discovery. Within a decade of the introduction of penicillin, its effectiveness was severely compromised by resistance. How did this resistance evolve? Penicillin acts by interfering with the synthesis of the cell wall of the bacterium. But, a genetic change in the DNA coding for the molecules that make the cell wall may alter them so that these synthetic enzymes maintain their essential function but are no longer a target for penicillin. Theoretically and experimentally, this could occur by a classical mutation, but in fact the mechanism that was most relevant to the rapid eclipse of the utility of penicillin against *S. aureus* involved the acquisition of novel DNA from an external source rather than the occurrence of an intrinsic mutation to *S. aureus*. There are no absolute barriers to the importation of DNA in bacteria and as demonstrated in 1963 [3], bacterial cells import antibiotic resistance genes (penicillinases) on plasmids or episomes, small circular pieces of DNA that are independent genetic elements, that can replicate using their own copying mechanism. Plasmids also have genes that mediate their transfer from one bacterium to another. They provide added survival value without disturbing the function and fitness of the bacterium in which they reside. This is an extraordinarily successful evolutionary strategy. Plasmid genes can evolve independently without affecting the host cell viability; their effects can be refined over time; plasmids can integrate and then excise from the main genome of the bacterium and finally they can carry other genes of potential benefit to bacterial survival. So, where did the plasmid-borne resistance genes for *S. aureus* come from in the first place? This is uncertain but the identical DNA found in the plasmids that confer methicillin resistance on *S. aureus* has been found in soil bacteria [4]. At any rate, it took merely a few years for the evolution of penicillin resistance through plasmid-borne enzymes that degrade penicillin to reach a point where all hospital- and most community-acquired *S. aureus* was penicillin resistant. Heritable variation and natural selection, as articulated by Darwin, underlie our current crisis with respect to antimicrobial resistance to drug treatment not only in virulent bacteria but in many viral, fungal and parasitic pathogens.

Microbes not only have dominated life on earth, but are completely essential to the maintenance of all other living organisms. But, microbial diseases remain the most important cause of death and disability on the planet through their ability to infect humans and other multicellular organisms (e.g. insects, plants, birds, reptiles and mammals). Thus, microbial infections have enormous consequences for human welfare. The capacity of microbes to engage with multicellular organisms and the strategies that they deploy are truly remarkable and provide yet another facet of the importance of Darwinian ideas in the field of microbial diseases. Microbes use every trick in the book and provide another brilliant example of the mechanisms of adaptation that depend on heritable variation and natural selection.

The extraordinary subtlety of the interplay of microbes and their hosts often prompts metaphors that attribute “smart behaviour” to microbes. For example, the interactions between hosts and pathogens are often characterised as “microbial arms wars” [5]. Of course, the metaphor of a war (a purposeful interaction), howbeit colourful, is misleading; evolution is a “blind” process in that it occurs through

selection of genetic variations (mutations). Mutations occur without respect for their utility. So what do we actually mean when we refer to the "smart behaviour" or "genetic intelligence" of microbes? The evolution of *fitness* (see later) comes down to the selective forces that shape on the one hand genetic mechanisms for underpinning the fidelity of the replication of microbial genomes and on the other hand the genetic mechanisms that have evolved to generate variations. In other words, there is a trade-off between maintaining the essential genetic information of an organism and generating sufficient genetic diversity to facilitate adaptation to a changing environment. This balance between genome stability and variation is subtle, dynamic, ever changing and, over billions of years, so intricate as to give the appearance of "intelligent design". But of course, as Darwin first articulated so eloquently, the complexity of adaptation and of evolved functions (consider, for example, bacterial chemotaxis) is the result of natural selection over long periods of time. This dynamic interaction between host and microbe is of course extremely complex but, from the perspective of the microbe, can be reduced to a Darwinian simplicity. A microbe either survives within the host or the host prevails; fitness can be defined and quantified as follows: in a population of bacteria within a host, each bacterial cell, on average, must generate at least one progeny or the bacterium is extinguished (Fig. 5). Fitness can be given a value that indexes, on average, how many progeny cells are derived from each progenitor cell. This index of fitness is often called the basic reproductive rate which, when it falls below 1, leads towards disappearance of the microbial population. On the other hand a rate of 1 or greater provides a numerical value of fitness. Broadly, evolution selects organisms with the highest basic reproductive rate although this is an over-simplification because the host environment may change, for example, through innate and acquired immune processes or when microbes spread from one host to another. As a result of these and many other variables, the fitness of organisms is not constant. Obviously, a microbe that

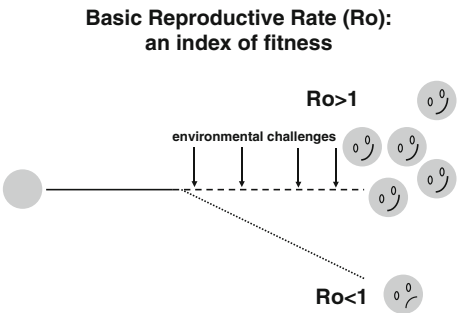

Fig. 5 Although complex, the biology of any organism (in this case a bacterial pathogen) can be reduced to a simplicity. Either the organism survives or it does not. Over time, any organism must face challenges that test its fitness. Fitness is reflected in the basic reproductive rate (Ro). The magnitude of Ro is given by the average number of surviving progeny in a population derived from each bacterial cell. Clearly, when Ro falls below 1, any given bacterial population will decline and will be extinguished. The higher Ro, the fitter are the bacteria that make up the population

is well adapted to one host and spreads to another may find that its former fitness is diminished. To adapt to changing circumstances, both host and microbe have evolved mechanisms for generating diversity [6], for example, antigenic diversity through enhanced mutation rates in microbes (Box 1) and antibody diversity through hyper-mutable events in B cells in animal hosts.

Box 1

Microbial fitness requires mechanisms for adapting to changing environments. In essence, two distinct mechanisms are used: gene regulation and gene variation. Gene regulation involves mechanisms by which cells sense the environment and transduce signals to alter the activity of specific genes. Gene variation includes a large number of mechanisms that are often broadly referred to as antigenic variation, a characteristic that is typical of many pathogens.

Pathogenic microbes face particularly stringent tests of their adaptive potential because infections often occur within a short time frame (hours). During this crucial period, these microbes encounter a plethora of challenges, for example, the host innate and adaptive immune repertoires. Given this race against time [7], bacteria have evolved a form of antigenic switching, called phase variation, in which a number of their surface structures undergo reversible and high-frequency on to off phenotypic switching. This is an example of bet-hedging. Although most variants are not useful, a few bacteria (it may only be one bacterium) may make the difference between survival and extinction. These phase variable genes have been called *contingency* genes [8]. Crucially, contingency genes are hyper-mutable so that switching occurs at rates of about one bacterium per thousand per cell division.

Consider a hypothetical genome of a pathogenic bacterium, comprising 2,000 genes of which 7 display phase variation. If each of seven contingency gene can switch at a frequency of 10^{-3} per bacterium per generation, a binary genetic switch (for example, A or A′, B or B′, . . ., G or G′) with corresponding phenotypes (a or a′, b or b′,. . ., g or g′) gives 128 different phenotypic possibilities. Suppose that a bacterium of phenotype a, b, c, d′, e, f, g is optimal for colonisation of a host's epithelial cells, but long-term colonisation is favoured by entry into host cells. Now further suppose that bacteria with the phenotype a′, b, c, d, e, f′, g′ (involving four switches from the original) are best able to invade and survive in the cells of the host: these bacteria will be selected as the adaptive phenotype. This is a rare event, i.e. would occur in about one in a trillion cells. But a population of cells with seven contingency genes will, on average, contain an individual of the requisite genotype when the population size is only 10,000. This phenotypic variation, which is stochastic with respect to the timing of switching but has a programmed genomic location,

allows a large repertoire of phenotypic solutions to be explored, while minimising deleterious effects on fitness. This combinatorial strategy, a classical Darwinian concept, exemplified by contingency loci is widespread in nature. It is of course characteristic of the repertoire of B cells in the adaptive immune response of animals including humans. Jacob spoke of: ". . . the mode of operation of the tinkerer. . . arranging various combinations so as to produce new objects of increasing complexity". The algorithm (tool-box) of the tinkerer also evolves by natural selection [9].

Microbes may have a decisive edge in these encounters. Their large numbers, rapid rates of replication and legacy of billions of years of evolution have allowed them to refine their fitness and to obtain a highly effective and subtle balance between the genetic mechanisms for maintaining replication fidelity and those for generating variability. So, infections are not going to go away and this is the bad news; but neither are microbes and this is good news. As mentioned earlier, multicellular organisms are absolutely and utterly dependent upon the world of microbes for the continuation of their life on earth. However, humans have, in a manner of speaking, played into the hands of the microbes. In the last century or so, civilisation has brought about the emergence of mega-cities, mass production and distribution of food, global travel and of course massive ecological changes, some of which result from natural disasters, but many of which we recognise to be man-made. We know that microbes are ancient and efficient replicators but humans have evolved cognitive intelligence. So, perhaps the arms race between microbes and humans really does come down to selection vs. prescription after all. I think I can guess which of the two Darwin would have put his money on!

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Human Herpesvirus 6

Charles G. Prober

Human herpesvirus 6 (HHV-6) is a member of the family Herpesviridae. This family of eight viruses includes four Alphaherpesvirinae (herpes simplex virus type 1 [HSV-1], herpes simplex virus type 2 [HSV-2], human herpesvirus 8 [HHV-8], and varicella-zoster virus [VZV]); one Gammaherpesvirinae (Epstein–Barr virus [EBV]); and three Betaherpesvirinae (cytomegalovirus [CMV], HHV-6, and human herpesvirus 7 [HHV-7]) [1]. Common physical traits of these clinically important DNA viruses include large size (150–200 nm), an icosahedral nucleocapsid encased in an envelope that has multiple surface projections, and a large number of structural proteins. Their genomes are linear and double-stranded, varying in size from 120 to 230 kb and specifying a large number of enzymes involved in nucleic acid metabolism. The intranuclear replication of herpesviruses is complex and destruction of the infected cells accompanies the production of progeny. A state of viral latency follows primary infection; the site of latency is different for each of the viruses. For example, HSV-1, HSV-2, and VZV establish latency in neuronal cells, HSV-1 favoring the trigeminal ganglia, HSV-2 the sacral ganglia, and VZV the posterior root ganglia. In contrast, CMV maintains latency in monocytes and macrophages, EBV in B lymphocytes, and HHV-6 and HHV-7 in T lymphocytes. Viral persistence in infected hosts is lifelong. Periodic reactivation can occur with each of the herpesviruses, thus they are "incurable."

The seroprevalence of each of the herpesviruses varies according to a number of demographic factors including age, race, socioeconomic status, and country of residence. Among healthy young adults in the United States, the seroprevalence of herpesviruses ranges from <10% for HHV-8 to almost 100% for VZV, HHV-6, and HHV-7 [2]. The seroprevalence for EBV, CMV, and HSV-1 range from 50 to 75% and the seroprevalence of HSV-2 is about 25%. Spread of infection usually occurs as a result of contact with body secretions containing virus. Infectious virus may be present in skin or mucosal lesions (e.g., HSV-1, HSV-2, and VZV); in blood and transplanted organs from previously infected donors (e.g., CMV and EBV);

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or in genital (e.g., HSV-2) or oral (e.g., HSV-1, EBV, CMV, HHV-6, and HHV-7) secretions from previously infected but asymptomatic individuals. Thus, susceptible contacts are at risk of contracting infection from persons who have acquired an herpesvirus, in the recent or distant past. Indeed, the majority of herpesvirus infections result from contact with asymptomatic individuals shedding virus as a result of viral reactivation of a distant infection.

The clinical manifestations of herpesvirus infections depend upon the specific virus, whether the infection is primary or reactivated, and the immunologic status of the host. In general, the severity of symptoms is greater with primary vs. reactivated infections and among those with compromised immune systems.

HHV-6 is genetically most closely related to the other Betaherpesvirinae, CMV and HHV-7. HHV-6 was originally named human B-lymphotrophic herpesvirus because it was first isolated from peripheral blood lymphocytes of patients with HIV and lymphoproliferative diseases in 1986 [3]. It subsequently was recognized as an herpesvirus and was named HHV-6 because it was the sixth member of the herpesvirus family. In 1988 the virus was isolated from the lymphocytes of infants with roseola [4] and it is now recognized as the prime cause of this common childhood infection.

HHV-6 infection is ubiquitous and the major mode of transmission appears to be respiratory and oral secretions. Primary infection is common during infancy and about 50% of all children have been infected by 15 months of age [5]. The peak incidence of infection occurs during the second 6 months of life, corresponding to the time of waning transplacental, neutralizing IgG antibodies [6]. Most children have been infected by 2 years of age. Following initial infection with HHV-6, active viral shedding persists for long periods of time; in many infants high copies of viral DNA are detectable in oral secretions for more than a year [5]. Following primary infection, HHV-6 becomes latent in the salivary glands, brain, and mononuclear cells or macrophages.

The most widely recognized clinical manifestation of infection with HHV-6 in normal infants is roseola (exanthema subitum). The first isolation of HHV-6 was from the blood of four Japanese infants with this exanthematous infection [4]. Although HHV-6 is the primary cause of roseola, it is estimated that only 10–30% of infants infected with this virus will exhibit the classic clinical manifestations of this illness [6]. These manifestations include the abrupt onset of high fever ($\geq$40°C), irritability, and a diffuse "rash of roses" eruption spreading from the trunk to the face and extremities. Typically, body temperature begins to normalize at the time of rash appearance. In addition to some combination of fever, irritability, and rash, HHV-6 infection in infants may be associated with other common signs and symptoms, including, in decreasing order of frequency, occipital cervical lymphadenopathy, inflamed tympanic membranes, signs of upper respiratory tract infection, cough, gastrointestinal complaints (especially diarrhea), and seizures [7]. HHV-6 infection is considered to be one of the most important etiologies of febrile seizures in infancy.

Uncommon but reported manifestations of primary HHV-6 infection in normal infants and children include a prolonged febrile illness; elevated liver function tests;

arthritis; peripheral blood abnormalities, including low platelet count and low total white blood cell count; hemophagocytosis; Gianotti–Crosti syndrome; large vessel arteritis; and encephalitis. HHV-6 DNA was isolated from the cerebrospinal fluid of 14 of 35 adults referred to the National Institutes of Health with encephalitis of undetermined etiology [8]. Other recent possible associations with HHV-6 infection in children include acute appendicitis [9]; respiratory failure [10]; and cardiomyopathy [11]. An association between infection with HHV-6 in adults and brain tumors has been proposed [12].

Although most reactivated HHV-6 infections are asymptomatic, in some immunosuppressed hosts, reactivated infection can be associated with a febrile illness associated with rash and bone marrow suppression; precipitation of graft-vs.-host disease (GVHD); and disseminated infection involving the lungs, liver, and CNS. The likelihood of clinical manifestations is greatest in the presence of substantial immunosuppression, including the use of anti-CD3 antibodies; solid organ transplant recipients; and co-infection with CMV or HHV-7. Overall, it is estimated that between 30 and 50% of bone marrow and solid organ transplant recipients experience reactivated HHV-6 infections, although most of these episodes of reactivation are asymptomatic. In one recent study of hematopoietic stem cell transplant recipients, the occurrence of HHV-6 infection increased the likelihood of acute GVHD and non-relapse-related mortality [13].

Diagnosis of HHV-6 infection, especially in normal infants, usually is based on clinical findings. Isolation of infectious virus (research settings) or the presence of HHV-6 antigens, nucleic acids, and antibodies may be detected but generally such testing is not necessary [14]. Furthermore, even when these tests are used, it may not be possible to distinguish recurrent from primary infection. Definitive diagnosis of primary infection requires both the isolation of the virus and the occurrence of seroconversion. HHV-6 DNA has been detected in the cerebrospinal fluid of children with primary infection, especially in the presence of seizures [15]. Persistence of HHV-6 DNA in the CSF of healthy children and adults also has been reported and its role in subsequent neurologic consequences, if any, debated [16].

The management of HHV-6 infections is supportive; the role of specific antiviral therapy is not well established. Agents that have been used on occasion based upon good in vitro activity against HHV-6 include, in order of decreasing in vitro activity, cidofovir, ganciclovir, and foscarnet [13].

In summary, the sixth member of the herpesvirus family is ubiquitous, infecting most children by 2 years of age. The most distinctive feature of early childhood infection is roseola and the virus is responsible for a substantial proportion of febrile illnesses early in life, including those that precipitate febrile seizures. Severe primary infections are uncommon among the immunocompetent, and reactivated infections are usually not associated with symptoms. However, among the immunosuppressed, reactivated infections can be severe and life threatening. Therapy is symptomatic and supportive. Although some antiviral agents have good in vitro activity against HHV-6, their therapeutic role is not yet established.

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Advances in the Diagnosis and Management of Central Venous Access Device Infections in Children

Asha Bowen and Jonathan Carapetis

Abstract Infection is a well-known complication of central venous access device (CVAD) use, with an incidence of 3–6 bloodstream infections per 1,000 catheter days in children. Prevention of CVAD infections has improved with new strategies including the use of chlorhexidine antisepsis, bundles, maximal sterile barriers for insertion, prophylactic locks, antibiotic impregnated catheters and tunnelling of long-term devices. Despite these strategies, catheter-related bloodstream infections (CRBSIs) continue to be an important health problem. New approaches to diagnosis include differential time to positivity and quantification of blood cultures and molecular diagnostics. The management of CRBSIs includes techniques for line salvage including ethanol, antibiotic, hydrochloric acid, taurolidine and urokinase locks. When these fail, line removal and antimicrobial therapy are recommended.

1 Introduction

Central venous access devices (CVADs), both temporary and permanent, provide more convenient and often safer venous access in paediatric patients than peripheral devices. CVADs are commonly used in neonates, children and adolescents for the intravenous delivery of life-saving fluids that include blood products, medications and nutrition.

Despite these obvious benefits, CVAD use is occasionally complicated by bloodstream infection. Other complications include thrombosis, mechanical occlusion or breakdown in line integrity; however, these are not the focus of this review. CVAD infection is a well-recognised health care-associated complication that may be life threatening. CVAD-related bloodstream infections (CRBSIs) result in increased morbidity, mortality, length of stay and health care costs. A recent study reported that CRBSIs in children are directly responsible for 2.4 deaths/1,000 patients [1].

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Adult studies have consistently shown increased length of stay with associated costs and other complications in patients with CRBSI [2]. Each CVAD infection in a short-term catheter in paediatric intensive care unit (PICU) patients is estimated to cost between US $36,000 and US $50,000 [3]. CVAD infectious complications occur in 5–26% of adult patients [4]. Similar rates have been observed in children and adolescents [5–7].

Management of CRBSI in children is at times extrapolated from adult studies. However, there are significant differences between adult and paediatric patients with respect to CVAD infection. These include differing underlying diagnoses; planning for a lifetime of CVAD use beginning in infancy (e.g. haemophilia patients and parenteral nutrition (PN)-dependent children); fewer feasible line insertion sites; increased likelihood of obstructive thrombus; differing aetiological organisms (e.g. increased risk of candidal line infections in newborns, increased risk of polymicrobial infections in children with chronic gut pathology); and the diagnostic uncertainty when there is reluctance to perform peripheral blood cultures. The use of non-paediatric evidence in this review will be limited to areas where there is an absence of good quality studies in children.

2 Types of Devices Used in Children for Central Venous Access

Central venous devices used in children and adolescents can be divided into those providing temporary access and surgically tunnelled devices that are considered to be semi-permanent or permanent. Devices used in children may be single or multiple lumen, with a recent meta-analysis finding no difference in infection rates between these [8].

2.1 Temporary CVAD

Temporary venous catheters are percutaneously inserted into either a peripheral vein (brachial, cephalic) and fed into a central vein or alternatively a larger, more proximal vein (jugular, subclavian, umbilical, femoral) is cannulated. These temporary lines usually remain in situ for a period of less than 2 weeks and can easily be removed if infection or occlusion ensues. Temporary lines are not the focus of this review, due to the relative ease of removal in the context of an infection.

2.2 Permanent, Tunnelled CVAD

In contrast, permanently sited, cuffed, silicone or polyurethane CVADs provide greater management challenges in the context of infection. The use of tunnelled Broviac®, Hickman® and Portacath® devices has increased in children and adolescents in recent years, since the first report of their use for prolonged PN in

1973 [9], due to the benefits of administering intravenous treatment over the long term without regular venepuncture and peripheral canullation. These devices are inserted into a larger, more proximal vein (jugular or subclavian) with the femoral site avoided if possible, due to increased infection risk. A non-randomised study in neonates, however, did not show a difference in infection rate between upper body and femoral insertion sites [10]. Permanent devices often remain in situ for months to years resulting in increased infection risk with CVAD longevity.

3 Pathogenesis

The modes of CVAD infection include contamination at time of insertion, migration of skin micro-organisms from the hub along the catheter, contamination of the hub when infusing treatment, contamination of the infusate at the time of administration of treatment or, rarely, haematogenous seeding from a distant site of infection [11]. Infections occurring within 10 days of catheter insertion are usually due to extraluminal migration of skin organisms, whereas those occurring 10 days or more after insertion have an increased likelihood of originating from contamination of the catheter hub or infusate.

The presence of a long-term intravascular device acts as a foreign body, providing a structure upon which a biofilm can develop and within which micro-organisms adhere and subsequently cause infection. The biofilm consists of host proteins (albumin, fibrinogen, fibrin, fibronectin, platelets), extracellular polysaccharide matrix and the micro-organisms [12, 13]. Biofilms protect organisms from antibiotics; in many organisms the mean inhibitory concentrations of antibiotics increase a hundred to a thousand-fold when the organisms are within a biofilm [14].

3.1 Common Organisms

Gram-positive bacteria predominate, accounting for up to 65% of all positive culture results [15, 16]. Of these, coagulase-negative staphylococci (CONS) are identified most frequently followed by enterococci, *Staphylococcus aureus* and *Streptococcus* species [16]. For confirmed CRBSI with Gram-positive organisms typically associated with skin contamination (*Corynebacterium*, *Propionibacterium*, CONS), at least two blood cultures collected at different time points must be positive [17].

Gram-negative enteric organisms cause approximately one-quarter of CRBSI, notably *Klebsiella* species, *Escherichia coli*, *Enterobacter* species and *Pseudomonas aeruginosa* [16]. Candidaemia accounts for one in ten CRBSIs, with the prominent yeast species including *albicans, parapsilosis, tropicalis* and *glabrata* [16]. Ten percent of total CRBSI episodes are polymicrobial with commonly identified organisms including CONS, enterococci, *Candida* and *Klebsiella* species [16].

4 Epidemiology

Rates of CVAD infection vary according to patient (underlying diagnosis, length of hospitalisation, immunodeficiency, age) and device factors (complications related to insertion, anatomical location, length of time CVAD has been in situ and number of lumens utilised) [18–20]. Infection risk increases particularly in patients less than 2 years of age, if the child has an underlying chronic disease, immunodeficiency or is on immunosuppressant therapy or PN or if the CVAD insertion is difficult or in a "dirty" site [21].

Thirty-six paediatric intensive care units (PICUs) in the USA contributing data to the national health care safety network have estimated a mean of 5.3–7.3 CRBSIs per 1,000 catheter days in PICU patients [22]. Contributing to these rates are the various patient groups admitted to PICUs, with differing patterns of CRBSI. For example, patients with respiratory illnesses have CRBSI rates of 2.1/1,000 catheter days, whereas patients with burns have rates as high as 30.2/1,000 catheter days [23]. Along with this, individual PICUs report rates as low as 0.9/1,000 catheter days (http://www.cincinnatichildrens.org/about/measures/system/patient-quality/cvc.htm, accessed 20 September 2009) demonstrating the variability that has been achieved with practice improvements, including bundles and insertion site hygiene [18].

CRBSI rates in neonates are particularly high at 11.3/1,000 catheter days [22], and children with immunodeficiency have similar rates of CRBSI. Haematology and oncology units report rates of CRBSI of 1.7–4.3/1,000 catheter days [24, 25], well below those in neonates; however, haematopoietic stem cell transplant recipients may not be included in these figures [25]. PN-dependent children are another risk group for CRBSI, and rates of infection vary dependent on whether the patient is hospitalised or cared for at home. Rates as low as 2.2/1,000 catheter days have been achieved with home PN with appropriate family education and support [26]. CRBSI rates for hospitalised PN-dependent children with chronic gastrointestinal disturbance and risk of translocation of enteric pathogens are higher. Parenteral nutrition is an independent risk factor for CRBSI, RR 3.3 (95% CI 1.3, 8.3) [27]. Surgically inserted haemodialysis catheters also place children at risk of CRBSI with rates of 0.58–0.71 per patient year reported [28, 29].

A significant advancement in reducing rates of CRBSI has been the use of tunnelled catheters. The rationale for their use is that tunnelling through subcutaneous tissues increases the distance between the insertion site and the vein, thus reducing the transmission of pathogens from the skin. A pooled analysis of different types of CVAD found that tunnelled, cuffed devices resulted in a mean of 1.2 (95% CI 1.0, 1.3) CRBSIs per 1,000 device-days compared to 2.3 (95% CI 2.0, 2.4) for standard devices, although these data were largely from adult studies [30]. Similarly, a meta-analysis of adult studies found that tunnelling reduced confirmed CRBSI sepsis by 44% (RR 0.56, 95% CI 0.31, 1.0), although most of the benefit came from one study of internal jugular lines, and the confidence interval for the pooled subclavian studies crossed one [31]. One paediatric randomised, controlled trial in 98 critically ill children found a significant reduction in line colonisation using tunnelling of

femoral CVADs. There was no significant reduction in CRBSI, but there were only five CRBSIs during the study [32].

CRBSIs have a significant impact on patient quality of life and utilisation of hospital resources (bed days, theatre time, new lines, antimicrobials) [2]. Consequently, investment in monitoring of CRBSI, supervision and bundles for insertion, improvements in catheter care (chlorhexidine) and new strategies for line salvage have become important hospital priorities [18, 19].

5 Diagnosis

Most bloodstream infections in children in affluent countries are associated with an intravascular device [33, 34]. CRBSI is suspected in a febrile child (temperature >38°C) with a CVAD in situ, without focal features or exit site infection [35]. The gold standard for diagnosis of CRBSI is isolation of a micro-organism from catheter tip culture. This diagnosis follows CVAD removal; however, this is not always necessary for appropriate management, so tip culture may not be available. Indeed, most CVADs removed because a clinical diagnosis of CRBSI is made, are subsequently determined to be non-infected on CVAD tip culture [11, 36, 37].

Therefore, the clinician usually requires a diagnosis of CRBSI to be made while the catheter remains in situ. There is no consensus definition of a paediatric CRBSI, although Randolph et al. offer useful recommendations on definite, probable and possible CRBSI in children [22] (Table 1).

To confirm the suspicion of a CRBSI, blood cultures should be drawn through the CVAD. A surrogate definition often used is that of fever and positive CVAD

Table 1 Recommendations on defining catheter-related bloodstream infection in children

	Recommendations
Possible	CVAD in situ in patient with fever, in association with • positive CVAD tip culture *and* fever that resolves when CVAD is removed; *or* • ≥1 positive BC with a common skin contaminant organism *and* no other source for the bacteraemia other than the CVAD
Probable	CVAD in situ in patient with fever, in association with • positive CVAD tip culture and resolution of symptoms within 48 h of CVAD removal; *or* • ≥2 positive BC with a common skin contaminant organism *and* no other source for the bacteraemia other than the CVAD
Definite	One peripheral positive BC in association with • positive quantitative CVAD culture with same organism; *or* • simultaneous positive quantitative BC; *or* • differential time to positivity of >2 h; *or* • pus from exit site growing same organism as BC

Adapted from Table 1 in Randolph et al. [22]
BC, blood culture; CVAD, central venous access device; ≥, greater than or equal to; >, greater than

blood cultures, with no other discernable focus of infection [17]. However, in a child with multiple risk factors for infection (as is often the case when a CVAD is in situ), a single positive blood culture in a febrile child is poorly specific for a diagnosis of CRBSI. Consequently, the diagnosis of paediatric CRBSI is challenging and may result in the overtreatment of CRBSI and the unnecessary removal of CVADs.

More accurate methods include quantitative blood cultures – either a fivefold difference or more in CFU count between simultaneous centrally and peripherally drawn cultures or an absolute count of $\geq$100 CFU/mL from centrally drawn cultures. However, this technique is labour intensive in the laboratory and thus not widely available. Other techniques including acridine orange leucocyte cytospin [36, 38], molecular diagnosis [39] or endoluminal brush quantitative cultures are also either labour intensive, not widely available or (in the case of the endoluminal brush) too dangerous to recommend routinely.

But a further method, differential time to positivity of central and peripheral blood cultures, has high predictive value and has also been studied in children [40–46]. In one study in immunocompromised children, a differential of 2 h or more (i.e. the CVAD culture signalling positive at least 2 h before the peripheral culture) gave a positive predictive value for CRBSI of 100% (95% CI 63, 100) and a negative predictive value of 96% (95% CI 78, 100) [43]. In most hospitals that use modern, automated blood culture systems, this technique should be possible. However, it requires well-trained medical staff who routinely perform simultaneous peripheral and CVAD cultures and who also aim for optimal volumes of blood (and, importantly, identical blood volumes from each site). Unfortunately, reluctance on behalf of patients, parents and paediatricians means that in most centres, simultaneous peripheral and central blood cultures are usually not taken [39] as would commonly occur in adult patients.

6 Prevention

The measures recommended by the US Healthcare Infection Control Practices Advisory Committee (HICPAC) to be based on good evidence include education of health staff about insertion technique and CVAD maintenance, institutional monitoring of CRBSI rates, good hand hygiene, having a dedicated infusion treatment team, use of sterile, semi-permeable dressings, avoidance of femoral insertion sites and removal of lines as soon as possible [47]. Newer strategies also recommended by HICPAC include cutaneous antisepsis using 2% chlorhexidine, use of maximal sterile barriers, use of chlorhexidine-impregnated sponges over the insertion site, antimicrobial catheters and antibiotic locks. Locks will be considered later in this review. Antimicrobial catheters – usually coated or impregnated with silver or antibiotics – have been extensively studied. In two pooled analyses, silver-impregnated catheters significantly reduced the incidence of CRBSI [48–50], and a pooled analysis of minocycline–rifampicin-coated catheters also found

dramatic reductions in CRBSI rates [48, 49]. However, these studies used adult data and focused on catheters left in situ for relatively short periods, so there is no evidence that impregnated or coated catheters are effective for long-term use.

7 Management

The mainstay of treatment is systemic antimicrobial therapy. In addition, decisions must be made about whether to remove or attempt to salvage the catheter. It is also important to offer appropriate supportive therapy as needed (e.g. intravenous fluids, inotropes, blood products) and to reduce or even cease PN during the early treatment period if feasible. The treatment and prevention of CVAD infection has been augmented in recent times through a range of new strategies, mainly in the form of lock therapies.

7.1 Antimicrobial Therapy

After diagnostic specimens have been collected for suspected CRBSI, empiric antimicrobial therapy is commenced [34]. This is based on an evaluation of patient risk factors (neutropenia, underlying diagnosis, previous CRBSI pathogens, features of sepsis) and CVAD-related factors (exit site or tunnel infection, length of insertion, need for ongoing CVAD utilisation and location of CVAD). Empiric therapy should broadly cover Gram-positive and Gram-negative bacterial pathogens based on the common infective organisms (see Common Organisms). Antifungal therapy may be added if risk factors for fungal infection are known (neutropenia, PN, neonates) or in the context of ongoing fevers despite broad spectrum Gram-positive and Gram-negative antibiotics.

When blood and other culture (e.g. exit site, tip if catheter removed) and antimicrobial susceptibility results are available, antimicrobial therapy should be directed to cover the known pathogens [19, 34]. However, positive culture results will not always be available (see Diagnosis) and in this setting, empiric treatment may continue for 7–14 days [34].

CRBSI is a serious complication of intravascular devices and recommendations often include prompt removal of the CVAD, particularly in the context of septic shock. The indications for CVAD removal have reduced over the past decade, particularly with the availability of measures to augment systemic antimicrobial treatment, such as lock therapy (see New Strategies). CVAD salvage may be considered early in the diagnosis if the patient is stable. The indications for line removal are summarised in Table 2.

The duration of therapy for CRBSI partly depends on whether the CVAD is removed or if there is an attempt to salvage it. Recent reviews of adult CRBSI [19, 34] recommend length of treatment based on the infecting organism (Table 3).

Table 2 Indications for removal of infected CVADs

Short-term lines
Most suspected of being infected, particularly if purulence at insertion site
Long-term lines
Organism-independent reasons
Persistent exit site infection
Tunnel infection
Complications (e.g. endocarditis, septic thrombosis)
Bacteraemia or candidaemia persists >3 days on treatment
Infection with the following organisms
Corynebacterium jeikeium
Bacillus spp.
Stenotrophomonas spp.
Burkholderia cepacia
Pseudomonas spp.
Filamentous fungi
Malassezia spp.
Mycobacterium spp.

Table 3 Length of therapy for CRBSI based on organism cultured

Organism	Minimum length of systemic antimicrobial therapy
Coagulase-negative *Staphylococcus*	7 days
S. aureus	14 days
Gram-negative bacilli	10–14 days
Candida species	14 days
Polymicrobial infections	14 days

7.2 New Strategies

A range of antimicrobial and antiseptic solutions instilled into the line have been tried in recent years. All of these have been used both to prevent CVAD infection (e.g. by regular, prophylactic instillation) and to augment systemic antibiotic therapy as treatment of CVAD infection.

8 Antimicrobial Lock Therapy (ALT)

Antimicrobial solutions in high concentrations are instilled into CVADs, in combination with systemic antimicrobials (when infection is being treated, rather than for prevention), in an attempt to decontaminate the intraluminal surface of the device [51]. This is an adjunctive therapy, as prolonged courses of systemic antimicrobials (7–21 days) fail in up to 30% of treatments, resulting in catheter removal [34, 52] due to the inability of systemic antibiotics to penetrate the organisms enmeshed in

the biofilm [12]. This technique also has the potential to deal with catheter infections that occur via colonisation of the hub [19, 34]. In vitro models have demonstrated efficacy of such an approach with a wide variety of antimicrobial classes [15, 17, 51, 53–55].

The antimicrobial lock technique has become more widely used in the treatment of CRBSI, due to the potential to extend the lifespan of a CVAD [56, 57]. ALT, in combination with systemic antibiotics, has been accepted by the Infectious Diseases Society of America (IDSA) for treatment of CRBSI in stable patients when CVAD salvage is a priority [34]. The technique has considerable paediatric application, particularly for children who may have limited vascular access sites available [58]. Various antimicrobial locks have been trialled in adult patients for the treatment of CRBSI with inconsistent results [19, 59–61].

A meta-analysis of vancomycin containing lock or flush solutions in predominantly paediatric patients demonstrated a reduction in the incidence of bloodstream infections with a risk ratio of 0.34 (95% CI 0.12,0.98; p=0.04) for those using true lock solutions [62]. Other antibiotics and antifungals have also been published in paediatric case reports and case series. Table 4 summarises the results of these studies. Most of the trials of antimicrobial locks combine an anticoagulant such as heparin or EDTA with the antimicrobial in order to improve antibiotic penetration into the biofilm and prevent thrombus formation occluding the CVAD [11].

9 Ethanol Locks

Ethanol is an effective disinfectant against bacteria and fungi and leads to sterilisation of many organisms in biofilm. Concentrations of more than 40% are needed to inhibit bacterial growth in established biofilms, and more than 70% ethanol exposure for 2 h or more is needed to kill the bacteria in established biofilms of many Gram-positive and Gram-negative bacteria and fungi [67]. Ethanol locks have been trialled in predominantly paediatric patients. Ethanol has been used in the treatment of CRBSI due to both the antimicrobial and fibrin degradation properties. There have been two paediatric case series [68, 69] published and a number of small trials presented at conferences [70]. There is a randomised controlled trial underway in children with malignancy which, when published, may provide greater insights into the real benefit of this technique (www.clinicaltrials.gov, NCT00471679 accessed 6 September 2010: Trial currently suspended).

10 Hydrochloric Acid Locks

Hydrochloric acid reduces pH, which denatures proteins and disrupts biofilms. Hydrochloric acid locks have also been considered for the management of CRBSI for the same reasons as ethanol [71]. A small case series has been published demonstrating efficacy of this technique; however, more studies are needed to alleviate concerns regarding safety [71].

Table 4 Trials of antibiotic lock therapy published in children

Antibiotic	Type of trial	Number of episodes	Results	Details
Teicoplanin (0.3 mL, 40 mg) + sodium heparin [63]	Prospective case series	20 episodes in 13 PN-dependent children	17/20 (85%) CVADs salvaged Shortened duration of hospitalisation at 5 days (cf15)	CONS or *S. aureus* 12 h locks for 15 days ALT aspirated
Vancomycin, amikacin, or AmphoB [64]	Case series	11 children, 12 episodes	10/12 (83%) CVADs salvaged	CONS, GNB, *Candida*
Ampicillin (3 mL, 15 mg) [65]	Case report	1 PN-dependent child with Broviac CRBSI	BC negative at days 1, 3 and 9 after starting ALT BC negative at 0, 2 and 4 weeks after stopping ALT	*E. coli and Enterococcus durans* both sensitive to ampicillin 6 h locks for 9 days ALT aspirated
Linezolid (2 mg/mL) + heparin [58]	Case report	1 PN-dependent child	Daily BC negative and at 3 weeks from end of Rx CVAD salvaged	*S. epidermidis*, sensitive to linezolid 8 h locks for 20 days
Placebo: heparin GP: vancomycin 0.5 mg/mL GN: ceftazidime 0.5 mg/mL [17]	DBPCRCT, adults and children	44 analysed by modified ITT[a] –21 ALT –23 Placebo	At 24 weeks, failure to cure CRBSI occurred in 7/21 (33%) of ALT and 13/23 (57%) of placebo. p=0.10	CONS (61%), other GP (16%), GN (23%) 8–12 h locks for 7–14 days
Vancomycin 25 μg/mL [66]	DBRCT, prevention	85 neonates	2/42 (5%) in Rx group developed CRBSI vs. 13/43 (30%) in placebo	20–60 min locks daily
Vancomycin 25 μg/mL flush or lock solution [62]	Meta-analysis of 6 paediatric trials	462 patients, 345 children	23% CVADs removed in Rx group vs. 96/261 (37%) in placebo Protective with *RR*=0.49 (95% CI 0.26–0.95), p=0.03 Vancomycin lock *RR*=0.34 (95% CI 0.12–0.98), p=0.04	Both vancomycin flushes and locks included in study

mL, millilitre; mg, milligram; PN, parenteral nutrition; BC, blood culture; CVAD, central venous access device; CONS, coagulase-negative *Staphylococcus*; GP, Gram positive; GN, Gram negative; DBPCRCT, double blind placebo-controlled randomised controlled trial; Rx, treatment; CRBSI, catheter-related bloodstream infection; ALT, antibiotic lock therapy; ITT, intention to treat; DBRCT, double blind randomised controlled trial; RR, relative risk; CI, confidence interval; h, hour; μg, micrograms

[a]Trial closed early due to failure to recruit enough patients

11 Taurolidine Citrate Locks (TauroLock)

Taurolidine is an amino acid with broad spectrum antimicrobial activity and has been shown in vitro to inhibit the formation of CVAD biofilm. Small studies in adult patients including one randomised controlled trial have been published for prevention and treatment of CRBSI [72]. Recently a single centre, prospective, cohort study to prevent CRBSI in paediatric cancer patients has been published [73] showing a statistically significant decline in the proportion of CONS CRBSI; however, a corresponding increase in the number of *E. coli* infections occurred. A prospective, randomised controlled trial for the prevention of CRBSI in children with tunnelled CVADs is underway (www.clinicaltrials.gov NCT00735813, accessed 6 September 2010). There are no published studies utilising taurolidine in the treatment of paediatric CRBSI.

12 Urokinase Locks and Infusions

Urokinase, in conjunction with antimicrobials, is used in the adjuvant treatment of CRSBI. The theoretical benefit of adding urokinase to the treatment regimen is based on degradation of fibrin and biofilm [74, 75]. Urokinase is widely used in paediatric haematology/oncology to unblock occluded catheters and prophylactically to prevent CRBSI. Simon et al. summarise the available evidence on prophylactic urokinase locks [75]. A randomised controlled trial in 74 children using urokinase flushes was underpowered to show a difference between placebo and urokinase in prevention of CRBSI [76]. A multicentre, randomised, placebo-controlled trial of 1 h urokinase locks every 2 weeks, involving 577 children with cancer, showed a reduction in infectious complications (1.2 vs. 2.2 events/1,000 utilisation days, p=0.05) [77].

However, the use of urokinase in the treatment of CRBSI is less frequent. There are no prospective randomised controlled trials utilising urokinase in the treatment of paediatric CRBSI. A case series of 97 children showed successful treatment of CRBSI in 88% of episodes with fewer than 5% of CVADs requiring removal [74].

13 Conclusions

The utilisation of central venous access devices in children with various underlying diagnoses continues to increase due to the benefits of convenient intravascular access with reduced pain for children. However, the most common complication of such a device is infection, the consequences of which, in terms of morbidity, mortality and cost, are high. A range of strategies are available to reduce CRBSI rates, and those based on good evidence should be used wherever possible. Often management strategies for paediatric CRBSI are inferred from adult studies, highlighting the need for better evidence in children. Strategies for prevention or treatment of CVAD infection that may not be widely practiced, but for

which the evidence for effectiveness is accumulating, include the use of chlorhexidine (rather than povidone-iodine) antisepsis, maximal sterile barriers for CVAD insertion and handling, tunnelled catheters and ports for long-term use wherever possible, use of vancomycin or ethanol prophylactic locks for prevention of infection in long-term catheters, antibiotic- or silver-impregnated catheters for short-term use, use of difference in time to detection of peripheral and central blood cultures for diagnosis, wider use of attempts at salvaging rather than removing infected lines and augmenting systemic antibiotic therapy with antibiotic or ethanol locks for treatment.

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Moraxella catarrhalis – Pathogen or Commensal?

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Abstract *Moraxella catarrhalis* is an exclusively human commensal and mucosal pathogen. Its role as a disease-causing organism has long been questioned. Today, it is recognized as one of the major causes of acute otitis media in children, and its relative frequency of isolation from both the nasopharynx and the middle ear cavity has increased since the introduction of the heptavalent pneumococcal conjugate vaccine, which is associated with a shift in the composition of the nasopharyngeal flora in infants and young children. Although otitis media caused by *M. catarrhalis* is generally believed to be mild in comparison with pneumococcal disease, numerous putative virulence factors have now been identified and it has been shown that several surface components of *M. catarrhalis* induce mucosal inflammation. In adults with chronic obstructive pulmonary disease (COPD), *M. catarrhalis* is now a well-established trigger of approximately 10% of acute inflammatory exacerbations.

Although the so-called cold shock response is a well-described bacterial stress response in species such as *Escherichia coli*, *Bacillus subtilis* or – more recently – *Staphylococcus aureus*, *M. catarrhalis* is the only typical nasopharyngeal pathogen in which this response has been investigated. Indeed, a 3-h 26°C cold shock, which may occur physiologically, when humans inspire cold air for prolonged periods of time, increases epithelial cell adherence and enhances proinflammatory host responses and may thus contribute to the symptoms referred to as common cold, which typically are attributed to viral infections.

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1 Introduction

Moraxella catarrhalis is exclusively a human pathogen and commensal of the upper respiratory tract. All attempts to establish long-term respiratory tract colonization in non-human primates or other vertebrates have failed. *Moraxella catarrhalis* is a gram-negative, aerobic, variably piliated, nonmotile, strongly autoagglutinating and non-encapsulated diplococcus, which microscopically resembles *Neisseria meningitidis* and *Neisseria gonorrhoeae* but is more closely related genetically to *Acinetobacter spp.* and the Pseudomonadales [1]. All other members of the genus *Moraxella* are gram-negative rods. The molecular basis of the exquisite tropism of *M. catarrhalis* for humans has not entirely been elucidated. Some data indicate that the organisms' iron-uptake apparatus requires a specific ligand–receptor interaction between human iron transport proteins (e.g., lactoferrin and transferrin) and specific binding proteins on the bacterial surface (e.g., lactoferrin-binding protein B) [2].

Formerly known as *Micrococcus catarrhalis* (first described in 1896), and renamed *Neisseria catarrhalis* (1963) and *Branhamella catarrhalis* (1970), *M. catarrhalis* was moved to the Genus *Moraxella* in 1984 (Class 1 γ-proteobacteria, order *Pseudomonadales*, family *Moraxellaceae*, Genus *Moraxella*) and had long been considered a non-pathogenic commensal of the nasopharynx. Its role as disease-causing organism has only recently been recognized and its pathophysiological mechanisms are still poorly understood. Nonetheless, several lines of evidence now indicate that *M. catarrhalis* causes mucosal infections in immunocompetent children (mainly otitis media) [3] and adults (acute exacerbations of chronic obstructive pulmonary disease) [4]. Invasive disease (e.g., bacteraemia, endocarditis, meningitis, arthritis) is extremely rare and almost exclusively occurs in immunocompromised individuals [5].

2 Phylogenetic Evidence for Virulence

The species *M. catarrhalis* consists of two major phylogenetic lineages. This was first emphasized by Bootsma et al. [6] and was recently described in detail by Wirth et al. [7]. Differentiation can easily be made by DNA sequence analysis of the 16S rDNA gene [6]. The phylogenetically older subpopulation (type 2) is believed to have existed long before the emergence of *Homo sapiens*, while the phylogenetically younger subpopulation is believed to have emerged approximately 4 million years ago, together with *H. sapiens*. Interestingly, the latter subpopulation is associated with the expression of phenotypic traits characteristically associated with bacterial virulence. These include resistance to human complement and adherence to human epithelial cells. This subpopulation is also naturally transformation competent [8] leading to frequent homologous recombination and thus reduced genetic diversity as a result of clonal selection (Table 1) [7]. Thus, it appears that type 1 strains are closely adapted to the human host. Convincing clinical evidence supporting the notion that type 1 strains are more commonly associated with disease as opposed to

Table 1 Characteristics of the two major phylogenetic subpopulations of *M. catarrhalis*

	Subpopulation	
	Complement sensitive (16S rDNA type 2)	Complement resistant (16S rDNA type 1)
Age	~50 million years	~4 million years
Genetic diversity	Extensive	Low
Homologous recombination	Rare	Frequent
Virulence factor expression	Rare	Frequent

asymptomatic colonization is currently lacking, but there is circumstantial evidence described below.

3 Expression of Virulence Factors

3.1 Complement Resistance

Type 1 strains are typically complement resistant in vitro. Exposure to human complement does not affect the growth of these strains, while complement-sensitive strains (mainly type 2) are killed by human complement within 60–120 min of exposure. Complement resistance is believed to be mediated mainly by expression of the UspA2 outer membrane protein (OMP), although other OMPs (e.g., CopB [9]) are also essential for this phenotype. UspA2 is a trimeric autotransporter protein, expressed abundantly which binds the complement modulator serum protein vitronectin and which inhibits assembly of the C5–C9 membrane attack complex [10]. It has also been demonstrated that *M. catarrhalis* OM vesicles are capable of protecting complement-sensitive *Haemophilus influenzae* against the bactericidal effect of complement, mainly by binding/inactivation of vitronectin, C4bp, C3 and factor H [11]. Complement resistance is believed to be a virulence factor because several clinical studies have indicated that this phenotype is associated more commonly with disease-causing isolates than with colonizing isolates [12, 13].

3.2 Adherence to Human Epithelial Cells

Bacterial virulence of mucosal pathogens is generally believed to depend on the organisms' capacity to bind to the epithelial layer of the host's mucosal surface. Complement-resistant type 1 strains generally express UspA1, a major *M. catarrhalis* adhesin, which is closely related to UspA2 [14] and which binds human epithelial cells via cell-bound fibronectin or the CEACAM-1 [15, 16] adhesion molecule displayed on various respiratory epithelial cell types. Interestingly, specific domains of UspA1 function as binding sites for fibronectin or CEACAM-1 [15, 16]. Type 2 strains typically do not express UspA1 on their surface [17], although they carry the *uspA*1 gene on their chromosome. Thus, type 1 strains

Table 2 Currently known adhesins of *M. catarrhalis* and their respective cellular ligands

Ligand	Cell line	Host cell receptor	References
UspA1	HEp-2	Not determined	[19]
UspA1	Chang	Fibronectin/α5β1	[20]
UspA2H	Chang	Not determined	[21]
UspA1	A549	CEACAM1	[22]
Hag/MID	A549	Not determined	[23]
Hag/MID	Human middle ear cells	Not determined	[23]
McaP	A549	Not determined	[24]
McaP	Chang	Not determined	[24]
OMP CD	A549	Not determined	[25]
LOS	Chang	Not determined	[26]
LOS	HeLa	Not determined	[26]

express adhesins, which enable their close interaction with the respiratory tract epithelial surface. In addition, UspA1 has also been shown to be essential for the internalization of *M. catarrhalis* cells into nonprofessional phagocytes [18].

Moraxella catarrhalis is known to display various adhesins specific for receptors expressed by different respiratory tract cell lines (Table 2). This redundancy may allow the organism to colonize the middle ear, the oro- and nasopharynx, as well as the bronchial surface and alveolar space of the human respiratory tract.

3.3 Colonization and Immune Response

Up to 80% of children have been colonized at least once with *M. catarrhalis* by the time they reach the age of 2 years [27]. Thereafter colonization frequency drops continuously to <10% in older children and healthy adults. It increases again in the elderly [28]. Colonization is not prevented by the appearance of mucosal (salivary) IgA directed against the major surface immunogens [25, 29]. However, there is a strong correlation between the disappearance of mucosal *M. catarrhalis* and the appearance of bactericidal serum anti-*M. catarrhalis* IgG1 and IgG3, which are mainly directed against the UspA OMP [30–32]. These data indicate that systemic humoral immunity recognizes immunodominant *M. catarrhalis* surface antigens and eliminates surface carriage by the development of bactericidal IgG antibodies and, presumably, complement-mediated killing.

However more recent evidence indicates that *M. catarrhalis* may evade humoral immunity by (i) intracellular location in epithelial cells (Fig. 1) [18, 33] and by submucosal location in pharyngeal lymphoid tissue (Fig. 2) [34]. The clinical relevance of these observations has not been elucidated, but it appears possible that *M. catarrhalis*, similarly to *H. influenzae*, *Staphylococcus aureus* and *Streptococcus pyogenes*, may be capable of entering an intracellular niche which protects the organism against the host's immune system. Preliminary data suggest that neither

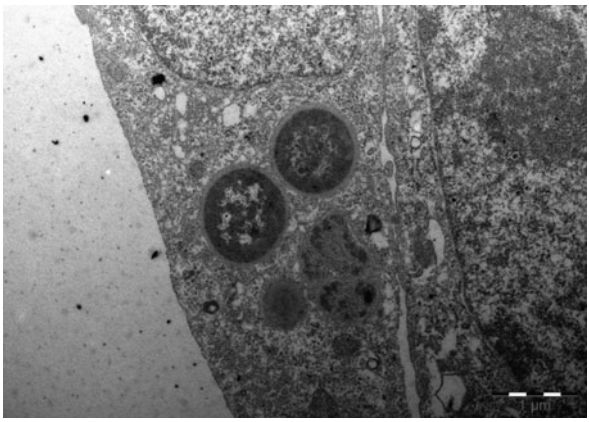

Fig. 1 Transmission electron micrograph of a Detroit 562 pharyngeal cell harbouring two *M. catarrhalis* cells 3 h after infection of a cellular monolayer with *M. catarrhalis* strain O35E and subsequent killing of all extracellular bacteria using 200 μg/ml of gentamicin

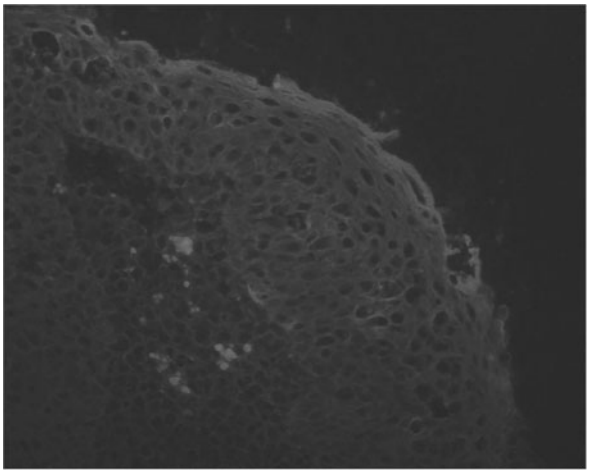

Fig. 2 Immunohistochemistry of a human tonsil showing subepithelial aggregates of *M. catarrhalis*. Squamous cell epithelium is stained with rabbit anti-human cytokeratin and goat anti-rabbit Cy3. *Moraxella catarrhalis* is stained with the monoclonal mouse monoclonal antibody 24B5 (a gift from Dr. E Hansen, Dallas, TX) and goat anti-mouse Alexa 488 and appears light

phase variation nor antigenic variation plays a substantial role in this phenomenon (author's unpublished data).

3.4 Biofilm Formation

A virulence mechanism employed by many bacterial species is the expression of biofilms, which protect individual bacteria from the detrimental effects of host immune mechanisms and antimicrobial substances. Biofilm formation in *M. catarrhalis* has not been investigated in detail, but some data clearly indicate that some clinical isolates produce biofilms in vitro [35, 36] and also in vivo on the middle ear mucosal surface [37].

3.5 *Cellular Invasion*

As stated above, recent evidence indicates that *M. catarrhalis* is capable of invading human respiratory tract cells in vitro and that this capacity is dependent on the expression of UspA1 and lipooligosaccharide [18, 33]. However, studies investigating long-term persistence have not been conducted to date. Thus, it remains unclear whether invasion is a means of evading the immune system as well as the effects of extracellular antimicrobial agents. Further studies will be needed to address these issues in detail.

3.6 *Proinflammatory Activity of* Moraxella catarrhalis

By contrast, it is now well established that both *M. catarrhalis* whole cells and outer membrane preparations induce strong proinflammatory stimulation in epithelial cells (interleukin-8) [38] and peripheral blood mononuclear cells (IL-1β, IL-6, TNF-α) [39]. These observations suggest that the presence of *M. catarrhalis* may cause inflammation and, consequently, clinical symptoms of respiratory tract disease in otherwise sterile locations.

4 Cold Shock Response of *Moraxella catarrhalis*

When humans inspire cold air (e.g., –1°C) for prolonged periods of time, their nasopharyngeal temperature drops to approximately 26°C within several minutes of the beginning of the exposure [40]. In an in vitro model, this "cold shock" affects the resident *M. catarrhalis* flora in several ways. Abundance of mRNA transcripts of the UspA1 adhesin increases with reduced temperature and reaches a maximum at 26°C [34], an effect possibly explained by a prolonged mRNA half-life at decreased temperature [41]. Consequently, more UspA1 adhesin is expressed on the bacterial surface at 26°C and adherence to epithelial cells such as Chang conjunctival cells [34], Detroit 562 pharyngeal cells and HEp2 laryngeal cells is enhanced (Fig. 3) [41]. Enhanced adherence translates into increased mucosal surface density, a phenomenon shown to be associated with an increased likelihood of acute otitis media in children [42]. On a molecular level, increased surface expression of

Fig. 3 Cold shock increases the outer membrane protein (OMP)-mediated release of proinflammatory cytokine IL-8 in Detroit 562 epithelial cells, which were incubated for 16 h with increasing doses of heat-inactivated strain O35E (**a**) and the strains 300,415, 420 (**b**) exposed to 26°C or to 37°C or stimulated with outer membrane vesicles isolated from *M. catarrhalis* exposed to 26°C or 37°C (**c**). IL-8 secretion in the supernatants was measured by ELISA. In each case, results from one representative experiment of either two or three replicates are shown. Results are expressed as mean ± 1 SD of duplicate wells. *, $P<0.05$ (two-way analysis of variance) at 26°C vs. 37°C. MOI stands for multiplicity of infections and indicates the ratio between bacteria inoculated and epithelial cells in a given well.

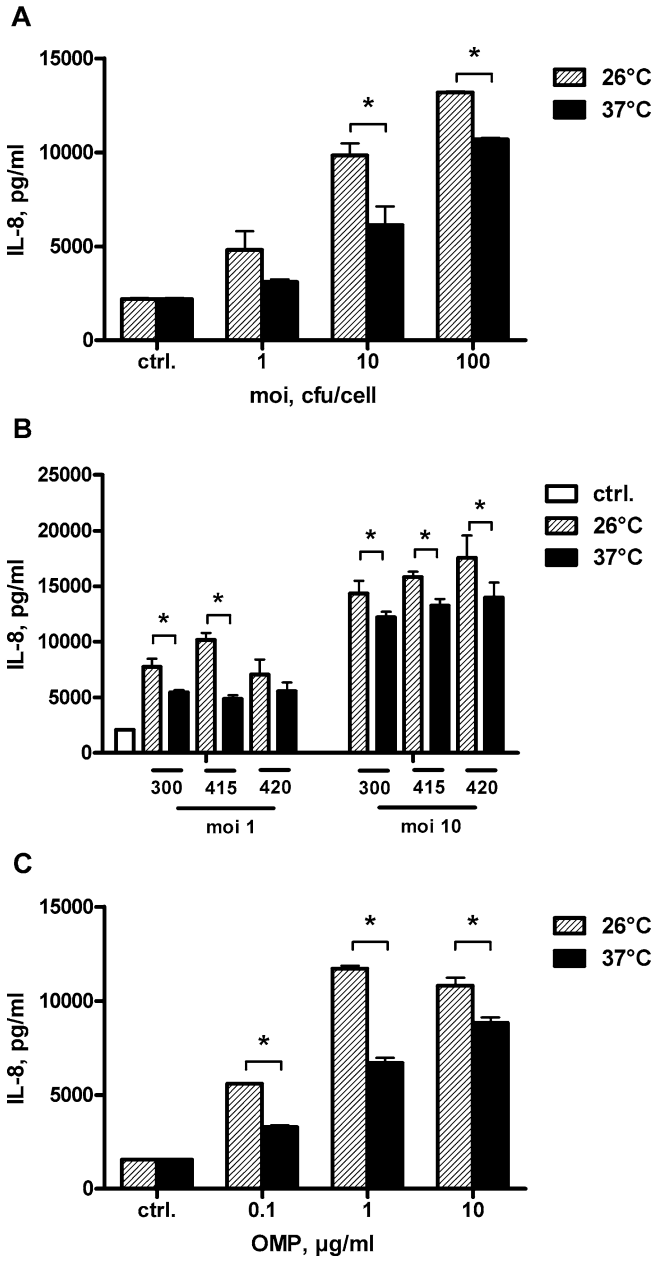

Fig. 3 (Continued)

UspA1 results in enhanced binding of fibronectin, which mediates binding to epithelial cells via α5β1 integrin [41]. The UspA1 cold shock phenomenon occurs in both phylogenetic lineages and is accompanied by increased expression of typical "cold

shock proteins" such as *recA* [34]. Genomic analyses of the cold shock response of *M. catarrhalis* have not been conducted to date. Unpublished data indicate that a limited number of OMPs are upregulated at 26°C and that others, e.g., haemagglutinin (which is an adhesin for A549 lung cells) or the m35 [43] outer membrane porin, are upregulated at 37°C.

Also of potential clinical relevance is the finding that *M. catarrhalis* undergoing a 3-h cold shock at 26°C induces a markedly enhanced proinflammatory immune response in comparison with bacteria held at 37°C. Both whole bacteria and outer membrane preparations induced a significantly enhanced release of IL-8 from Detroit 562 pharyngeal cells in one study (Fig. 3) [41].

5 Summary

The data summarized here emphasize that *M. catarrhalis* is a commensal and pathogen which is well adapted to the human respiratory tract niche, that it usually behaves as non-pathogenic commensal, but under certain circumstances it can become pathogenic. Such opportunities may include viral co-infections, augmented mucosal density of the organism (which might theoretically occur, for example, as a result of colonisation replacement phenomena secondary to pneumococcal vaccination) or – as specifically discussed in this article – exposure of the pharynx to cold air. More research is needed to understand the modes of transition from commensal to pathogen better. However, overall, the behaviour of *M. catarrhalis* resembles in many ways that of non-typable *H. influenzae*, with which it shares the nasopharyngeal habitat.

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Anaerobic Infections in Children

Itzhak Brook

Abstract Anaerobic bacteria commonly cause infection in children. Anaerobes are the most predominant components of the normal human skin and mucous membranes bacterial flora and are therefore a common cause of bacterial infections of endogenous origin. Because of their fastidious nature, they are difficult to isolate from infectious sites and are often overlooked. Anaerobic infections can occur in all body sites, including the central nervous system, oral cavity, head and neck, chest, abdomen, pelvis, skin, and soft tissues. They colonize the newborn after delivery and have been recovered from several types of neonatal infections. These include cellulitis of the site of fetal monitoring, neonatal aspiration pneumonia, bacteremia, conjunctivitis, omphalitis, and infant botulism. The failure to direct adequate therapy against these organisms may lead to clinical failures. Their isolation requires appropriate methods of collection, transportation, and cultivation of specimens. Treatment of anaerobic infection is complicated by the slow growth of these organisms, by their polymicrobial nature, and by the growing resistance of anaerobic bacteria to antimicrobials. Antimicrobial therapy is often the only form of therapy required, whereas in others it is an important adjunct to a surgical approach. Because anaerobic bacteria generally are recovered mixed with aerobic organisms, the choice of appropriate antimicrobial agents should provide for adequate coverage of both types of pathogen.

1 Introduction

Infections due to anaerobic bacteria are common in children and may be serious and life threatening. The recent increased recovery of these organisms from children has led to greater appreciation of the role anaerobes play in pediatric infections. Anaerobic infections can occur in all body sites, including the central nervous system, oral cavity, head and neck, chest, abdomen, pelvis, skin, and soft

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tissues. Anaerobes colonize the newborn after delivery and have been recovered from several types of neonatal infections. These include cellulitis of the site of fetal monitoring, neonatal aspiration pneumonia, bacteremia, conjunctivitis, omphalitis, and infant botulism.

Anaerobes are the predominant components of the normal human skin and mucous membranes bacterial flora [1, 2] and are therefore a common cause of bacterial infections of endogenous origin. Because of their fastidious nature, these organisms are difficult to isolate from infectious sites and are often overlooked. Their exact frequency is difficult to ascertain because of the inconsistent use of adequate methods for their isolation and identification. The failure to direct adequate therapy against these organisms may lead to clinical failures. Their isolation requires appropriate methods of collection, transportation, and cultivation of specimens [3–5]. Treatment of anaerobic infection is complicated by the slow growth of these organisms, by their polymicrobial nature, and by the growing resistance of anaerobic bacteria to antimicrobials.

2 Microbiology

The clinically important anaerobic bacteria are six genera of Gram-negative rods (*Bacteroides, Prevotella, Porphyromonas, Fusobacterium, Bilophila*, and *Sutterella*), Gram-positive cocci (primarily *Peptostreptococcus*), Gram-positive spore-forming (*Clostridium*) and non-spore-forming bacilli (*Actinomyces, Propionibacterium, Eubacterium, Lactobacillus*, and *Bifidobacterium*), and Gram-negative cocci (mainly *Veillonella*) (Table 1) [5, 6]. The frequency of recovery of anaerobic strains differs in various infectious sites (Table 2). Mixed infections caused by numerous aerobic and anaerobic organisms are observed commonly in clinical situations [3, 4].

The taxonomy of anaerobic bacteria has changed in recent years because of their improved characterization using genetic studies [5, 7]. The ability to differentiate between similar strains enables better characterization of type of infection and predicted antimicrobial susceptibility. The frequency of recovery of anaerobic strains differs in various infectious sites.

2.1 Gram-Positive Spore-Forming Bacilli

Anaerobic spore-forming bacilli belong to the genus *Clostridium*. The clostridia found most frequently in clinical infections are *Clostridium perfringens, Clostridium septicum, Clostridium ramosum, Clostridium novyi, Clostridium sordellii, Clostridium histolyticum, Clostridium fallax, Clostridium bifermentans*, and *Clostridium innocuum*.

C. perfringens, the most commonly recovered clostridial isolate, is an inhabitant of soil and of intestinal contents of humans and animals and is the most

Table 1 Classification of predominant recovered anaerobic bacteria

Gram-positive cocci
Peptostreptococcus spp., *P. magnus, P. asaccharolyticus, P. prevotii, P. intermedius, P. anaerobius, P. micros*
Microaerophilic streptococci (not true anaerobes)
Gram-positive (non-spore-forming) bacilli
P. acnes
Propionibacterium propionicum
Eubacterium lentum
Bifidobacterium eriksonii
Bifidobacterium dentium
Actinomyces species: *A. isrealii, A. naeslundii, A. viscosus, A. odontolyticus, A. meyerii*
Arachnia propionica
Gram-positive (spore-forming) bacilli
Clostridium species: *C. perfringens, C. ramosum, C. septicum, C. novyi, C. histolytica, C. sporogenes, C. difficile, C. bifermentans, C. butyricum, C. innocuum, C. sordellii, C. botulinum, C. tetani*
Gram-negative bacilli
Bacteroides fragilis group: *B. fragilis, B. thetaiotaomicron, B. distasonis, B. vulgatus, B. ovatus, B. uniformis*
Other *Bacteroides*: *B. gracilis, B. ureolyticus*
Pigmented *Prevotella* spp. *P. melaninogenica, P. intermedia, P. denticola, P. loescheii, P. corporis, P. nigrescens*
Other *Prevotella* spp.: *P. oris, P. buccae, P. oralis* group, (*P. oralis, P. buccalis, P. veroralis*), *P. bivia, P. disiens*
Porphyromonas spp: *P. asaccharolytica, P. gingivalis, P. endodontalis*
Fusobacterium species: *F. nucleatum, F. necrophorum, F. gonidiaformans, F. naviforme, F. mortiferum, F. varium*

frequently encountered histotoxic clostridial species and elaborates a number of necrotizing extracellular toxins [8] and can cause a devastating illness with high mortality. Clostridial bacteremia is associated with extensive tissue necrosis, hemolytic anemia, and renal failure.

Recovery of *C. septicum* has been often found associated with malignancy. *Clostridium botulinum* (types A and B) is usually associated with food poisoning and rarely with wound infections. Infant botulism occurs with types A, B, and F [8]. Disease caused by *C. botulinum* usually is an intoxication produced by ingestion of contaminated food (uncooked meat, poorly processed fish, and improperly canned vegetables), containing a highly potent neurotoxin [9]. The polypeptide neurotoxin is relatively heat labile, and food containing this toxin may be rendered innocuous by exposure to 100°C for 10 min.

Clostridium difficile has been incriminated as the causative agent of antibiotic-associated and spontaneous diarrhea and colitis [10]. *Clostridium tetani* is found in soil and rarely is isolated from human feces. Infections caused by this bacillus are a result of soil contamination of wounds with *C. tetani* spores [9] that germinate and produce neurotoxin.

Table 2 Anaerobic bacteria most frequently encountered in clinical specimens

Organism	Infectious site
Gram-positive cocci	
Peptostreptococcus sp.	Respiratory tract, intraabdominal, and soft tissue infections
Microaerophilic streptococci[a]	Sinusitis, brain abscesses
Gram-positive (non-spore-forming) bacilli	
Actinomyces sp.	Intracranial abscesses, chronic mastoiditis, aspiration pneumonia, head and neck infections
P. acnes	Shunt infections (cardiac, intracranial), infections associated with foreign body
Bifidobacterium sp.	Chronic otitis media, cervical lymphadenitis, abdominal infections
Gram-positive (spore-forming) bacilli	
Clostridium sp.	
C. perfringens	Soft tissue infection, sepsis, food poisoning
C. septicum	Sepsis, neutropenic enterocolitis
C. difficile	Colitis, antibiotic-associated diarrheal disease
C. botulinum	Botulism
C. tetani	Tetanus
C. ramosum	Soft tissue infections
Gram-negative bacilli	
B. fragilis group (*B. fragilis*, *B. thetaiotamicron*)	Intraabdominal and female genital tract infections, sepsis, neonatal infections
Pigmented *Prevotella* and *Porphyromonas*	Orofacial infections, aspiration pneumonia, periodontitis
P. oralis	Orofacial infections
P. oris-buccae	Orofacial infections, intraabdominal infections
B. bivius, B. disiens	Female genital tract infections
Fusobacterium sp.	
F. nucleatum	Orofacial and respiratory tract infections, brain abscesses, bacteremia
F. necrophorum	Aspiration pneumonia, bacteremia

[a]Not obligate anaerobes.

2.2 Gram-Positive Non-Spore-Forming Bacilli

Anaerobic, Gram-positive, non-spore-forming rods comprise part of the microflora of the gingival crevices, the gastrointestinal tract, the vagina, and the skin. Several distinct genera are recognized: *Actinomyces, Arachnia, Bifidobacterium, Propionibacterium, Eubacterium,* and *Lactobacillus*.

Actinomyces israelii and *Actinomyces naeslundii* have been recovered from intracranial abscesses, chronic mastoiditis, aspiration pneumonia, and peritonitis [4, 6]. Actinomycosis occurs most commonly in the tissues of the face and neck, lungs, pleura, and ileocecal regions. Bone, pericardial, and anorectal lesions are less common, but virtually any tissue may be invaded; a disseminated, bacteremic form has been described.

Propionibacterium ordinarily is not a pathogen but can be found in association with implanted cardiac or neurogenic shunt prostheses [11] or as a cause of endocarditis on previously damaged valves. *Propionibacterium acnes* and *Propionibacterium granulosum*, the two most common species, may be isolated from blood cultures but are associated only rarely with bacteremia or endocarditis. Because they are part of the normal skin flora, they are common laboratory contaminants. *P. acnes* may play a role in the pathogenesis of acne vulgaris.

2.3 Gram-Negative Bacilli

Bacteroides spp. The species of Bacteroidaceae that occur with greatest frequency in clinical specimens belong to the *B. fragilis* group. These organisms are resistant to penicillins mostly through the production of beta-lactamase. The group includes *B. fragilis* (the most commonly recovered member), *Bacteroides distasonis*, *Bacteroides ovatus*, *Bacteroides thetaiotaomicron*, and *Bacteroides vulgatus*. They are part of the normal gastrointestinal flora [2] and predominate in intra-abdominal infections and infections that originate from that flora (i.e., perirectal abscesses and decubitus ulcers) [4, 6].

Pigmented *Prevotella* (*Prevotella melaninogenica* and *Prevotella intermedia*) and *Porphyromonas* (*Porphyromonas asaccharolytica*) and non-pigmented *Prevotella* (*Prevotella oralis*, *Prevotella oris*) are part of the normal oral and vaginal flora and the predominant Gram-negative anaerobic species isolated from respiratory infections and their complications. These include aspiration pneumonia, lung abscess, chronic otitis media, chronic sinusitis, abscesses around the oral cavity, human bites, paronychia, brain abscesses, and osteomyelitis [12]. *Prevotella bivia* and *Prevotella disiens* are important isolates in obstetrical and gynecological infections.

Fusobacterium species seen most often in clinical infections are *Fusobacterium nucleatum, Fusobacterium necrophorum, Fusobacterium mortiferum,* and *Fusobacterium varium*. *F. nucleatum* is the predominant *Fusobacterium* from clinical specimens, often associated with oral, pulmonary, and intracranial infections [13]. They are often isolated from abscesses, obstetrical and gynecological infections, blood, and wounds.

The growing resistance of Gram-negative anaerobic bacilli to penicillins has been noticed in the last decade [14]. These include the pigmented *Prevotella* and *Porphyromonas, Fusobacterium* spp., *P. oralis, B. disiens*, and *B. bivius*. The main mechanism of resistance is through the production of the enzyme beta-lactamase. Complete identification and susceptibility testing and ability to produce beta-lactamase by members of the *B. fragilis* group as well as other Gram-negative anaerobic bacilli are factors of practical importance when making choices between antimicrobials for the therapy of pediatric infections involving these organisms.

The recovery rate of the different anaerobic Gram-negative bacilli in infected sites is similar to their distribution in the normal flora [4, 6]. *B. fragilis* group was more often isolated in sites proximal to the gastrointestinal tract (abdomen, bile),

pigmented *Prevotella* spp. were more prevalent in infections proximal to the oral cavity (bones, sinuses, chest), and *P. bivius* and *P. disiens* were more often isolated in obstetric and gynecologic infections. Knowledge of this common mode of distribution allows for logical empiric choice of antimicrobials adequate for the therapy of infections in these sites.

2.4 Gram-Positive Cocci

The species most commonly isolated are *Peptostreptococcus magnus*, *Peptostreptococcus asaccharolyticus*, *Peptostreptococcus anaerobius*, *Peptostreptococcus prevotii*, and *Peptostreptococcus micros*. These organisms are part of the normal flora of the mouth, upper respiratory tract, intestinal tract, vagina, and skin.

These organisms are also predominant isolates in all types of respiratory infections, including chronic sinusitis, mastoiditis, acute and chronic otitis media, aspiration pneumonia, lung abscess, necrotizing and subcutaneous and soft tissue infections [15]. They generally are recovered mixed with other aerobic or anaerobic organisms, but in many cases they are the only pathogens recovered. This may be of particular significance in cases of bacteremia or acute otitis media. Microaerophilic streptococci are of particular importance in chronic sinusitis and brain abscesses [16].

2.5 Gram-Negative Cocci

There are three species described as anaerobic Gram-negative cocci: *Veillonella, Acidaminococcus*, and *Megasphaera*. There are two described species of *Veillonella* and only one each of the other two genera. The veillonellae are the most frequently involved of the three species and are part of the normal flora of the mouth, vagina, and the small intestine of some persons. Although they rarely are isolated from clinical infections, these organisms have been recovered occasionally from almost every type of anaerobic infection [17].

3 Pathogenicity and Virulence

3.1 Anaerobes as Normal Flora

The human body mucosal and epithelial surfaces are colonized with aerobic and anaerobic microorganisms [1, 2]. The organisms at the different sites tend to belong to certain major bacterial species and their presence in that location is predictable. The relative and total counts of organisms can be affected by various factors, such as age, diet, anatomic variations, illness, hospitalization, and antimicrobial therapy. However, the predictable pattern of bacterial flora remains stable through life, despite their subjection to perturbing factors. Anaerobes outnumber aerobic

Table 3 Normal flora

Site	Number of organisms/gram		Predominant anaerobic bacteria
	Aerobes	Anaerobes	
Skin			*P. acnes* *Peptostreptococcus* sp.
Mouth/upper respiratory tract	$10^{8–9}$	$10^{9–11}$	Pigment *Prevotella* and *Porphyromonas* spp. *Fusobacterium* spp. *Peptostreptococcus* spp. *Actinomyces* spp.
Gastrointestinal tract			
Upper	$10^{2–5}$	$10^{3–7}$	*B. fragilis* group *Clostridium* spp.
Lower	$10^{5–9}$	$10^{10–12}$	*Peptostreptococcus* spp. *Bifidobacterium* spp. *Eubacterium* spp.
Female genital tract	10^{8}	10^{9}	*Peptostreptococcus* spp. *P. bivia* *P. disiens*

bacteria in all mucosal surfaces, and certain organisms predominate in the different sites (Table 3).

Knowledge of the composition of the flora at certain sites is useful for predicting which organisms may be involved in an infection adjacent to that site and can assist in the selection of a logical antimicrobial therapy, even before the exact microbial etiology of the infection is known.

The anaerobic microflora of the total body skin is largely made up of the genus *Propionibacterium* [11] and to a lesser extent *Peptostreptococcus* spp. The perineum and lower extremity may harbor members of the colonic and vaginal flora. The microflora of the upper airways including oral cavity, nasopharynx, and oropharynx is complex and contains many kinds of obligate anaerobes. The ratio of anaerobic bacteria to aerobic bacteria in saliva is approximately 10:1. The total count of anaerobic bacteria in the saliva and elsewhere in the oral cavity reaches 10^{7}–10^{8}/mL.

The gastrointestinal flora varies in bacterial concentration at different levels. The stomach acidity accounts for the reduction in the number of organisms that are swallowed from the oropharynx. The stomach, duodenum, jejunum, and proximal ileum normally contain relatively few bacteria. However, the flora becomes more complex, and the number of different bacterial species increases in the distal portion of the gastrointestinal tract. However, interruption in intestinal motility may result in an increase in the number of anaerobic and aerobic bacteria. The bacterial counts in the small intestine are relatively low, with total counts of 10^{2}–10^{5} organisms/mL. The organisms that predominate up to the ileocecal valve are Gram-positive facultative, while below that structure *Bacteroides* organisms (mostly *B. fragilis*, *Peptostreptococcus*, and *Clostridium* spp.) and coliform bacteria are the major isolates [2]. The mean number of bacteria in the colon is approximately 10^{12}

bacteria/g fecal material. Approximately 99.9% of these bacteria are anaerobic (ratio of aerobes to anaerobes is 1 to 1,000–10,000). In the colon 300–400 different species or types of bacteria can be found.

The female genital flora comprises a mixture of aerobic and anaerobic flora. However, the concentration and type of bacteria is less stable than that of the gastrointestinal flora and can be influenced by antibiotic therapy, pregnancy, and gynecologic surgery. A concentration of 10^8/mL organisms is found in the reproductive years. Changes occur in the number of organisms at the various stages of the menstrual cycle [18, 19]. The predominant aerobic organisms are *Lactobacillus*, and the predominant anaerobic bacteria are *Lactobacillus, Peptostreptococcus, Prevotella*, and *Bacteroides* spp. Bacterial vaginosis is associated with an increase in the number of anaerobic flora and a decrease in the concentration of lactobacilli [19].

Most infections due to anaerobic bacteria originate from the endogenous mucosal membrane and skin flora. Anaerobes belonging to the indigenous flora of the oral cavity can be recovered from various infections adjacent to that area, such as cervical lymphadenitis; subcutaneous abscesses and burns in proximity to the oral cavity; human and animal bites; paronychia; tonsillar and retropharyngeal abscesses; chronic sinusitis; chronic otitis media; periodontal abscess; thyroiditis; aspiration pneumonia; and bacteremia associated with one of the above infections [4, 6]. The predominant anaerobes recovered in these infections are *Prevotella* and *Porphyromonas,* and *B. oralis*, *Fusobacterium*, and *Peptostreptococcus* spp. which are all part of the normal flora of the mucous surfaces of the oropharynx (Table 4).

A similar correlation exists in infections associated with the gastrointestinal tract. Such infections include peritonitis following rupture of appendix, liver abscess, abscess and wounds near the anus, intraabdominal abscess, and bacteremia associated with any of these infections [4, 6]. The anaerobes that predominate in these infections are *B. fragilis* group, clostridia, and *Peptostreptococcus* spp.

Another site with a correlation between the normal flora and the anaerobic bacteria recovered from infected sites is the genitourinary tract. The infections involved are amnionitis, septic abortion, and other pelvic inflammations [4, 6]. The anaerobes usually recovered from these sites are species of anaerobic Gram-negative bacteria and *Peptostreptococcus* spp. Organisms belonging to the vaginal–cervical flora are also important pathogens of neonatal infections. They can be acquired by the newborn prior to delivery in the presence of amnionitis, or during passage through the birth canal.

3.2 Conditions Predisposing to Anaerobic Infection

The clinical situations that predispose to anaerobic infections include exposure of the sterile body sites to high inoculum of indigenous mucous membrane flora. Poor blood supply and tissue necrosis lower the oxidation–reduction potential and favor the growth of anaerobic bacteria. Any condition that lowers the blood supply to an affected area of the body can predispose to anaerobic infection. Therefore, trauma,

Table 4 Recovery of anaerobic bacteria in infectious sites

Infection	*Peptostreptococcus* sp.	*Clostridium* sp.	*B. fragilis* group	*Pigmented Prevotella* and *Porphyromonas*	*P. bivius* *P. disien*	*Fusobacterium* sp.
Bacteremia	1	1	2	1	0	1
Central nervous system	2	1	1	2	0	1
Head and neck	3	1	1	3	0	3
Thoracic	2	1	1	3	0	3
Abdominal	3	3	3	1	1	1
Obstetric-gynecology	3	2	1	1	2	1
Skin and soft tissue	2	1	2	2	1	1

Frequency of recovery in anaerobic infections: 0, none; 1, rare (1–33%); 2, common (34–66%); 3, very common (67–100%).

foreign body, malignancy, surgery, edema, shock, colitis, and vascular disease may predispose to anaerobic infection. Previous infection with aerobic or facultative organisms also may make the local tissue conditions more favorable for the growth of anaerobic bacteria. The human defense mechanisms also may be impaired by anaerobic conditions and anaerobic bacteria.

Suppuration, abscess formation, thrombophlebitis, and gangrenous destruction of tissue associated with gas formation are the hallmark of anaerobic infection. Anaerobes are especially common in chronic infections, and they are commonly seen after therapy with antimicrobials that fail to eradicate them (i.e., aminoglycosides, trimethoprim-sulfamethoxazole, and the older quinolones).

Certain infections are very likely to involve anaerobes as important pathogens and their presence should always be assumed. Such infections include brain abscess, oral or dental infections, human or animal bites, aspiration pneumonia and lung abscesses, peritonitis following perforation of viscus, amnionitis, endometritis, septic abortions, tubo-ovarian abscess, abscesses in and around the oral and rectal areas, and pus-forming necrotizing infections of soft tissue, muscle, and tumors.

3.3 Virulence Factors

Anaerobes contribute to the severity of infection through their synergy with their aerobic counterpart and with each other [20]. Anaerobic bacteria require more time than aerobic bacteria to become virulent. This is because some of the major virulence factors of certain anaerobes (i.e. the production of a capsule by *Bacteroides*) are expressed only after the infection has become chronic [21].

Anaerobes possess several important virulence factors, including the presence of surface structures such as capsule polysaccharide or lipopolysaccharide, production of superoxide dismutase and catalase, immunoglobulin proteases, coagulation promoting and spreading factors (such as hyaluronidase, collagenase, and fibrinolysin), and adherence factors [22]. Other factors that enhance the virulence of anaerobes include mucosal damage, oxidation–reduction potential drop, and the presence of hemoglobin or blood in an infected site.

An indirect pathogenic role of some anaerobes is their ability to produce the enzyme beta-lactamase. Beta-lactamase-producing bacteria can be involved directly in the infection and protect not only themselves but also other penicillin-susceptible organisms from the activity of penicillins. This can occur when the enzyme is secreted into the infected tissue or abscess fluid in sufficient quantities to degrade the beta-lactam ring of penicillin or cephalosporin before it can kill the susceptible bacteria [23].

4 Diagnostic Microbiology

4.1 Collection of Specimens for Anaerobic Bacteria

The proper management of anaerobic infection depends on appropriate documentation of the bacteria causing the infection. Without such an approach, the patient

may be exposed to inappropriate, costly, and undesirable antimicrobial agents with adverse side effects. Certain or all of the anaerobes may not be recovered when the specimen is not promptly placed under anaerobic conditions for transport to the laboratory. If contamination of the specimen with normal flora occurs, anaerobes may be recovered that are not related with the patient's illness.

The essential elements requiring the cooperation of the physician and the microbiology laboratory for appropriate documentation of anaerobic infection are the collection of appropriate specimens, the expeditious transportation, and careful laboratory processing.

Appropriate cultures for anaerobic bacteria are especially important in mixed aerobic and anaerobic infections [5]. Techniques or media that are inadequate for isolation of anaerobic bacteria can lead to the assumption that the aerobic organism(s) recovered are the sole pathogens. This may cause the clinician to direct therapy toward only those aerobic organisms.

Specimens should be obtained free of contamination so that normal flora organisms are excluded. Because indigenous anaerobes often are present on the surfaces of skin and mucous membranes in large numbers, even minimal contamination of a specimen with normal flora can give misleading results. Specimens should therefore be classified as acceptable or unacceptable according to their acceptability for anaerobic culture. Appropriate specimens for anaerobic cultures should be obtained using a technique that bypasses the normal flora. Unacceptable or inappropriate specimens can yield normal flora also and therefore have no diagnostic value.

Acceptable specimens (Table 5) include blood specimens; aspirates of body fluids (pleural, pericardial, cerebrospinal, peritoneal, and joint fluids); urine collected by percutaneous suprapubic bladder aspiration; abscess contents; deep wound aspirates; and specimens collected by special techniques, such as transtracheal aspirates or direct lung puncture. Lower respiratory tract specimens are difficult to obtain without contamination with indigenous flora. Double lumen catheter bronchial

Table 5 Methods for collection of specimen for anaerobic bacteria

Infection site	Methods
Abscess or body cavity	Aspiration by syringe and needle Incised abscesses – syringe or swab (less desirable); specimen obtained during surgery after cleansing the skin
Tissue or bone	Surgical specimen using tissue biopsy or curette
Sinuses or mucus surface abscesses	Aspiration after decontamination or surgical specimen
Ear	Aspiration after decontamination of ear canal and membrane; in perforation: cleanse ear canal and aspirate through perforation
Pulmonary	Transtracheal aspiration, lung puncture, bronchoscopic aspirate[a]
Pleural	Thoracentesis
Urinary tract	Suprapubic bladder aspiration
Female genital tract	Culdocentesis following decontamination, surgical specimen Transabdominal needle aspirate of uterus intrauterine brush[a]

[a]Using double lumen catheter and quantitative culture.

brushing and bronchial lavage, cultured quantitatively, can be useful. Specimens obtained from normally sterile sites may be collected after thorough skin decontamination as is the case for the collection of blood, spinal, joint, or peritoneal fluids.

4.2 Transportation of Specimens

Specimens should be transported to the microbiology laboratory promptly. Various transport devices are available that generate oxygen-free environment using a mixture of carbon dioxide, hydrogen, and nitrogen, that contain an indicator to illustrate aerobic condition. Specimens should be placed into an anaerobic transporter as soon as possible after their collection. Aspirates of liquid specimen or tissue are always preferred to swabs. Liquid specimens may be inoculated into an anaerobic transport vial. A plastic or glass syringe and needle also may be used for transport. After collection, all air should be expelled from the syringe and the needle tip should be inserted into a sterile rubber stopper. No more than 30 min should elapse before the specimen is plated, because air gradually diffuses through the plastic syringe wall.

Swabs or tissue specimens can be transported in an anaerobic jar or in a Petri dish placed in a sealed plastic bag that can be rendered anaerobic by a catalyzer.

4.3 Laboratory Diagnosis

Laboratory diagnosis of anaerobic infections starts with the examination of a Gram-stained smear of the specimen. This can reveal important preliminary information about the types of bacteria present, suggest empiric therapy, and serve as a quality control on the final culture results. The laboratory should be able to isolate all of the morphological types in the approximate ratio in which they are seen.

Detailed procedures of the methods for cultivation of anaerobes can be found in microbiology manuals [5]. Cultures should be placed immediately under anaerobic conditions and incubated for 48 h or longer. An additional period of 36–46 h is generally needed to completely identify the anaerobic bacteria to a species level, using biochemical tests. Kits of these biochemical tests are commercially available. Gas liquid chromatography of metabolites can be employed to assist in the identification of anaerobes. Nucleic acid probes and polymerase chain reaction (PCR) methods are being developed for rapid identification of anaerobic bacteria. Identification of an anaerobe to a species level is often cumbersome, expensive, and time consuming, taking up to 72 h. The decision of what level of speciation is adequate for identifying an anaerobic organism is often controversial.

Occasionally, identification of an organism can provide the diagnosis, as is the case with *C. difficile* in a patient with colitis or *C. botulism* in infants with botulism. Identifying the *B. fragilis* group that is more often causing bacteremia and septic complications has significant prognostic value.

Identification of an anaerobe is also helpful in selecting what antibiotic to use to treat species whose antibiotic susceptibility is predictable. Until the late 1970s, most clinically significant anaerobes except *B. fragilis* group were susceptible to penicillin [14]. Therefore, extensively speciating and antibiotic susceptibility testing were generally unnecessary. In the last decade, however, there is more variability in antimicrobial susceptibility patterns that necessitate more extensive speciation as well as antimicrobial susceptibility testing for some anaerobic bacteria. Organisms that should be identified include isolates from sterile body sites (i.e., blood, cerebrospinal fluid, and joint), those with particular epidemiological or prognostic significance (e.g., *C. difficile*), and organisms with variable of unique susceptibility

4.4 Antimicrobial Susceptibility Testing

The antimicrobial susceptibility of anaerobes has become less predictable over the last decade, as resistance to several antimicrobial agents especially by Gram-negative bacilli has increased. Screening of anaerobic Gram-negative bacilli isolates (particularly *Prevotella*, *Bacteroides*, and *Fusobacterium* species) for production of beta-lactamase may be important. This can provide information about their penicillin susceptibility. However, occasional resistance to beta-lactam antibiotics can occur through other mechanisms.

Routine susceptibility testing of all anaerobic isolates is time consuming and often unnecessary. Susceptibility testing should be limited to organisms isolated from blood cultures, bone, central nervous system, and serious infections when isolated in pure culture from properly collected specimens. Antibiotics tested should include penicillin, a broad-spectrum penicillin, a penicillin plus a beta-lactamase inhibitor, clindamycin, chloramphenicol, a newer quinolone, a second-generation cephalosporin (e.g., cefoxitin), metronidazole, and a carbapenem (e.g., imipenem). The recommended method by the National Committee for Clinical Laboratory Standards (NCCLS) includes agar microbroth and macrobroth dilution [24]. Newer methods include the E-test and the spiral gradient endpoint system.

5 Prevention

The appropriate therapy of acute infections can prevent the development of chronic infections where anaerobes predominate. In settings where anaerobic infections are expected, such as intraabdominal and wound infection following surgery, antimicrobial prophylaxis can reduce the risk of such infection. Prophylactic therapy prior to surgery is given when the surgical site is expected to be contaminated by the normal flora of the mucous membrane at the operated site. Cefazolin is effective in surgical prophylaxis in sites distant from the oral or rectal areas. Cefoxitin is the drug of choice in procedures that involve the oral, rectal, or vulvovaginal areas

because its spectrum extends to both the aerobic and anaerobic flora likely to be encountered.

Prevention and early therapy of conditions that may lead to anaerobic infection can reduce their rate. Aspiration pneumonia and its complication can be prevented by reducing the aspiration of oral flora by improving patient's neurological status, repeated suctioning of oral secretion, improving oral hygiene, and maintaining lower stomach pH. Skin and soft tissue infections can be prevented by irrigation and debridement of wounds and necrotic tissue, drainage of pus, and improvement of blood supply.

6 Clinical Infections

6.1 Central Nervous System Infections

Anaerobic bacteria can cause a variety of intracranial infections. They often induce brain abscess, subdural empyema, and infrequently cause epidural abscess and meningitis. The main source of brain abscess is an adjacent, generally chronic infection in the ears, mastoids, sinuses, oropharynx, teeth, or lungs [25]. Ear or mastoid infection tends to spread to the temporal lobe or cerebellum, while sinusitis often causes abscess of the frontal lobe. Hematogenous spread often occurs after dental, oropharyngeal, or pulmonary infection. Rarely bacteremia of another origin or endocarditis can lead to such infection.

Meningitis is rare and can follow respiratory infection or be a complication of a cerebrospinal fluid shunt. Shunt infections are generally caused by skin flora such as *P. acnes* [11], or in instances of ventriculoperitoneal shunts that perforate the gut by anaerobes of enteric origin (i.e., *B. fragilis*) [26]. *C. perfringens* can cause brain abscesses and meningitis following head injuries or after intracranial surgery [27].

The anaerobic bacteria generally recovered from brain abscesses that complicate respiratory and dental infections include *Prevotella*, *Porphyromonas*, *Bacteroides, Fusobacterium*, and *Peptostreptococcus* spp. Microaerophilic and other streptococci are also often isolated. Actinomyces is less frequently encountered.

Encephalitis often precedes abscess formation, it then progresses to liquification, pus formation, and eventually to fibrous encapsulation [28]. At the stage of encephalitis, antimicrobial therapy accompanied by measures to control the increase in the intracranial pressure and can prevent the abscess formation. Once an abscess has formed, surgical excision or drainage may be needed, combined with a long course of antibiotics (4–8 weeks). Some neurosurgeons advocate complete abscess evacuation while others advocate repeated aspirations as indicated [29]. In cases with multiple abscesses or in those with abscesses in essential brain areas, repeated aspirations are preferred to complete excision. High-dose antibiotics for an extended period may represent an alternative approach in this group of patients and can replace surgical drainage in many other cases.

A long course of antimicrobial treatment of the brain abscess is required because of the prolonged time needed for brain tissue to repair and close the abscess space

[30]. Because of the difficulty involved in the penetration of various antimicrobial agents through the blood–brain barrier, the choice of antibiotics is limited. The antimicrobials advocated for these infections are metronidazole, penicillins, and chloramphenicol.

6.2 Head and Neck Infections

Anaerobic bacteria can be isolated from a variety of these infections and predominate more in the chronic form of these infections. These include chronic otitis media, sinusitis, and mastoiditis; tonsillar, peritonsillar, and retropharyngeal abscesses; all deep neck space infections, thyroiditis, odontogenic infections, and post-surgical and non-surgical head and neck wounds and abscesses. The organisms that are predominant in these infections, *Prevotella*, *Porphyromonas*, *Bacteroides, Fusobacterium*, and *Peptostreptococcus* spp., are all members of the oropharyngeal flora (Table 6).

Most dental infections involve anaerobes. These include endodontal pulpitis and periodontal (gingivitis and periodontitis) infections, periapical and dental abscesses, and perimandibular space infection [31, 32]. Pulpitis may progress to an abscess and eventually involve the mandible and other neck spaces. In addition to the above-mentioned organisms, microaerophilic streptococci and *Streptococcus salivarius* can also be involved.

Vincent's angina (or trench mouth) is a distinct form of ulcerative gingivitis. The causative organisms include *Fusobacterium* species and anaerobic spirochetes; however, definitive studies using anaerobic microbiologic methods remain to be performed.

Deep neck infections generally follow oral, dental, and pharyngeal infections and are generally polymicrobial, involving the anaerobes that caused the primary infections. Mediastinitis following perforation of the esophagus or extension of retropharyngeal abscess or cellulitis, or abscess of dental origin, is most likely to involve mixed aerobic anaerobic infection [33, 34].

Anaerobes have been isolated in 5–15% of patients with acute otitis [35] and 42% of culture-positive aspirates of patients with serous otitis [36]. The predominant isolates in acute otitis were *Peptostreptococcus* spp. and *P. acnes*, and Gram-negative anaerobic bacilli were found in serous otitis media.

Anaerobes were recovered in about 50% of the patients with chronic suppurative otitis media [4, 6, 37, 38] and those with cholesteatoma [39, 40]. The variability in the rate of isolation of anaerobes in these studies may be attributed to differences in the geographic locations and to laboratory methodologies. The predominant anaerobes recovered were Gram-negative bacilli and *Peptostreptococci*, and the aerobes were *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Many of these organisms can produce beta-lactamase and might have contributed to the high failure rate of beta-lactam antibiotics in the therapy of this infection. Anaerobic bacteria were generally recovered, mixed with aerobic bacteria, and the number of isolates per

Table 6 Aerobic and anaerobic bacteria isolated in upper respiratory tract infections

Type of infection	Aerobic and facultative aerobic organisms	Anaerobic organisms
Otitis media: acute	*S. pneumoniae* *Haemophilus influenzae*[a] *M. catarrhalis*[a]	*Peptostreptococcus* spp.
Otitis media: chronic and mastoiditis	*S. aureus*[a]	Pigmented *Prevotella* and *Porphyromonas* spp.
	E. coli[a]	*Bacteroides* spp.[a]
	Klebsiella pneumoniae[a]	*Fusobacterium* spp.[a]
	Pseudomonas aeruginosa[a]	
	Peptostreptococcus spp.	
Peritonsillar and retropharyngeal abscess	*S. pyogenes*	*Fusobacterium* spp.[a]
	S. aureus[a]	*Pigmented Prevotella* and *Porphyromonas* spp.[a]
	S. pneumoniae	
Recurrent tonsillitis	*S. pyogenes*	*Fusobacterium* spp.[a]
	H. influenzae[a]	
	S. aureus[a]	
Suppurative thyroiditis	*S. pyogenes*	*Pigmented Prevotella* and *Porphyromonas* spp.[a]
	S. aureus[a]	
Sinusitis: acute	*H. influenzae*[a]	*Peptostreptococcus* spp.
	S. pneumoniae	
	M. catarrhalis[a]	
Sinusitis: chronic	*S. aureus*[a]	*B. fragilis* group[a]
	S. pneumoniae	*Pigmented Prevotella* and *Porphyromonas* spp.[a]
	H. influenza[a]	
Cervical lymphadenitis	*S. aureus*[a]	*Pigmeted Prevotella* and *Porphyromonas* spp.[a]
	Mycobacterium spp.	*Peptostreptococcus* spp.
Postoperative infection disrupting oral mucosa	*Staphyloccus* spp.[a]	*Fusobacterium* spp.[a]
	Enterobacteriaceae[a]	*Bacteroides* spp.[a]
	Staphylococcus spp.[a]	*Pigmented Prevotella* and *Porphyromonas* spp.[a]
		Peptostreptococcus spp.
Deep neck species	*Streptococcus* spp.	*Bacteroides* spp.[a]
	Staphylococcus spp.[a]	*Fusobacterium* spp.[a]
		Peptostreptococcus spp.[a]
Odontogenic complications	*Streptococcus* spp.	*Pigmented Prevotella* and *Porphyromonas* spp.[a]
	Staphylococcus spp.[a]	*Peptostreptococcus* spp.
Oropharyngeal: Vincent's angina	*Streptococcus* spp.	*F. necrophorum*[a]
Necrotizing ulcerative gingivitis	*Staphylococcus* spp.[a]	*Spirochetes*

[a]Organisms that have the potential of producing beta-lactamase.

specimen ranged between two and six. Anaerobes were isolated from 23 of 24 (96%) specimens of chronic mastoiditis [41] and from most patients with intracranial abscesses that complicate chronic suppurative otitis media [4, 6].

Anaerobic bacteria are often isolated from infected cholesteatomas [39, 40]. Cholesteatoma that often accompanies chronic suppurative otitis media can enhance the absorption of bone, that is enhanced by organic acids produced by anaerobic bacteria [40]. Since cholesteatoma associated with chronic suppurative otitis media contains bacteria similar to those isolated from chronically infected ears, the cholesteatoma may serve as a nidus of the chronic infection.

6.2.1 Sinusitis

In the acute stage of sinusitis the most common pathogens are similar to those recovered in otitis media: *Streptococcus pneumoniae*, *H. influenzae*, and *Moraxella catarrhalis*. Anaerobic organisms become involved as the infection turns chronic and the levels of tissue oxygen decline [42]. Although anaerobes are generally isolated from only about 10% of patients with acute sinusitis (generally in maxillary sinusitis secondary to periodontal infection), they can be isolated from up to 67% of patients with chronic infection [4, 6]. An average of three anaerobes per sinus aspirate was recovered in patients with chronic sinusitis [43].

The infection may spread via anastomosing veins or contiguously to the central nervous system. Intracranial complications include orbital cellulitis [44], meningitis, cavernous sinus thrombosis, and epidural, subdural, and brain abscesses [4, 6].

6.2.2 Parotitis

Acute suppurative parotitis is generally caused by *S. aureus, Streptococcus* species, and, rarely, aerobic Gram-negative bacteria. Anaerobes, mostly *Peptostreptococcus*, *Bacteroides*, and pigmented *Prevotella* and *Porphyromonas* species, have also been recognized as an important cause of this infection [45]. Empiric antibiotic therapy should be directed against both aerobic and anaerobic bacteria. Surgical drainage may be indicated when pus has formed.

6.2.3 Cervical Lymphadenitis

The most common causes in children are viruses. The organisms that cause acute unilateral infection associated with facial trauma or impetigo are *S. aureus* and *Streptococcus pyogenes*. Cat scratch and mycobacterial infections are important in chronic infections. Anaerobic bacteria have been isolated in about 25% of the infections, often in pure culture [46]; the predominant anaerobes were *Fusobacterium* and *Peptostreptococcus* species. The recovery of anaerobes was often associated with a primary dental, periodontal, or tonsillar infection.

6.2.4 Thyroiditis

Anaerobic bacteria such as anaerobic Gram-negative bacilli and *Peptostreptococcus* species have been identified as causative agents in thyroiditis [4, 6, 47]. *Eikenella corrodens* and *Actinomyces* species have also been reported.

6.2.5 Infected Cysts

Thyroglossal duct cysts, cystic hygromas, branchial cleft cysts, laryngoceles, and dermoid cysts can become inflamed and cause local infection. The organisms that can cause secondary infection of these cysts can originate from either the skin or the oropharynx [48].

6.2.6 Wound Infection After Head and Neck Surgery

These infections are related to the exposure of the surgical site to the oropharyngeal flora and the degree of compromise of the surgical site. Postsurgical head and neck wounds are generally infected by polymicrobial aerobic and anaerobic flora; the number of isolates varies from one to nine (average six) [49]. The most frequently recovered isolates are *Peptostreptococcus* species, *S. aureus*, anaerobic Gram-negative bacilli (i.e., *Bacteroides* species), *Fusobacterium* species, and enteric Gram-negative rods. The presence of polymicrobial flora in postsurgical wounds warrants the use of antimicrobials effective against these organisms in the prophylaxis and therapy of this infection [50].

6.2.7 Tonsillitis

Indirect evidence supports the involvement of anaerobes in acute and chronic tonsillitis. The evidence is mainly derived from studies that show the major role of anaerobes in complications of tonsillitis. The organisms associated with the infection are *Fusobacterium* spp., Gram-negative anaerobic bacilli, and *Peptostreptococcus* spp. Polymicrobial aerobic flora and anaerobic flora predominate in peritonsillar and retropharyngeal abscesses [4, 6, 51]. These organisms can be isolated from 25% of suppurative cervical lymph nodes and are mostly associated with the presence of dental or tonsillar infections [46]. Anaerobic organisms have been associated with thrombophlebitis of the internal jugular veins, which often causes postanginal sepsis [4, 6].

The pathogenic role of anaerobes in the acute inflammatory process in the tonsils is also supported by several clinical observations: their recovery in tonsillar or retropharyngeal abscesses in many cases without any aerobic bacteria [51], the isolation of anaerobes from tonsils in Vincent's angina [4, 6], the recovery of encapsulated pigmented *Prevotella* and *Porphyromonas* species in acutely inflamed tonsils, the isolation of anaerobes from the core of recurrently inflamed non-group A beta-hemolytic streptococcal (GABHS) (*S. pyogenes*) tonsils [52], and the response to antibiotics in patients with non-GABHS tonsillitis [53, 54]. Furthermore, immune

response against *P. intermedia* can be detected in patients with non-GABHS tonsillitis [55]; an immune response can also be detected against *P. intermedia* and *F. nucleatum* in patients who recovered from peritonsillar cellulitis or abscesses [56] and infectious mononucleosis [57].

Therapy with metronidazole alleviated the symptoms of tonsillar hypertrophy and shortened the duration of fever in patients with infectious mononucleosis [53]. Because metronidazole has no antiviral or aerobic antibacterial efficacy, suppression of the oral anaerobic flora may contribute to diminishing the inflammation induced by the Epstein–Barr virus. This is supported by the increased recovery of *P. intermedia* and *F. nucleatum* during the acute phases of infectious mononucleosis [58].

Anaerobes have been isolated from the cores of tonsils of children with recurrent GABHS [59] and non-GABHS tonsillitis [52] and peritonsillar and retropharyngeal abscesses. Beta-lactamase-producing aerobic and anaerobic bacteria were recovered from 75% of tonsils of children with recurrent GABHS tonsillitis [23, 59, 60] and from 40% of those with non-GABHS tonsillitis [52]. Similar organisms were recovered from patients with adenoiditis and adenoid hypertrophy [61].

Recurrent pharyngotonsillitis and penicillin failure to eradicate the GABHS can be a serious clinical problem. One explanation for penicillin failure is that repeated administrations result in selection of beta-lactamase-producing bacteria [23]. The recovery of these bacteria in more than three-quarters of the patients with recurrent GABHS tonsillitis [23, 59, 60], the ability to measure beta-lactamase activity in the core of these tonsils [62], and the response of patients to antimicrobials effective against beta-lactamase-producing bacteria (i.e., clindamycin or amoxicillin plus clavulanic acid) [23, 63, 64] support the role of these beta-lactamase-producing aerobic and anaerobic organisms in the inability of penicillin to eradicate GABHS tonsillitis.

6.3 Pleuropulmonary Infections

Aspiration of oropharyngeal secretions or gastric contents and severe periodontal or gingival disease are the most prevalent risk factors for developing anaerobic pleuropulmonary infection. The infection can progress from pneumonitis into necrotizing pneumonia and pulmonary abscess, with or without empyema. The lesions tend to form in the dependent pulmonary segments, either the superior segments of the lower lobes or the posterior segments of the upper lobes. The infection is generally polymicrobial where the causative organisms of community-acquired infection in 60–80% of cases are members of oropharyngeal flora (Table 7). The predominant anaerobes are *Prevotella*, *Porphyromonas*, *Fusobacterium*, and *Peptostreptococcus* spp., and the aerobic organisms are alpha hemolytic streptococci and microaerophilic streptococci [65]. Anaerobes can be recovered in about a third of children with nosocomial-acquired aspiration pneumonia and pneumonia associated with tracheostomy with and without mechanical ventilation [66] where

Table 7 Aerobic and anaerobic bacteria isolated in different infections

Type of infection	Aerobic and facultative aerobic organisms	Anaerobic organisms
Pleuropulmonary	*Streptococcus viridans*	Pigmented *Prevotella* (*P. denticola, P. melaninogenica, P. intermedia, P. nigrescens, P. loescheii*)
	S. aureus[a]	Nonpigmented *Prevotella* (*P. oris, P. buccae, P. oralis*)
	Enterobacteriaceae[a]	*F. nucleatum* (subsp. *nucleatum*, *polymorphum*)
	P. aeruginosa	*Peptostreptococcus* (*P. micros, P. anaerobius, P. magnus*)
		B. fragilis group
		Non-spore-forming Gram-positive rods (*Actinomyces, Eubacterium, Lactobacillus*)
Intra-abdominal	Intra-abdominal	*B. fragilis* group
	E. coli	*B. wadsworthia*
	Streptococcus (*viridans* group and group D)	*Peptostreptococcus* (especially *P. micros*)
	P. aeruginosa	*Clostridium* spp.
Female genital tract	*Streptococcus* (groups A, B, and others)	*Peptostreptococcus* spp.
	E. coli	*Prevotella* (especially *P. bivia, P. disiens*)
	K. pneumoniae	*B. fragilis* group
	N. gonorrhoeae (in sexually active patients)	*Clostridium* (especially *C. perfringens*)
	Chlamydia (in sexually active patients)	*Actinomyces, Eubacterium* (in intrauterine contraceptive device-associated infections)
	M. hominis (in postpartum patients)	
Skin and soft tissue	*S. aureus*	*Peptostreptococcus* (*P. magnus, P. micros, P. asaccharolyticus*)
	Streptococcus (*S. milleri* group, groups A, B viridans group)	Pigmented *Prevotella* spp.
	Enterococcus spp.[b]	*Actinomyces* spp.
	Enterobacteriaceae[b]	*F. nucleatum*
	P. aeruginosa[a]	*B. fragilis* group[b]
		Clostridium spp.[b]

[a]Recovered in hospital-acquired infection.
[b]After exposure to colonic flora.

they are generally recovered mixed with Enterobacteriaceae, *Pseudomonas* spp., and *S. aureus*. Specimens for culture should be obtained in a fashion that will avoid their contamination by the oral flora. They can be obtained using bronchoalveolar lavage, bronchoscopy via bronchial brush protected in a double lumen-plugged catheter (using quantitative cultures in the last two methods), percutaneous transtracheal aspiration, lung biopsy, and thoracentesis (of empyema fluid). Treatment of these infections include pleural space drainage (in the presence of empyema), and antimicrobials effective against the anaerobic and aerobic bacteria.

6.4 Intra-Abdominal Infections

Secondary peritonitis and intra-abdominal abscesses usually occur because of the entry of enteric bacteria into the peritoneal cavity through a defect in the wall of the intestine or other viscus as a result of obstruction, infarction, or direct trauma. Perforated appendicitis, inflammatory bowel disease with perforation, and gastrointestinal surgery often are associated with polymicrobial synergistic infections caused by aerobic and anaerobic bacteria, where the number of isolates can average 12 (two-thirds are generally anaerobes) (Table 7). Characteristically, the more types of bacteria that can be isolated, the graver the morbidity. The initial infection that follows perforation is peritonitis. The specific microorganisms involved in peritonitis generally are those of the normal flora of the gastrointestinal tract where anaerobic bacteria outnumber aerobes in the ratio 1:1,000–1:10,000 [2]. Of about 400 bacterial species that make the flora, only the virulent ones survive in the peritoneal cavity to cause the infection. The more distal the perforation is in the gastrointestinal tract, the more numerous are the types and number of organisms that spill into the peritoneal cavity.

The predominant aerobic and facultatives are *Escherichia coli*, *Streptococcus* spp. (including *Enterococcus* spp.), and the most frequently encountered anaerobes are the *B. fragilis* group, *Peptostreptococcus* spp., and *Clostridium* spp. [67].

Intra-abdominal infections are typically biphase, where in the initial stages a generalized peritonitis occurs, which is primarily associated with *E. coli* sepsis, and a later phase, where the infection is contained, and intra-abdominal abscesses emerge where *B. fragilis* can be recovered.

Appropriate management of mixed intra-abdominal infections requires the administration of antimicrobials effective against both aerobic and anaerobic components of the infection [4, 6] as well as surgical correction and drainage of pus [8]. Single and easily accessible abscesses can be drained percutaneously, thus avoiding a surgical procedure. The outcome of the infection depends on a variety of factors that include the patient's general condition, the site of performation, the bacteriology of the infection, and the antimicrobial chosen for therapy. The principle of using antimicrobial coverage effective against both aerobic and anaerobic offenders involved in intra-abdominal infections has become the cornerstone of practice and has been confirmed by numerous studies [68].

Therapy should cover Enterobacteriaceae and anaerobes (mainly *B. fragilis* group) and can be achieved by combination or single-agent therapy. Single-agent therapy provides the advantage of avoiding the ototoxicity and nephrotoxicity of aminoglycosides and is less expensive. However, a single agent may not be effective against hospital-acquired resistant bacteria, and the use of a single agent is devoid of antibacterial synergy, which may be important in an immunocompromised host. Combination of therapy can be made of anti-Enterobacteriaceae agent such as an aminoglycoside, a quinolone (in children older than 16 years) or a third-generation cephalosporin, plus anti-anaerobic agent such as clindamycin, metronidazole, or cefoxitin. Single-agent therapy includes a carbapenem (i.e., imipenem and meropenem) or a penicillin plus a beta-lactamase inhibitor (i.e.,

ticarcillin-clavulanate). The need to direct therapy against *Enterococcus* sp. is controversial and some advocate drugs such as ampicillin or vancomycin.

Antimicrobial prophylaxis prior to colonic surgery can reduce the rate of post-surgical wound infection [69]. Therapy includes either oral preparation such as erythromycin and neomycin or parenteral antimicrobial such as cefoxitin.

6.5 Female Genital Tract Infection

These infections can occur in sexually active adolescent females. Genital tract infection involving anaerobes are polymicrobial and include bacterial vaginosis, soft tissue perineal and vulvar and Bartholin gland abscesses, endometritis, pymetra, salpingitis, tubo-ovarian abscesses, adnexal abscess, pelvic inflammatory disease that may include pelvic cellulitis and abscess, amnionitis, septic pelvic thrombophlebitis, intrauterine device-associated infection, septic abortion, and post-surgical obstetric and gynecologic infections (Table 7) [4, 6]. Obtaining proper cultures can be difficult, and avoiding their contamination by the normal genital flora can be achieved by utilization of culdocentesis, laparoscopy, or quantitative endometrial cultures of transcervical samples using a telescoping catheter.

The predominant anaerobic bacteria include *P. bivia* and *P. disiens*, *Peptostreptococcus* spp., *Porphyromonas* spp., and *Clostridium* spp. *Actinomyces* spp. and *Eubacterium nodature* are commonly isolated in infections associated with intrauterine devices. *Mobiluncus* spp. may be involved with bacterial vaginosis [4, 6, 70]. The aerobic organisms also isolated mixed with these anaerobes include Enterobacteriaceae, *Streptococcus* spp. (including group A and B), *Neisseria gonorhoeae* and Chlamydia (in sexually active females), and *Mycoplasma hominis*.

Management of polymicrobial pelvic infection include the use of antimicrobials effective against all potential aerobic and anaerobic pathogens and coverage against sexually transmissible pathogens. The regimens include doxycycline or a macrolide in combination with cefoxitin, cefotetan, clindamycin, or metronidazole.

6.6 Skin and Soft Tissue Infections

Skin and soft tissue infections that can involve anaerobes include superficial infections such as infected cutaneous ulcers, cellulitis, secondary diaper rash, gastrostomy or tracheostomy site wounds, infected subcutaneous sebaceous or inclusion cysts, eczema, scabies or kerion infections, paronychiae, hidradenitis suppurativa, and pyoderma. Subcutaneous tissue infections and post-surgical wound infection that may also include skin involvement include cutaneous and subcutaneous abscesses, decubitus ulcers, breast abscess, bite wound, anaerobic cellulitis and gas gangrene, bacterial synergistic gangrene, infected pilonidal cyst or sinus, and burn wound infection. Deeper situated anaerobic soft tissue infections are necrotizing fasciitis, necrotizing synergistic cellulitis, gas gangrene, and crepitus cellulitis

[71]. These infections can involve only the fascia and/or the muscle surrounded by the fascia (inducing myositis and myonecrosis).

The organisms recovered from soft tissue infections vary according to the type of infections (Table 7). However, the location and the circumstances leading to the infection influence the organisms involved. Cultures often contain several bacterial species that frequently originate from the "normal flora" of the adjacent region.

Wounds and subcutaneous tissue infections and abscesses of the rectal area (decubitus ulcer, perisacral abscess) originate from the gut flora tend to yield organisms found in the colon [4, 6, 72]. These include *B. fragilis* group, *Clostridium* spp., Enterobacteriaceae, and *Enterococci*. In contrast, specimens obtained from sites in and around the oropharynx, or originating from that site, generally contain members of the oral flora (i.e., paronychia and bites). These include pigmented *Prevotella* and *Porphyromonas* spp., *Fusobacterium* spp., and *Peptostreptococcus*. Skin flora organisms such as *S. aureus* and *Streptococcus* spp. or nosocomially acquired organisms (Gram-negative aerobic bacilli) can be isolated at all body sites. In addition to oral flora, human bite infections often contain *Eikenella* and animal bite harbor *Pasteurella multicida* [73].

Infections involving anaerobes are usually polymicrobial and can be complicated by osteomyelitis or bacteremia [74, 75]. Deep tissue infections such as necrotizing cellulitis, fasciitis, and myositis often involve *Clostridium* spp., *S. pyogenes*, and/or polymicrobic combination of aerobic and anaerobic bacteria. They often contain gas in the tissues and putrid-like pus of gray thin quality and can be associated with high rate of bacteremia and mortality [75].

Management of deep-seated soft tissue infection includes surgical de-bridement, drainage and vigorous surgical management. Improvement of oxygenation of the involved tissues through enhancement of blood supply when indicated and administration of HBO especially in clostridial infection may be helpful.

6.7 Osteomyelitis and Septic Arthritis

Anaerobes are especially notable in oesteomyelitis of the long bones after trauma and fracture, osteomyelitis related to peripheral vascular disease, and decubitus ulcers and osteomyelitis of cranial and facial bones. Most of the infections are polymicrobial [74].

Anaerobic osteomyelitis of cranial and facial bones is often secondary to spread of infection from a contiguous soft-tissue source or from sinus, ear, or dental infection. Pelvic osteomyelitis has been related to spread of anaerobes from decubitus ulcers [6]. Osteomyelitis of long bones is generally due to hematogenic spread, trauma, or the presence of a prosthetic device.

Anaerobic streptococci [15] and *Bacteroides* species are the most common organisms at all sites, including bites and cranial infection [73]. Pigmented *Prevotella* and *Porphyromonas* species are especially prevalent in skull and bite infections [12], whereas members of the *B. fragilis* group were associated with

vascular disease or neuropathy. *Fusobacterium* species, which are members of the oral flora, were most frequently isolated from bites and from cranial and facial infections [13]. *Clostridium* species are often found in long bones, especially in association with wound contamination after trauma. Because clostridial species are inhabitants of the lower gastrointestinal tract, they may contaminate compound fractures of the lower extremities.

Septic arthritis due to anaerobic bacteria is uncommon. The role of anaerobes in joint infection was especially obvious in arthritis following hematogenous and contiguous spread of infection, in trauma, and in arthritis associated with prosthetic joints [76]. Most cases of septic arthritis due to anaerobes are monomicrobial.

6.8 Bacteremia

The incidence of anaerobes in bacteremia is 5–15% [75, 77]. *B. fragilis* group is the most prevalent blood culture isolate accounting for over three-quarters of the anaerobic isolates. Other common isolates include *Clostridium* spp., *Peptostreptococcus* spp., *Fusobacterium* spp., and *P. acnes*.

Specific organisms involved in anaerobic bacteremia largely depend on the portal of entry and underlying disease. The predominance of certain isolates in conjunction with the specific sources is related to the origin of the primary infection and the endogenous flora at the anatomic sites. Recovery of *B. fragilis* group organisms and clostridia was mostly associated with a gastrointestinal source, pigmented *Prevotella* and *Porphyromonas* and *Fusobacterium* with oropharynx and pulmonary sources, fusobacteria with the female genital tract, *P. acnes* with foreign body, and peptostreptococci with all sources but especially with oropharyngeal, pulmonary, and female genital tract sources.

The factors predisposing to bacteremia due to anaerobes include neoplasms, hematologic disorders, organ transplant, recent gastrointestinal, obstetric or gynecologic surgery, intestinal obstruction, decubitus ulcers, dental extraction, the newborn age, sickle cell disease, diabetes mellitus, postsplenectomy, and the use of cytotoxic agents or corticosteroids [4, 6].

The clinical presentation of anaerobic bacteremia is similar to aerobic infection except for the signs of infection observed at the infection's port of entry. It commonly includes fever, chills, hypotension, leukocytosis, shock, disseminated intravascular coagulation, and anemia are less infrequent. Features more typical of anaerobic infection include metastatic lesions, hyperbilirubinemia, and suppurative thrombophlebitis. Mortality rate varies between 5 and 10% and is improved with early and appropriate antimicrobial therapy and resolution, when present, of the primary infection.

6.9 Neonatal Infection

The newborn's exposure to the mother's vaginal flora that contains polymicrobial bacterial flora can be associated with the development of anaerobic infection.

These include cellulitis of the site of fetal monitoring (due to *Bacteroides* spp.) [78], neonatal aspiration pneumonia (due to *Bacterodes* spp.) [79], bacteremia [80], conjunctivitis (due to clostridium) [81], omphalitis (due to mixed flora) [82], and infant botulism [83]. Clostridial species may play a role in necrotizing enterocolitis [84].

Management of these infection requires treating the underlying condition(s) and administration of age-adjusted dosages of proper antimicrobial agents.

7 Management

The recovery from an anaerobic infection depends on prompt and proper management. The principles guiding the management of anaerobic infections include neutralizing the toxins produced by anaerobes, preventing their local proliferation by changing the environment, and hampering their spread into healthy tissues.

Toxin neutralization by specific antitoxins may be employed, especially in infections caused by *Clostridium* sp. (tetanus and botulism). Environmental control is achieved by debriding of necrotic tissue, draining the pus, improving circulation, alleviating obstructions, and increasing the tissue oxygenation. Certain types of adjunct therapy such as hyperbaric oxygen (HBO) may also be useful. Antimicrobials' primary role is in limiting the local and systemic spread of the organism. Antimicrobial therapy is in many patients the only form of therapy required, whereas in others it is an important adjunct to a surgical approach.

7.1 Hyperbaric Oxygen

There is controversy whether HBO should be used in infection of spore-forming Gram-positive anaerobic rods. There are several uncontrolled reports that demonstrated efficacy in individual cases [4, 6], however, because no well-controlled studies are available, the use of HBO is unproven. Using HBO in conjunction with other therapeutic measures is not contraindicated except when it may delay the execution of other essential procedures. Topical application of oxygen-releasing compounds may also be useful as an adjunct to other procedures.

7.2 Surgical Therapy

In many cases, surgical therapy is the most important and sometimes the only form of treatment required, whereas in others, surgical therapy is an important adjunct to a medical approach. Surgery is important in draining abscesses, debriding necrotic tissues, decompressing closed space-infections, relieving obstructions, and correcting underlying pathology. When surgical drainage is not used, the infection may persist and serious complications may develop.

7.3 Antimicrobial Therapy

Appropriate management of mixed aerobic and anaerobic infections requires the administration of agents effective against both types of organisms. A number of factors should be considered when choosing appropriate antimicrobial agents. They should be effective against all target organism(s), induce little or no resistance, achieve sufficient levels in the infected site, have safety record and appropriate dosage schedules for children, and have minimal toxicity and maximum stability.

Antimicrobials often fail to cure the infection. Among the reasons for this are the development of bacterial resistance, achievement of insufficient tissue levels, incompatible drug interaction, and the development of an abscess. The environment of an abscess is detrimental to many antibiotics. The abscess capsule interferes with the penetration of drugs, and the low pH and the presence of binding proteins or inactivating enzymes (i.e., beta-lactamase) may impair their activity. The low pH and the anaerobic environment within the abscess are especially unfavorable for the aminoglycosides and quinolones. An acidic pH, high osmolarity, and an anaerobic environment can also develop in an infection site in the absence of an abscess.

When choosing antimicrobials to treat mixed infections, their aerobic and anaerobic antibacterial spectrum (Table 8) and their availability in oral or parenteral form should be considered (Table 9). Some antimicrobials have a limited range of activity. For example, metronidazole is active only against anaerobes and therefore cannot be administered as a single agent for the therapy of mixed infections. Others (i.e., imipenem) have wide spectra of activity against Enterobacteriaceae and anaerobes.

Antimicrobial selection is simplified when reliable culture results are available. However, this may be difficult to achieve because of the problems in obtaining appropriate specimens in anaerobic infections. For this reason, many patients are treated empirically on the basis of suspected rather than known pathogens. Fortunately, the types of anaerobes involved in many anaerobic infections and their antimicrobial susceptibility patterns tend to be predictable, although they may vary in a particular hospital. Some anaerobic bacteria, however, have become resistant to antimicrobial agents or may become so while a patient is receiving therapy.

The susceptibility of the *B. fragilis* group to the frequently used antimicrobial drugs was studied systemically over the past several years. Surveys showed no to minimal resistance to chloramphenicol, metronidazole, imipenem, and the combinations or a penicillin and beta-lactamase inhibitors. However, resistance to other agents varied and the rate differs among various medical centers and generally increases with extensive use of some antimicrobial agents (penicillins, cephalosporins, and clindamycin).

Factors other than susceptibility patterns also influence the choice of antimicrobial therapy. These include the pharmacologic characteristics of the various drugs, their toxicity, their effect on the normal flora, and bactericidal activity. Although identification of the infecting organisms and their antimicrobial susceptibility may

Table 8 Susceptibility of anaerobic bacteria to antimicrobial agents

Bacteria	Pencillin	A penicillin and a beta-lactamase inhibitor	Ureido- and carobxy-penicillin	Cefoxitin	Chloramphenicol	Clindamycin	Macrolydes	Metronidazole	Carbapenems
Peptostreptococcus sp.	4	4	3	3	3	3	2–3	2	3
Fusobacterium sp.	3–4	3–4	3	3	3	2–3	1	3	3
B. fragilis group	1	4	2–3	3	3	3–4	1–2	4	4
Prevotella and *Porphyromonas* sp.	1–3	4	2–3	3	3	3–4	2–3	4	4
C. perfringens	4	4	3	3	3	3	3	3	3
Clostridium sp.	3	3	3	2–3	3	2	2	3	3
Actinomyces sp.	4	4	3	3	3	3	3	1	3

Degrees of activity: 1, minimal; 2, moderate; 3, good; 4, excellent.

Table 9 Antimicrobial recommended for the therapy of site-specific anaerobic infections

	Surgical prophylaxis	Parenteral	Oral
Intracranial	(1) Penicillin (2) Vancomycin	(1) Metronidazole (4) (2) Chloramphenicol	(1) Metronidazole (4) (2) Chloramphenicol
Dental	(1) Penicillin (2) Erythromycin	(1) Clindamycin (2) Metronidazole (4), chloramphenicol	(1) Clindamycin, amoxicillin + CA (2) Metronidazole (4), chloramphenicol
Upper respiratory tract	(1) Cefoxitin (2) Clindamycin	(1) Clindamycin (2) Chloramphenicol, metronidazole (4)	(1) Clindamycin, amoxicillin + CA (2) Chloramphenicol, metronidazole (5)
Pulmonary	NA	(1) Clindamycin (5) (2) Chloramphenicol, ticarcillin + CA, ampicillin + SU (6), imipenem	(1) Clindamycin (8) (2) Chloramphenicol, metronidazole (5), amoxicillin + CA
Abdominal	(1) Cefoxitin (2) Clindamycin (3)	(1) Clindamycin (3), cefoxitin (3), metronidazole (3) (2) Imipenem, ticarcillin + CA	(1) Clindamycin (8), metronidazole (8) (2) Chloramphenicol, amoxicillin + CA
Pelvic	(1) Cefoxitin (2) Doxycycline	(1) Cefoxitin (6), clindamycin (3) (2) Ticarcillin + CA (6), ampicillin + SU (6), metronidazole (6)	(1) Clindamycin (6) (2) Amoxicillin + CA (6), metronidazole (6)
Skin	(1) Cefazolin (7) (2) Vancomycin	(1) Clindamycin, cefoxitin (2) Metronidazole (4) + methicillin	(1) Clindamycin, amoxicillin + CA (2) Metronidazole (5)
Bone and joint	(1) Cefazolin (7) (2) Vancomycin	(1) Clindamycin, imipenem (2) Chloramphenicol, metronidazole (4), ticarcillin + CA	(1) Clindamycin (2) Chloramphenicol, metronidazole (4)
Bacteremia with BLPB	NA	(1) Imipenem, metronidazole (2) Cefoxitin, ticarcillin + CA	(1) Clindamycin, metronidazole (2) Chloramphenicol, amoxicillin + CA
Bacteremia with non-BLPB	NA	(1) Penicillin (2) Clindamycin, metronidazole, cefoxitin	(1) Penicillin (2) Metronidazole, chloramphenicol, clindamycin

1, drug(s) of choice; 2, alternative drugs; 3, plus aminoglycoside; 4, plus penicillin; 5, plus a macrolide (i.e., erythromycin); 6, plus doxycycline; 7, in location proximal to the reactal and oral areas use cefoxitin; 8, plus a quinolone (only in adults); NA, not applicable; CA, clavulanic acid; SU, sulbactam; BLPB, beta-lactamase-producing bacteria.

be needed for selection of optimal therapy, the clinical setting and Gram-stain preparation of the specimen may suggest what types of anaerobes are present in the infection as well as the nature of the infectious process.

Because anaerobic bacteria generally are recovered mixed with aerobic organisms, selection of proper therapy becomes more complicated. In the treatment of mixed infection, the choice of the appropriate antimicrobial agents should provide for adequate coverage of most of the pathogens.

7.4 Antimicrobial Agents

Some classes of agents possess poor activity against anaerobes. These include the aminoglycosides, the monobactams, and the older quinolones. Antimicrobials suitable to control anaerobic infections are summarized in Tables 9 and 10 and discussed in more detail below [85, 86].

Table 10 Antimicrobial drugs of choice for anaerobic bacteria

	First	Alternate
Peptostreptococcus sp.	Penicillin	Clindamycin, chloramphenicol, cephalosporins
Clostridium sp.	Penicillin	Metronidazole, chloramphenicol, cefoxitin, clindamycin
C. difficile	Vancomycin	Metronidazole, bacitracin
Gram-negative bacilli[a] (BL–)	Penicillin	Metronidazole, clindamycin, chloramphenicol
Gram-negative bacilli[a] (BL+)	Metronidazole, imipenem, a penicillin and beta-lactamase inhibitor, clindamycin	Cefoxitin, chloramphenicol, piperacillin

BL, beta-lactamase.
[a]*B. fragilis* group; *Prevotella* spp., *Porphyromonas* spp., *Fusobacterium* spp.

7.4.1 Penicillins

Penicillin G is the drug of choice against most non-beta-lactamase-producing organisms. These include anaerobic streptococci, *Clostridium* spp., non-sporulating anaerobic bacilli, and most non-beta-lactamase-producing Gram-negative anaerobic rods (i.e., *Bacteroides, Fusobacterium, Prevotella,* and *Porphyromonas* sp.). However, in addition to the *B. fragilis* group, which is known to be resistant to the drug, many other anaerobic Gram-negative rods are showing increased resistance. These include *Fusobacterium*, pigmented *Prevotella* and *Porphyromonas* (common in orofacial and respiratory infections), *P. bivia* and *P. disiens* (prevalent in female genital infections), *Bilophila wadsworthia*, and *Bacteroides splanchinus*.

Resistance to penicillin of some *Clostridium* spp. (*C. ramosum*, *C. clostridioforme*, and *C. butylicum*) through production of beta-lactamase was also noted.

The presence of penicillin-resistant bacteria in an infectious site has important implications for antimicrobial therapy. Many penicillin-resistant anaerobic bacteria can produce enzymes that degrade penicillins or cephalosporins. When such organisms are present in a localized infection, they can release the enzyme into the environment, thus degrading the beta-lactam antibiotic in the area of the infection. Therefore, these organisms may protect not only themselves but also penicillin-sensitive pathogens. Penicillin therapy directed against a susceptible pathogen might therefore be rendered ineffective by the presence of these bacteria [23].

The combinations of beta-lactamase inhibitors (such as clavulanic acid, sulbactam, or tazobactam) plus a beta-lactam antibiotic (ampicillin, amoxicillin, ticarcillin, or piperacillin) can overcome this phenomenon in organisms that produce a beta-lactamase that can be bound by the inhibitor. However, if resistance is due to other mechanisms, blockage of the enzyme beta-lactamase will not prevent resistance. Other mechanisms of resistance are alteration in the porin canal through which the antimicrobial penetrates into the bacteria and changes in the penicillin-binding protein that inhibits introduction of the drug into the cell.

The semisynthetic penicillins, carbenicillin, ticarcillin, piperacillin, and mezlocillin are generally administered in large quantities to achieve high serum concentrations. These drugs have good activity against Gram-negative enterics and most anaerobes in these concentrations. However, these drugs are not entirely resistant to the beta-lactamase produced by Gram-negative anaerobic bacilli.

7.4.2 Cephalosporins

The activity of cephalosporins varies against *Bacteroides* sp. First-generation cephalosporins activity against anaerobes is similar to that of penicillin G, although on a weight basis they are less active. Most strains of the *B. fragilis* group and many *Prevotella* and *Porphyromonas* are resistant because of cephalosporinase production. Cefoxitin is relatively resistant to this enzyme and is the most effective cephalosporin against the *B. fragilis* group and is often used for the therapy and prophylaxis of mixed infections. However, 5–15% of *B. fragilis* group may be resistant, reflecting hospital use pattern. With the exception of *C. perfringens*, cefoxitin is relatively inactive against most species of *Clostridium* (including *C. difficile*). Cefotetan and cefmetazole (also second-generation cephalosporins), have a longer half-life, are as effective as cefoxitin against *B. fragilis*, but have poor efficacy against other members of the *B. fragilis* group (i.e., *B. thetaiotaomicron*). Third-generation cephalosporins are inferior to cefoxitin against *Bacteroides* sp.

7.4.3 Carbapenem (imipenem, meropenem)

The beta-lactam carbapenem have excellent activity against a broad spectrum of aerobic bacteria and anaerobic bacteria, including beta-lactamase-producing *Bacteroides* sp., Enterobacteriaceae, and *Pseudomonas.*

7.4.4 Chloramphenicol

Chloramphenicol has excellent in vitro activity against most anaerobes, and resistance is rare. The drug also is effective against many Enterobacteriaceae and Gram-positive cocci. However, the experience of using this drug in intra-abdominal sepsis was disappointing. The rare but fatal aplastic anemia, the dose-dependent leukopenia, gray syndrome in newborns, and patients with impaired hepatic glucuronidation limit its use.

7.4.5 Clindamycin and Lincomycin

Clindamycin and lincomycin are effective against anaerobes and have good activity against aerobic Gram-positive cocci. Clindamycin has the broader coverage against anaerobes, including beta-lactamase producing *Bacteroides* sp. Resistance of *B. fragilis* group is 5–10%, and some *Clostridium* sp. other than *C. perfringens* are resistant. Antibiotic-associated colitis due to *C. difficile* was first described following clindamycin therapy. However, colitis has been associated with other antimicrobials, and more cases are reported annually following penicillins and cephalosporin therapy than after clindamycin therapy.

7.4.6 Metronidazole

Metronidazole has excellent activity against anaerobes; however, it is not effective against aerobic bacteria. Microaerophilic streptococci*, P. acnes,* and *Actinomyces* sp. often are also resistant. Concern was raised about the carcinogenic and mutagenic effects of this drug; however, these effects were shown only in one species of mice and were never substantiated in other animals or humans [4, 6].

7.4.7 Macrolids (Erythromycin, Azithromycin, Clarithromycin)

Macrolids have moderate-to-good in vitro activity against anaerobic bacteria other than *B. fragilis* and fusobacteria. They are active against *Prevotella* and *Porphyromonas* sp., microaerophilic and anaerobic streptococci, Gram-positive non-spore-forming anaerobic bacilli, and certain clostridia. They show relatively good activity against *C. perfringens* and are poor or inconsistent against Gram-negative anaerobic bacilli.

7.4.8 Glycopeptides (Vancomycin, Teicoplanin)

Vancomycin is effective against all Gram-positive anaerobes (including *C. difficile*) but is inactive against Gram-negative bacilli.

7.4.9 Tetracyclines

Tetracycline is of limited use because of resistance to it by all types of anaerobes including *B. fragilis* group. The newer tetracycline analogues, doxycycline and minocycline, are more active than the parent compound. The use of tetracyclines is not recommended under 8 years of age because of their adverse effect on teeth and bone.

7.4.10 Quinolones

The older quinolones (ciprofloxacin, ofloxacin) are less active than the newer one (trovafloxacin, clinafloxacin) against the *B. fragilis* group. However, the use of the quinolones is limited in growing children because of their possible adverse effects on the cartilage.

7.5 Choice of Antimicrobial Agents

The parenteral antimicrobials that can be used in most infectious sites are clindamycin, metronidazole, chloramphenicol, cefoxitin, a penicillin (i.e., ticarcillin or ampicillin) combined with a beta-lactamase inhibitor (i.e., clavulanic acid or sulbactam), and a carbapenem (i.e., imipenem). An anti-Gram-negative enteric agent (i.e., aminoglycoside) is generally added to clindamycin, metronidazole, and, occasionally, cefoxitin when treating intra-abdominal infections to provide coverage for enteric bacteria. Failure of therapy in intra-abdominal infections has been noticed more often with chloramphenicol and, therefore, this drug is not recommended. Penicillin is added to metronidazole in the therapy of intracranial, pulmonary, and dental infections to cover for microaerophilic streptococci, *Actinomyces* sp., and *Arachnia* sp. A macrolide (i.e., erythromycin) is added to metronidazole in upper respiratory infections to treat *S. aureus* and aerobic streptococci. Penicillin is added to clindamycin to supplement its coverage against *Peptostreptococcus* sp. and other Gram-positive anaerobic organisms (Table 10). Doxycycline is added to most regimens in the treatment of pelvic infections to provide therapy for chlamydia and mycoplasma.

Penicillin is still the drug of choice for bacteremia caused by non-beta-lactamase-producing bacteria. However, other agents should be used for the therapy of bacteremia caused by beta-lactamase-producing bacteria.

The duration of therapy for strict anaerobic infections, which are often chronic, is generally longer than for infections due to aerobic and facultative anaerobes. Oral therapy is often substituted for parenteral therapy after an initial period. The agents available for oral therapy are limited and include clindamycin, amoxicillin plus clavulanic acid, chloramphenicol, and metronidazole.

Clinical judgment, personal experience, safety, and patient compliance should direct the physician in the choice of the appropriate antimicrobial agents. Duration of treatment also must be individualized, depending on the response. In some cases,

such as lung abscesses, treatment may be required for as long as 6–8 weeks but can often be shortened with proper surgical drainage.

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Encephalitis Diagnosis and Management in the Real World

Sarah S. Long

Abstract Significant advances, especially in microbiologic diagnostics and brain imaging, have broadened our understanding of the etiology, pathogenesis, and natural history of acute encephalitis. In some instances this had led to specific therapies and preventive measures. The clinical hallmark of acute encephalitis is the triad of fever, headache, and altered mental status. Common neurologic features include disorientation or depressed level of consciousness; disturbances of behavior, speech, or executive function; and diffuse or focal neurologic signs such as cranial nerve dysfunction, hemiparesis, or seizures. These features distinguish the unusual patient with encephalitis from the more commonly encountered patient with uncomplicated meningitis who has fever, headache, and nuchal rigidity but lacks abnormal global or focal neurologic signs. Etiologies of acute encephalitis are myriad. Most are viral infections. Table 1 shows a robust but not exhaustive list of viral etiologies. Beyond this there is another robust but not exhaustive list (Table 2) of important considerations in the differential diagnosis. These include infectious agents (bacterial, fungal, parasitic, and amebic) as well as non-infectious etiologies (parainfectious, post-infectious, autoimmune, neoplastic, cerebrovascular, systemic, and other conditions). The challenge for the clinician is to rapidly hone the list and make critical management decisions by considering the specific features of the setting of the patient's illness, host susceptibility, clinical and neurologic findings, and results of laboratory and imaging studies.

1 Approach to Focusing Possible Etiologies

1.1 Pathogenesis

Most cases of acute encephalitis are due to viruses (Table 1). Tropism of different viruses for different cell types as well as the balance of the pathogen

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Table 1 Viruses that cause acute encephalitis

Herpesviridae	Reoviridae
Herpes simplex	Colorado tick fever
Varicella zoster	Picornaviridae
Cytomegalovirus	Echovirus
Epstein–Barr	Coxsackie
Human herpesvirus 6	Poliovirus
Bunyaviridae	Enterovirus 71
California serogroup	Other enteroviruses
La Crosse	Retroviridae
Toscana	Human immunodeficiency virus
Togaviridae	Papovaviridae
Eastern equine encephalitis	JC, BK
Western equine encephalitis	Orthomyxoviridae
Venezulean equine encephalitis	Influenza A and B
Chikungunya	Paramyxoviridae
Flaviviridae	Rubeola (Measles/SSPE)
Japanese encephalitis	Mumps
St. Louis encephalitis	Nipah
West Nile	*Other*
Tick-borne encephalitis	Adenovirus
Powassan/Deer tick	Lymphocytic choriomeningitis
Dengue	Rabies
	Parvovirus B19

damage-response framework determines clinical findings [1, 2]. Bunyaviridae, Togaviridae, Flaviviridae, and Reoviridae are neurotropic, i.e., although the infection is unusual, infection is likely to lead to central nervous system infection. Others, such as herpesviruses, West Nile virus, human immunodeficiency virus (HIV), parvovirus B19, measles, and mumps virus, cause frequent enough infections that their modest neurotropism leads to the infrequent consequence of CNS dissemination and gives them importance on the list. Additionally, some neurotropic viruses, such as enteroviruses, lymphocytic choriomeningitis virus, and poliovirus usually lead to aseptic meningitis but occasionally can cause encephalitis. Finally, rabies virus is a unique example in which human infection inevitably results in central nervous system infection in the absence of systemic infection. Cellular mechanisms of damage that lead to clinical encephalitis are as diverse as associated agents and are not addressed in this chapter [1].

The same principles of tropism apply to non-viral infectious causes of acute encephalitis listed in Table 2. Some bacteria that are not uncommon causes of systemic infection also are neurotropic, such as *Neisseria meningitidis*, *Haemophilus influenzae*, and *Rickettsia* species. Although CNS site of infection usually leads to clinical meningitis, encephalitis can occur occasionally. Other less neurotropic agents are so prevalent that the occasional manifestation as encephalitis makes them important considerations. *Borrelia burgdorferi*, parasites, and ameba are such examples. *Bartonella henselae* is unique on this list, both as an under-recognized cause

Table 2 Non-viral infectious and non-infectious causes of acute encephalitis-like syndromes

Non-viral infectious	Non-infectious
Bacteria	Parainfectious and Autoimmune
Meningitis	Reye syndrome
Brain abscess	Acute disseminated encephalomyelitis
Parameningeal abscess	Acute necrotizing encephalopathy
Venous sinus thrombosis	Neoplasia
B. burgdorferi	Primary or metastatic
B. henselae	Paraneoplastic disease
Bacterial toxins	Cerebrovascular
Rickettsia/*Ehrlichia*/*Anaplasma* spp.	Ischemic stroke
M. pneumoniae	Subdural/epidural hematoma
Fungi	Vasculitis
Meningitis	Systemic
Brain abscess	Metabolic conditions
Parasites	Connective tissue disorders
Toxoplasma gondii	Drug intoxication
Plasmodium spp.	Other
Cysticercosis	Epilepsy
B. procyonis	Head injury
Angiostrongylus/*Gnathostoma* spp.	Confusion migraine
Ameba	
N. fowleri	
Acanthamoeba spp.	
B. mandrillaris	

of acute onset of status epilepticus and stupor or coma and because the clinical syndrome is encephalopathy rather than true encephalitis (absent cellular necrosis and cerebrospinal fluid [CSF] pleocytosis) [3].

1.2 Prioritizing Treatable Etiologies

Focussing on the primary clinical question of which causes of acute encephalitis have possible treatments, the major virus entries in Table 1 disappear, leaving a skeletal few as treatable causes of viral encephalitis shown in Table 3. The herpesviruses, and especially herpes simplex virus (HSV), tops the list with randomized, placebo-controlled trials of antiviral therapy providing evidence of life-saving, morbidity-reducing efficacy [4]. The possibility of HSV in all cases of acute encephalitis drives empiric therapy with acyclovir. There are only single or series of case reports or open label trials for other possibly treatable causes, such as intravenous ribavirin for La Crosse virus [5] and Nipah virus [6], interferon-alpha for West Nile virus [7], interferon alpha-2B for St. Louis encephalitis virus, and drug-induced coma, ribavirin, and neurotransmitter modulation (Milwaukee protocol) for rabies [8–10]. Efficacy of intravenous immunoglobulin containing high anti-West Nile virus antibody titers has been studied in a randomized, placebo-controlled trial in patients with West Nile neuroinvasive disease; results are pending.

Table 3 Treatable or possibly treatable viruses that cause encephalitis[a]

Herpesviridae	Reoviridae
Herpes simplex	Picornaviridae
Varicella zoster	Retroviridae
Cytomegalovirus	Human immunodeficiency virus
Epstein–Barr	Papovaviridae
Human herpesvirus 6	JC, BK
Bunyaviridae	Orthomyxoviridae
La Crosse	Influenza A and B
Togaviridae	Paramyxoviridae
Flaviviridae	Nipah
St. Louis encephalitis	Other
West Nile	Rabies

[a]Evidence from randomized, placebo-controlled trial exists only for herpes simplex virus.

In contrast to viral etiologies of acute encephalitis, there are specific anti-infective therapies and other potentially life-saving management for most infectious and non-infectious causes on the list of non-viral encephalitis-like syndromes in Table 2 that translate to treatable non-viral encephalitis considerations as shown in Table 4. Empiric therapy for encephalitis almost always includes anti-bacterial therapy. Anti-infective therapy has no proven efficacy in *B. henselae* or *Mycoplasma pneumoniae* encephalopathy/encephalitis. This table of differential diagnoses must

Table 4 Treatable or possibly treatable non-viral infectious and non-infectious causes of acute encephalitis-like syndromes[a]

Non-viral infectious	Non-infectious
Bacteria	Parainfectious and autoimmune
Meningitis	Reye syndrome
Brain abscess	Acute disseminated encephalomyelitis
Parameningeal abscess	Acute necrotizing encephalopathy
Venous sinus thrombosis	Neoplasia
B. burgdorferi	Primary or metastatic
Bacterial toxins	Paraneoplastic disease
Rickettsia/*Ehrlichia*/*Anaplasma* spp.	Cerebrovascular
Fungi	Ischemic stroke
Meningitis	Subdural/epidural hematoma
Brain abscess	Vasculitis
Parasites	Systemic
Toxoplasma gondii	Metabolic conditions
Plasmodium spp.	Connective tissue disorders
Cysticercosis	Drug intoxication
B. procyonis	Other
Angiostrongylus/*Gnathostoma* spp.	Epilepsy
Ameba	Head injury
N. fowleri	Confusion migraine
Acanthamoeba spp.	
B. mandrillaris	

[a]Treatable includes drug therapies and other medical and surgical management.

be considered early and repeatedly in the course of management of patients with acute encephalitis. Brain imaging and examination of CSF and clinical course are most useful in guiding clinical management for the non-infectious cause of encephalitis most frequently encountered by a pediatric infectious diseases subspecialist – acute disseminated encephalomyelitis (ADEM) (discussed below).

1.3 Etiologically Focussed Prevention of Acute Encephalitis

Prevention is not in the scope of this chapter. However, before leaving the lists of etiologies and treatable causes of acute viral encephalitis, one recognizes that the highly successful approach of vaccine prevention of bacterial meningitis is not possible currently for most viral encephalitides. Table 5 shows in italics the vaccine preventable viral encephalitides – varicella zoster virus, Japanese encephalitis virus (IXIARO, licensed by the US Food and Drug Administration March 30, 2009 as the only available JE vaccine in the United States), tick-borne encephalitis virus (two vaccines licensed in Europe) [11], poliovirus, influenza, measles, mumps, and rabies. It is noteworthy that prior to universal immunization in childhood in the United States, measles and mumps viruses were the most common causes of encephalitis and post-infectious encephalitis, and measles virus was the cause of subacute sclerosing panencephalitis. These viruses still cause substantial CNS disease where immunization is not universal.

Table 5 also shows (in standard font) avoidable causes of acute encephalitis. This list is lengthy. Methods of control for the arboviruses predominantly are reduction or avoidance of the virus' vector or the amplifying host. It is important then to attempt to identify outbreaks of arboviral infections and emerging infections specifically to

Table 5 Vaccine preventable and avoidable viruses that cause encephalitis[a]

Herpesviridae	Reoviridae
Varicella zoster	Colorado tick fever
Bunyaviridae	Picornaviridae
California serogroup	*Poliovirus*
La Crosse	Retroviridae
Toscana	Human immunodeficiency virus
Togaviridae	Papovaviridae
Eastern equine encephalitis	Orthomyxoviridae
Western equine encephalitis	*Influenza A* and *B*
Venezulean equine encephalitis	Paramyxoviridae
Chikungunya	*Rubeola* (*Measles/SSPE*)
Flaviviridae	*Mumps*
Japanese encephalitis	Nipah
St. Louis encephalitis	Other
West Nile	*Rabies*
Tick-borne encephalitis	
Powassan/Deer tick	
Dengue	

[a]Vaccine preventable viruses are shown in italics; avoidable viruses are shown in standard font.

implement optimal control measures, to use appropriate therapies if available, and to understand burden of disease as the first step in vaccine development. The lesson from the Nipah virus outbreak in Malaysia is an example of identification of the causative agent informing control [12]. From September 1998 to May 1999, 265 cases of encephalitis with 105 deaths occurred. Because the outbreak initially was attributed to Japanese encephalitis virus, mosquito control measures were implemented but were ineffective and the epidemic spread. Only when the Nipah virus was identified from a case, did it become clear that close contact with infected pigs was the mechanism of acquisition; pig culling halted the epidemic. In the extended story, typical of such epidemics, fruiting failure due to drought and fires in Indonesia drove the fruit bats (the natural reservoir host for Nipah virus) from forest habitat to farming areas, where bat excretions contaminated pig swill.

1.4 Season, Geography, and Epidemiology of Viral Encephalitis

Taken together, clues provided by intersections of certain characteristics of the host, the season of illness, geography, and epidemiologic setting as well as specific neurologic abnormalities help hone a long list of possibilities, eliminating many and elevating certain pathogens for specific consideration in each patient (Fig. 1).

Table 6 provides an example of virus considerations in which geography and season are not highly relevant, as viruses are common or ubiquitous and not seasonal. A healthy immunologically competent host can develop encephalitis from most pathogens shown, although immunocompromised individuals are unduly susceptible to CNS disease due to cytomegalovirus, JC virus, and varicella zoster virus. Certain etiologic possibilities become almost certainties with careful delineation of the epidemiologic setting and clinical extra-CNS manifestations (e.g., history of animal bite or exposure to bat for rabies, findings of clinical manifestations compatible

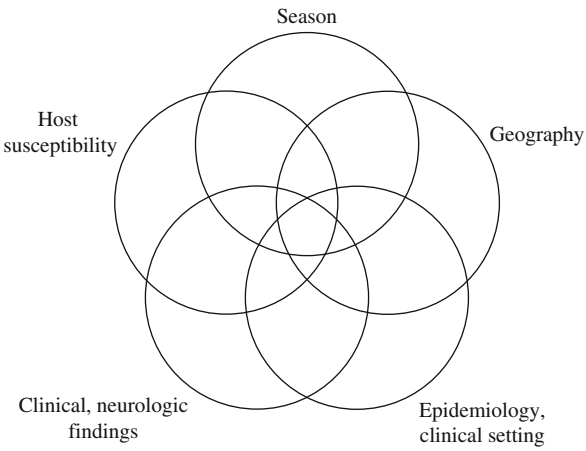

Fig. 1 Schematic shows the intersecting clues regarding the patient and the environment that help focus possible causes of viral encephalitis

Table 6 Viral etiologies of encephalitis for which season and geography are not relevant

Virus	Host	Epidemiology/Setting	Clinical
Herpes simplex virus	Any	Any	Focal neurologic signs, seizures, temporal lobe predilection
Varicella zoster virus	Any, compromised	Vesicular rash	Stroke, ataxia, transverse myelitis
Cytomegalovirus	Compromised, any	HIV, transplant recipient	Periventricular predilection, lumbosacral polyradiculitis
Human immunodeficiency virus	Any	Seroconversion syndrome	Meningoencephalitis
JC virus	Compromised	HIV, transplant recipient	Focal neurologic signs, multifocal MRI lesions
Rabies virus	Any	Animal bite, bat exposure	Autonomic and limbic abnormalities, paralysis

with mononucleosis-like seroconversion syndrome for HIV). Nonspecific neurologic findings of decreased sensorium can be the only signs for most etiologies, but the presence of certain neurologic laboratory or imaging findings, if present, heighten certain possibilities (e.g., focal neurologic abnormalities, focal seizures and temporal lobe predilection for HSV, or autonomic and mood abnormalities due to limbic predilection for rabies virus).

Example pathogens shown in Table 7 share particular predilection for season, almost always summer and fall, with human infection being dependent on circulation of a vector, a neutral host or an amplifying host, and opportunity for

Table 7 Viral causes of encephalitis with seasonal and geographic predilection in the United States

Virus	Season	Geography	Epidemiology/ Exposure	Clinical Characteristics
Easter equine encephalitis	Summer/Fall	East, Gulf	Outdoors, swamp	Fulminant, seizures, coma
Western equine encephalitis	Summer/Fall	Midwest, West	Outdoors, rural	Headache, non-focal neurologic findings
St. Louis encephalitis	Summer/Fall	Nation	Outdoors, rural in West, urban in East	Headache, non-focal neurologic findings
La Crosse	Summer/Fall	Midwest, East	Outdoors, suburban wooded	Mild encephalitis, focal seizures
Enterovirus	Summer/Fall	Nation, Globe	Known epidemic community	Exanthem/enanthem, myopericarditis, acute flaccid paralysis

human exposure. Exposure, travel history, and knowledge of incubation period are important considerations. Enteroviruses are the obvious exception to vector-positive history. Enteroviral disease burden is seasonal, but infection follows human-to-human transmission; there is no extra-human natural or amplifying host or vector.

Clinical neurologic clues sometimes stand out to suggest an etiologic agent, such as fulminant course to coma for Eastern equine encephalitis, focal seizures but mild encephalitis for La Crosse virus, acute flaccid paralysis for tick-borne encephalitis virus and West Nile virus, and findings typical of hippocampal predilection for Powassan virus. Epidemiologic shifts of a few pathogens in Table 8 highlight infinite ecological and evolutionary complexities as well as our meager understanding of events that shape emergence and spread of arthropod-borne viruses. Toscana virus, a member of the family Bunyaviridae, was first registered in the international catalogue of arboviruses only in 1980, first isolated from CSF in 1983, and transmission subsequently was shown to be by the Phlebotomine sandfly. Currently endemic in the Siena province of Central Italy, the virus is responsible for at least 80% of local acute viral CNS infections in children throughout the summer and for global sporadic cases following travel to the area [13].

Table 8 Viral causes of acute encephalitis with recent geographic expansion

Virus	Geography	Epidemiology/exposure	Clinical
Toscana	Italy, Mediterranean	Rural, suburban	Non-focal neurologic findings, meningitis
Tick-borne encephalitis	Patchy endemic, Central Europe to Japan	Outdoors, forested, raw milk	Biphasic illness, transverse myelitis/radiculopathy
West Nile	Global	Outdoors, blood/organ transplant	Non-focal neurologic findings, meningitis, tremors, acute flaccid paralysis
Powassan	Northeast North America, Atlantic US, Russia, Wisconsin	Wooded	Non-focal neurologic findings, hippocampal abnormalities
Chikungunya	Tropical Asia, Africa and imported from Africa to Europe, US	Outdoors	Meningitis, polyneuropathy, myositis/arthritis

West Nile virus (WNV) historically has been considered to be among the least virulent of the Japanese serogroup of the family Flaviviridae. However, a virus subtype with greater epidemic potential and virulence emerged in the early 1990s, facilitating geographic expansion in Asia, the Middle East, and Mediterranean, with westward spread. WNV first appeared in the western hemisphere in 1999. Birds serve as the natural vertebrate host and *Culex* mosquitoes are the enzootic and epizootic vector. With a broad host and vector range, WNV moved sequentially westward in the United States with human cases reported from the northeast in 1999

to California by 2002. In recent epidemics of WNV, approximately 80% of cases are asymptomatic, 20% have dengue-like syndromes, and <1% have neuroinvasive disease (WNND) including encephalitis, meningitis, and polio-like flaccid paralysis [14]. Children are less likely than adults with WNV to have WNND, accounting for only 4% of such cases from 1999 to 2007 reported in the United States; unlike older adults with WNND, children and young adults are more likely to have meningitis than encephalitis [15].

Chikungunya virus (CHIKV) is a mosquito-borne alphavirus indigenous in the sylvatic cycle in tropical Africa and Asia where it causes endemic and epidemic high fever, disabling joint and muscle pain (chikungunya in Swahili meaning "that which bends up"), and often a maculopapular rash and gastrointestinal complaints. CNS infection can lead to meningoencephalitis or polyneuropathy. In 2005–2006, an explosive epidemic of CHIK fever occurred on islands in the Indian Ocean, affecting almost one-half of the population and resulting in an estimated millions of cases in the islands and in India. Previously transmitted by *Aedes aegypti* (an exclusive human feeder), a point mutation in the virus more recently has been shown to make the Asian tiger mosquito, *Aedes albopictus* (a multi-species feeder) an enhanced transmitter [16]. Epidemic CHIK fever in Italy in 2007, *Ae. albopictus* transmitted, and global spread of the mosquito during the past two decades is worrisome for broader endemic disease as well as infection following travel.

1.5 Studies of Causes of Acute Encephalitis

The California Encephalitis Project (CEP) was initiated in 1998 by the California Department of Health in collaboration with the Emerging Infections Program of the Centers for Disease Control and Prevention to study causative agents, disease trends, risk factors, and clinical features of encephalitis in California. Comprehensive results have been published following intensive evaluations (which evolved somewhat over the first decade of study) of more than 1,500 patients enrolled. Although the majority of study subjects have been adults, studies have enlightened understanding of relative importance of pathogens, clinical patterns of disease, and usefulness of diagnostic tests [17–22]. Enrollment consisted of subjects with encephalopathy (altered level of consciousness for at least 24 h, lethargy, or change in personality) or ataxia, plus ≥ 1 of the following: temperature ≥ 38°C, seizure, focal neurologic finding, CSF pleocytosis, abnormal EEG, or neuroimaging study. Patients were excluded who were <6 months of age, immunosuppressed, or had underlying neurologic conditions (http://ceip.us/encephalitis.htm). A summary list of diagnostic tests performed is shown in Table 9. Additional tests for 24 pathogens were performed on a case-by-case basis if history, examination, laboratory, or imaging findings suggested certain pathogens. Etiologies of infections were assigned as confirmed, probable, or possible by pre-fixed definitions; confirmed non-infectious causes also were defined; the remainder of cases was designated as of unknown cause. Infection was designated as *confirmed* when an agent well established as a

Table 9 Summary of diagnostic tests performed in the California encephalitis project

CSF	Herpesviruses PCR Mycoplasma PCR Enterovirus PCR Measles antibody Virus culture
Serum acute/convalescent antibody	Arboviruses × 3 Other viruses × 7 *Mycoplasma pneumoniae* *Chlamydophila pneumoniae*
Respiratory tract	Respiratory virus panel PCR Enterovirus PCR Mycoplasma PCR Virus culture
Selected additional tests	24 pathogens based on suspicion by history, examination, laboratory findings, or brain imaging

Modified from Glaser et al. [18].
PCR, polymerase chain reaction test.

cause of encephalitis was detected in CSF or brain by PCR or by an antibody test when PCR is not the diagnostic test of choice. Infection was designated as *probable* when an agent well established as a cause of encephalitis was not identified in CSF or brain but when there was strong evidence by culture or serologic evidence of infection. Infection was designated as *possible* when an agent well established as a cause of encephalitis was identified by suggestive but not conclusive testing of a specimen other than the CSF (e.g., enteroviruses, HSV-1, and influenza) or when an agent *not* well established as a cause of encephalitis was identified by strong serologic or culture-based evidence at a site other than CNS (e.g., adenovirus and *Mycoplasma*) [18].

Despite extensive investigation in >1,500 subjects, no etiology was found in >60% of encephalitis cases. Infection accounted for 28% of cases (confirmed/probable, 15% and possible, 13%). Table 10 shows infectious causes identified. Viruses accounted for approximately 70% of confirmed and probable causes, with enteroviruses and HSV-1 heading the list. One extremely important finding that impacts decisions for empiric therapy was that approximately 20% of subjects with infectious encephalitis-like clinical presentation had CNS bacterial infections. Enteroviruses, influenza, and adenoviruses headed the list of possible viral etiologies. In a prospective registry in Toronto, influenza infection was identified in 7% of children with acute encephalitis [23]. It is noteworthy that although the association of influenza with neurologic disease is well established, the virus is almost never identified in the CSF or brain; pathophysiology appears in some cases to be acute necrotizing encephalopathy (ANE) (discussed below) due to CNS cytokine dysregulation, and in others, infection-triggered ADEM.

Mycoplasma accounted for 47% of possible non-viral cases (and no probable or confirmed cases in the CEP). In fact, *Mycoplasma* was the most frequently identified pathogen associated with encephalitis in the CEP. Christie et al. [19] reported on the

Table 10 Infectious causes of encephalitis in California encephalitis project

Confirmed/probable causes		Possible causes	
Viruses		*Viruses*	
Enterovirus	17%	Enterovirus	14%
HSV-1	16%	Influenza	11%
Varicella zoster	9%	Adenovirus	7%
West Nile	8%	HSV-1	6%
Epstein–Barr	7%	Metapneumovirus	2%
HSV-2	2%	Other viruses	4%
SSPE	2%		
Other viruses	9%		
Non-virus causes		*Non-virus causes*	
Bacteria	20%	*Mycoplasma*	47%
Prion	7%	*Chlamydophila*	5%
Parasite	3%	Other non-viral agents	2%
Fungus	1%		

Modified from Glazer et al. [18].
HSV, herpes simplex virus; SSPE, subacute sclerosing panencephalitis.

84 patients ≤ 18 years of age with evidence of acute *Mycoplasma* infection among 1988 encephalitis subjects accrued in the CEP by 2007. Median age was 11 years; 70% had fever, 45% had respiratory tract symptoms, and 44% had gastrointestinal symptoms. CSF showed mild pleocytosis (median 33 cells, predominantly mononuclear in 67%), with median protein of 45 mg/dL and normal glucose concentration. Clinical findings were different from adults and were most similar in presentation and outcome to proven enterovirus encephalitis. Diagnosis was made solely by positive serum IgM test in 92%; only 2 of 39 who had acute and convalescent serum IgG tests performed had confirmation of acute *Mycoplasma* infection. PCR test on respiratory specimen was positive in 30%. Sixty-two of the 84 patients had CSF *Mycoplasma* tested by PCR; none was positive. One patient had CSF IgM antibody detected [19]. In another study, Christie et al. [24] investigated auto-antibodies to galactocerebroside (anti-GalC) in CEP patients with evidence of acute *Mycoplasma* infection, since anti-GalC previously had been associated with *Mycoplasma* CNS disease; 8 of 21 had CSF anti-GalC antibody found, indicating that this is not a specific marker for *Mycoplasma* CNS disease (or that *Mycoplasma* was not the cause of CNS disease). At present it is not clear whether and how *Mycoplasma* is associated with CNS disease in children. Limitations of nonspecificity of the serum IgM *Mycoplasma* antibody test and common occurrence of subclinical *Mycoplasma* infection in children raise doubt about this association. There is no evidence that treatment for *Mycoplasma* infection alters the course of CNS-associated symptoms.

Other large-scale investigations of causes of acute central nervous system infection have been reported, none as intensely investigated as in the CEP. Two recent studies were reported in 2004 [25] and 2007 [26] from upstate New York and Helsinki, respectively. Huang et al. [25] report a 6-year study of CSF or brain tissue samples submitted to the Clinical Virology Laboratory at the New York

State Department of Health from 3,465 patients with encephalitis or meningitis of unknown etiology. PCR and RT-PCR were performed for 12 viruses. For the 1,584 patients who met CEP-like criteria for acute encephalitis, 7.8% had a virus identified. Ranking of agents was identical to CEP ranking: enteroviruses, followed by HSV, VZV, and WNV [25]. Huttunen et al. [26] report a prospective 3-year study of 213 children admitted to the Hospital for Children and Adolescents, Helsinki University Hospital with suspicion of CNS infection. Extensive culture, PCR, and serologic testing were performed. Sixteen patients had encephalitis; most frequently confirmed etiologies were enteroviruses, human herpesvirus 6, and VZV. Additionally, nine patients had neuroborreliosis.

Although nonspecific neurologic symptom can be associated with any etiologic agent, specific neurologic findings are more commonly associated with certain pathogens. Examples include acute flaccid paralysis with enterovirus, tick-borne encephalitis virus, and WNV; seizures with HSV, La Crosse virus, Eastern equine encephalitis virus, and *B. henselae*; focal temporal abnormalities with HSV, VZV, and *Mycobacterium tuberculosis*; cerebellar abnormalities with Epstein–Barr virus, *Listeria monocytogenes*, and non-infectious etiologies; and hydrocephalus with bacteria, parasites, and thromboses. In the CEP, etiology was identified in approximately 75% of patients who had hydrocephalus, 63% who had focal temporal signs, 31% who had cerebellar signs, 64% who had seizure(s) with rapid recovery, and only 27% who had intractable seizures (a neurologic course with poor outcome more common in the pediatric age group than in adults in this study [21]).

1.6 Additional Noteworthy Diagnoses

1.6.1 Acute Disseminated Encephalomyelitis (ADEM)

Post-infectious encephalomyelitis was a term used when neurologic signs and symptoms followed a known infection (e.g., measles and varicella) or immunization (e.g., rabies vaccine made in neural tissue) [27]. There is no direct infection of neural cells, but there is widespread perivenular inflammation and demyelination localized in the white matter of the brain. PIEM is now rare. ADEM is PIEM, with similar neurologic signs and symptoms, with demyelination evident on MRI, usually following a nonspecific illness. A variety of viruses and vaccines have been temporally associated in case reports of ADEM. ADEM by definition has all of the following components: acute onset over days of a monophasic, multifocal, inflammatory, and demyelinating autoimmune CNS disorder [28]. It is not rare. In the CEP, 120 (9%) patients had multifocal white matter lesions; 23 (19%) of these patients had associated viral illnesses (enteroviruses, adenovirus, and influenza >2 cases each and 9 other viruses 1–2 cases each) and 14 (12%) had associated non-viral infections (*M. pneumoniae* in 6 cases). The remaining 7 patients (6%) had non-infectious etiologies (including malignancies and multiple sclerosis) and 63% had no inciting infection or associated condition found.

Table 11 Clinical and neuroimaging findings in 360 cases of acute disseminated encephalomyelitis[a]

Clinical finding		*Initial MRI site of lesions*	
Fever	48%	Bilateral	96%
Headache	36%	White matter	93%
Nausea/vomiting	32%	Brainstem/cerebellum	43%
Stiff neck	25%	Thalamus	27%
Neurologic finding		Basal ganglion	23%
Altered consciousness	63%	Spinal cord	36%
Motor signs	62%	*General MRI finding*	
Ataxia	38%	Gadolinum enhancement	26%
Cranial neuropathy	34%	Mass effect	16%
Seizures	29%	Hemorrhage	<1%
Visual loss	21%	*CSF Finding*	
Language impairment	20%	Mean white blood cells (WBCs)	49/mm^3
Sensory abnormality	6%	Mean protein	58 mg/dL
Movement disorder	5%	Oligoclonal bands present	6%
Peripheral nerve abnormality	0	CSF myelin basic protein positive	11%

Modified from Davis and Booss [29].
[a]Percent is percent of patients; for CSF, not all patients had all studies performed. CSF WBCs were predominantly mononuclear.

Davis and Booss reviewed 360 pediatric cases of ADEM [29]. Median age was 6.7 years (rare < 1 year), 60% were boys, >50% had history of preceding illness or immunization. Clinical, imaging, and CSF findings are shown in Table 11. Patients can come to medical attention acutely ill with fever, nausea, and vomiting or because of neurologic changes without systemic illness. In our experience, motor signs (weakness with spasticity or diminished reflexes) are present frequently with impaired attention and "executive" function and/or with limbic signs (autonomic dysregulation, hallucinations, and behavioral changes), which frequently are out of proportion to global depression of sensorium. MRI shows specific abnormalities with bilateral, usually asymmetric white matter lesions (lesions present in the thalamus and basal ganglion can be symmetric). ADEM is a clinical diagnosis with compatible MRI findings.

The pathogenesis of ADEM is thought to be molecular mimicry of microbes with myelin proteins. Theoretically, T lymphocytes respond to the microbe and by mimicry to myelin protein. Antigen-specific T or B lymphocytes or both gain access to the central nervous system, or antigen-presenting microglial cells encounter homologue myelin protein. Perivenulitis and demyelination ensue. Host-specific immune dysregulation rather than certain infectious agents has been speculated as possibly causative. No pathogen, RNA, or DNA is expected to be found in the CNS. ADEM must be differentiated from multiple sclerosis (MS). Diagnosing MS requires "dissemination in time and space," meaning affecting multiple areas of demyelination in a polyphasic course. Concerning factors for MS in patients with first episode, presumed ADEM are age > 12 years, optic neuritis, or involvement of the spinal cord [29]. New symptoms or signs within 3 months of diagnosis of

ADEM (and especially if associated with weaning of corticosteroid) are considered as part of the same ADEM event and not as fulfilling criteria for MS [28]. A recent review of ADEM, especially as differentiated from MS is a valuable resource [28].

Diagnosis is important as empiric corticosteroid therapy is first-line therapy and decreases time to recovery [30, 31]. Bilateral asymmetric white matter lesions are the sine qua non of ADEM. There can be a lag of days to weeks in MRI findings in some patients as well as a "mismatch" in other patients who have remarkable MRI findings and relatively few neurologic abnormalities. CSF usually is abnormal, but nonspecifically and frequently mildly (Table 9).

Therapy for ADEM has evolved from case reports and series rather than randomized, blinded placebo-controlled trials, but evidence of effect of immunosuppressive therapy is compelling – sudden remarkable improvement in some patients, decreased signs of CNS inflammation, decreased time to recovery, and relapses on weaning [28, 29]. Intravenous corticosteroid in doses of 20–30 mg/kg/day (maximum 1 g) for 3–5 days often results in dramatic clinical improvement. Oral prednisone at a dose of 1 mg/kg/day is then tapered over 14–21 days. Duration of initial and tapering doses has been lengthened after experience of frequent relapses after 5-day treatment/10-day taper regimen [28]. Immunoglobulin intravenously (IGIV) usually is given if corticosteroid response is not clinically remarkable; dose is 2 g/kg divided over 2–5 days. Plasma exchange usually is third-line therapy; it has been subject only of case reports, in which benefits appear to approximate that of IGIV.

1.6.2 Acute Necrotizing Encephalopathy (ANE)

Acute necrotizing encephalopathy is less frequent than ADEM. ANE is a distinct form of acute encephalopathy triggered by respiratory tract infections, especially viral infections such as influenza A and B [32]. First described as a novel disease by Mizuyuchi in 1995, the condition has been reported mainly from Japan, although increasingly ANE is recognized in multiple countries [33]. It is not a rare encounter among pediatric infectious diseases subspecialists in the United States. Affected children usually are 5 months to 10 years of age, previously healthy, who in the peak of a febrile respiratory tract illness have acute onset of seizures or changes in mental status or both; global suppression ranges from obtundation to coma. Specific neurologic abnormalities are not universal, but speech abnormalities and akinetic mutism as well as delirious behavior [34] may occur more commonly in ANE than in acute infectious encephalitis or ADEM. CSF is acellular, glucose concentration is normal, and protein concentration typically is normal or mildly elevated. Serum hepatic enzymes may be elevated, severity of which has been associated with poor prognosis in Japanese children. The hallmark of ANE is the MRI findings on T2-weighted images of high intensity signal diffusely and symmetrically in the periventricular white matter – characteristically affecting the thalamus, internal capsule, cerebellum, and brainstem in some cases. Changes are seen within 12 h after onset of coma.

Pathogenesis is thought to be cytokine dysregulation localized to the CNS. Autopsy of fatal cases shows apoptosis of neurons, with edema but no inflammatory infiltrate or vasculitis. No evidence of pathogen entry into the CNS is evident by culture, PCR, antibody test, or in situ hybridization tests on tissue. Possible role of non-steroidal anti-inflammatory medication in precipitation of ANE has been suggested. The same clinical syndrome following infection with different viruses also is compatible with predisposition due to host factors. Recent data suggest that some cases of ANE represent a familiar disorder or may be the result of an inherited predisposition due to a mutation in the *RANBP*2 gene [33].

ANE can be fatal, and outcome of survivors generally is poor. Efficacy of therapies has been anecdotal or evaluated only retrospectively in case series [35]. In such a series of 34 children with ANE, corticosteroid therapy within 24 h of onset was correlated with improved outcome (good outcome in 7 of 12 treated patients compared with none of 5 untreated patients). Children with brainstem involvement had poor outcome regardless of corticosteroid therapy. Intravenous administration of immunoglobulin (IGIV) had no apparent effect on the clinical course in this series.

1.7 Additional Noteworthy Pathogens

1.7.1 *B. burgdorferi*

B. burgdorferi is the most common tick-borne infection in the northeastern United States. Spirochetes can invade the central nervous system and cause subacute aseptic meningitis, acute facial nerve palsy, or other cranial nerve palsies. A prospective study of 177 children evaluated for neuroborreliosis in Sweden showed frequent fatigue, headache, fever, neck pain, and facial nerve palsy in confirmed cases [36]. Encephalopathy was not described in this series, 118 of whom had confirmed or probable Lyme disease. *B. burgdorferi* was not found in the CEP [18], not tested for in the New York state encephalitis study [25], and was identified in the Helsinki study in children with meningitis (not encephalitis) and facial nerve palsy [26]. Lyme encephalitis with altered consciousness, therefore, must be a rare disease. A useful clinical prediction model for Lyme meningitis, however, was proposed recently from clinical study of 50 children from a Lyme endemic area in Rhode Island [37]. The so-called rule of 7s would predict a < 10% probability of CNS Lyme disease if headache was present for < 7 days, cranial nerve 7 palsy was absent, and CSF had < 70 mononuclear cells/mm^3.

1.7.2 Parvovirus B19

A retrospective report of 81 cases of neurologic disease, including encephalitis, meningitis, stroke, and peripheral neuropathy, was published recently from an English and French literature review [38]. One-third of patients had altered immune status. Two-thirds of patients were < 18 years of age. Encephalitis, stroke, and seizures were more common heralding manifestations in children than in adults;

peripheral neuropathy (brachial plexus palsy and carpal tunnel syndrome) occurred in adults. Overall, rash was reported in 36% of patients. CSF was normal or showed modest mononuclear cell pleocytosis and elevated protein concentration. Diagnosis was confirmed by PCR testing on CSF sample (81%), serum (85%), or by specific antibody testing on CSF (33%). The methods of PCR and antibody testing were not reviewed. This review raises parvovirus B19 into considerations among causes of encephalitis, especially in children. It is noteworthy that in the CEP testing for parvovirus was performed only when rash or anemia was present (15 instances); no case of confirmed parvovirus etiology was reported [18]. Parvovirus B19 serology was performed in patients in the Helsinki study.

1.7.3 *Balamuthia mandrillaris*

In the >3,500 cases referred to the CEP, 10 patients (5 cases < 18 years of age) were found to have amebic encephalitis due to the free-living ameba *B. mandrillaris*. Presentations were nonspecific – headache, seizures, cranial nerve palsies, and lethargy – and would have been described as "encephalitis of unknown etiology" except for the CEP protocol. Nine cases were fatal. Neuroimaging was abnormal in all, with ringed enhancing focal lesions or hydrocephalitis or both [22].

1.8 Specific Clinical Neurologic Syndromes

Certain cardinal neurologic findings in patients with encephalitis make specific etiologic agents more likely to be confirmed. Table 8 shows some examples – from author's experience, case reports, and the CEP results. In the CEP, likelihood of confirming a cause varied substantially by neurologic finding: 75% confirmation for cases with hydrocephalus, 63% for focal temporal lobe signs, 31% for cerebellar signs, and only 28% for status epilepticus [18]. Acute flaccid paralysis cases were not tallied as such. The most important clinical conclusions from these data are that hydrocephalus should not be thought to be due to viral encephalitis or post-infectious ADEM without exclusion of treatable, more likely causes, e.g., bacterial, fungal, or parasitic infections or thromboses.

2 Approach to Empiric Management

Predicting precise etiologic agent of encephalitis in an individual case is extremely difficult. Empiric therapy is urgently necessary for the minority of infections that are treatable as outcome is dependent on severity of neurologic findings at commencement of effective therapy. Approach to choosing agents for empiric therapy can be based on consideration of categories of treatable infectious agents: common bacteria – *Streptococcus pneumoniae*, *H. influenzae*, *N. meningitidis*, and in the case of focal brain or parameningeal lesions, viridans *Streptococcus* and anaerobic bacteria, or

Staphylococcus aureus; uncommon bacteria – *L. monocytogenes* (which has propensity among bacteria to cause an encephalitis syndrome), *B. burgdorferi*; treatable virus – herpes simplex virus; mycobacteria – *M. tuberculosis*; fungi – *Cryptococcus neoformans*, other fungi in immunocompromised hosts; rickettsia – *Rickettsia rickettsiae, Ehrlichia*, and *Anaplasma* species; parasites and ameba – *Baylisascaris procyonis*, agents of neurocysticercosis, *Balamuthia* species, *Acanthamoeba*, and *Naegleria fowleri*.

Simple laboratory tests – complete blood count, sedimentation rate, C-reactive protein, serum chemistry panel – which frequently are helpful in categorizing systemic infectious diseases, are not discriminating for encephalitis. Cerebrospinal fluid analysis and MRI are the cornerstones for choosing empiric therapy and for pursuing confirmation of etiology to plan definitive therapy. It is noteworthy with regard to HSV encephalitis that first sampling of CSF may show negative PCR test results. When the unfolding clinical course is compatible with HSV encephalitis, it is our practice to continue acyclovir until plasma and a second CSF PCR test are negative. Clinical practice guidelines for management of patients with encephalitis have been published recently by the Infectious Diseases Society of America [39].

Figure 2 delineates the author's strategy for empiric therapy based on analysis of cerebrospinal fluid (CSF). Opening pressure is measured; increased intracranial pressure suggests that bacteria, mycobacteria, rickettsia, fungi, or parasites are

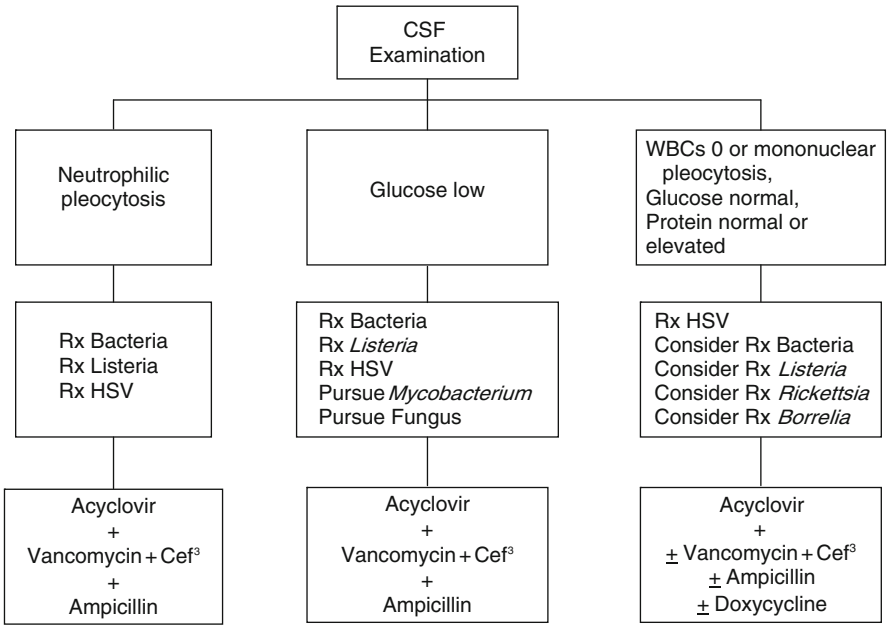

Fig. 2 Schematic depicts a strategy for considering empiric anti-infective therapies for children with acute encephalitis-like syndromes depending on CSF findings. Mtb, *M. tuberculosis*; Cef^3, third-generation cephalosporin

more likely etiologies than are viruses. Presence of eosinophils would cue pursuit of parasites such as *B. procyonis* [40] and *Angiostrongylus* and *Gnathostoma* species [41]. Pleocytosis predominantly comprised of neutrophils leads to empiric treatment for common meningitis pathogens – *S. pneumoniae*, *H. influenzae*, *N. meningitidis*, *L. monocytogenes*, and herpes simplex virus. Although HSV is not equally likely as causative, therapy should include high-dose acyclovir (60 mg/kg/day for neonates to 2- to 3-year-old children; 30 mg/kg/day for older children) for a patient with encephalitis regardless of age and CSF findings. Vancomycin plus a third-generation cephalosporin (usually cefotaxime or ceftriaxone) are given for usual pathogens of meningitis, which occasionally cause meningoencephalitis. Because *L. monocytogenes* is considered as having propensity among bacteria to cause encephalitis, ampicillin usually is given. Antibiotic therapy generally is discontinued when cultures of CSF and blood are negative, and imaging does not suggest an abscess.

Hypoglycorrhachia should lead to increased suspicion of bacteria, including *Listeria* and can occur with HSV encephalitis (especially when necrotizing). Empiric therapy should include therapy for these pathogens. Because hypoglycorachia (especially in the presence of modest mononuclear pleocytosis) is typical of mycobacterial and fungal CNS infection, these diagnoses should be considered specifically and pursued depending on the setting and likelihood of another diagnosis.

Patients with encephalitis-like syndromes with normal CSF or nonspecifically abnormal CSF, e.g., with mononuclear pleocytosis or elevated protein concentration, also should be treated for HSV infection. Treatments for bacterial causes, rickettsia, and neuroborreliosis are considered based on other findings, such as season and exposures for rickettsia and *Borrelia*; or rash, hyponatremia, thrombocytopenia for rickettsia.

Tests performed on CSF always will include Gram stain and culture, PCR test for HSV and enterovirus. Decisions to perform CSF PCR or IgM antibody test for *Borrelia*, WNV, and other herpesviruses; CSF antigen test for *Cryptococcus*; or serum antibody tests for other viruses, rickettsia, etc. depend on poor clinical "fit" for expected pathogens or specific clues in exposure history, clinical, or laboratory findings that raise suspicion for unusual pathogens. Table 12 may help focus testing.

Neuroimaging, especially MRI, has added immeasurably to management and to diagnostic approach in patients with encephalitis syndromes. Specificity of MRI for diagnosis is beyond the scope of this paper. Basic categorization of findings is useful: normal, predominant gray vs. white matter lesion(s), edema/hydrocephalus, focal vs. multifocal site, symmetric vs. asymmetric distribution, or specific abnormality, such as mass, abscess, calcification, ring lesion, hemorrhage, or meningeal enhancement. Empiric therapy is not limited because of MRI findings but certain findings trigger additional investigations or therapies. A few examples are edema/hydrocephalus for bacteria and fungi; asymmetric white matter abnormalities for ADEM; bilateral, symmetrical increased T2-weighted signal in perivascular deep white matter for ANE; and ring lesions for parasitosis. In patients with

Table 12 Focusing early tests for infectious agents of acute encephalitis

Should perform	Should consider
CSF	*CSF*
Gram stain and culture	*Borrelia* IgM antibody
HSV PCR	WNV IgM antibody
Enterovirus PCR	VZV IgM antibody
VZV PCR	*Serum*
Other sampling sites	EBV antibody
Virus culture/antigen tests	Encephalitis virus IgM antibodies
PCRs as appropriate on NP, stool, ± plasma	*Borrelia* antibody
	Rickettsia, etc. IgM antibody
	Freeze for additional/convalescent testing

HSV, herpes simplex virus; VZV, varicella zoster virus; PCR, polymerase chain reaction.

normal MRI at presentation whose condition deteriorates or whose diagnosis is unclear, repeated MRI can enlighten next steps in diagnosis and management. Electroencephalopathy, likewise, can show specific abnormalities such as sometimes can be seen in HSV encephalitis or subacute sclerosing panencephalitis or can confirm subclinical seizures, but mostly commonly is nonspecifically abnormal in acute encephalitis of multiple etiologies.

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Toxic Shock Syndrome – Evolution of an Emerging Disease

James K. Todd

1 Introduction

Toxic shock syndrome (TSS) was originally described in 1978 in seven patients: four females and three males who presented acutely with fever, hypotension, mucous membrane hyperemia, a scarlatiniform rash (that ultimately desquamated), and multiple organ system failure [1]. A unique *Staphylococcus aureus* strain, which produced a new epidermal toxin, was isolated from five of the children. By January 1980, 35 cases had been observed, of which 25 were in females with an average age of 28.2 years; only 10 were in males with an average age of 11.6 years [2]. Seven of 10 males had an identifiable focus of *S. aureus* infection, whereas 20 of 22 females had a watery vaginal discharge from which *S. aureus* was isolated. Shortly thereafter, cases of TSS were further associated with menstruation and the use of tampons. In September 1980 a particular tampon was linked by case–control study to TSS and was removed from the market [3]; however, cases of both menstrual and nonmenstrual TSS have continued to occur to this day and this syndrome has in fact expanded in its microbial and clinical spectrum of disease [4–6].

2 Epidemiology

A number of studies suggested that the incidence of TSS decreased after 1980 but that remains controversial [7, 8]. In Colorado, almost 25% of infectious shock cases can still be attributed to Gram-positive organisms and only 25% of cases are reported to authorities [4, 5]. Several active case ascertainment studies have suggested that the incidence has remained rather constant in females of menstruating age at the rate of one case per hundred thousand menstruating females at risk per year [4–6]. It is clear that young menstruating females are at higher risk of TSS but it is also clear (as manifest in its original description) that boys and older adults of either sex are also at risk [9, 10].

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3 Pathogenesis

Toxic shock syndrome toxin (TSST-1) was shown to be associated with TSS in menstruating females and additional staphylococcal strains producing one of a number of enterotoxins have also been associated with TSS due to focal infections in both males and females [11, 12]. These toxins act as superantigens which stimulate a bloom of V-beta subsets of lymphocytes that release large amounts of cytokines which in turn mediate capillary leakage resulting in the signs and symptoms of the disease [13]. At-risk patients have no pre-existing antibody to these toxins, whereas those who do have antibody seem to be protected [14, 15]. In addition, TSST-1-producing *S. aureus* strains have been shown to produce sufficient amounts of toxin only when growing under favorable growth conditions that include a neutral pH, high protein concentration, and high oxygen and carbon dioxide concentrations [2]. This may explain why the majority of adults seem to have antibody to TSST-1 in the absence of any history of actual disease; they have likely encountered the organism by nasal colonization which results in exposure to very low amounts of immunizing toxin. Even in the presence of all of these risk factors, it appears that host response is an important element of additional risk as certain patients seem to be genetically vulnerable to manifest an exaggerated hypotensive response to the toxin.

Figure 1 summarizes the current understanding of the pathogenesis of toxic shock syndrome due to *S. aureus*. A similar mechanism can be invoked for streptococcal toxic shock syndrome which has increased in its incidence over the past 20 years and appears to be caused by similar superantigen toxins: the pyrogenic exotoxins [16].

4 Clinical Findings

The clinical definitions for these two syndromes are shown in Tables 1 and 2. It should be emphasized that these definitions are, of necessity, quite strict and in all probability significantly underestimate the incidence of less severe disease (e.g., streptococcal or staphylococcal scarlet fever) [17].

At the time of initial presentation, it is often not possible to clinically distinguish staphylococcal TSS from streptococcal TSS. Fortunately, except for the antibiotic selection, management is similar. It is usually the case that an enabling focus of infection (e.g., menstruation plus tampon use, abscess) can be found in staphylococcal TSS, which is important because patients often do not improve unless it is adequately drained. This may be occult, a common site being sinus infection, which should be investigated if no other focus is found [18, 19]. Streptococcal TSS is more often associated with bacteremia or cellulitis/necrotizing fasciitis associated with *Streptococcus pyogenes* (group A *Streptococcus*) [16]. An interesting variant of TSS called neonatal toxic shock syndrome-like exanthematous disease (NTED) has been described in neonates in Japan associated with unique TSST-1-producing *S. aureus* strains [20]. The difference in epidemiological and clinical presentation may reflect differences in both host and organism genetics.

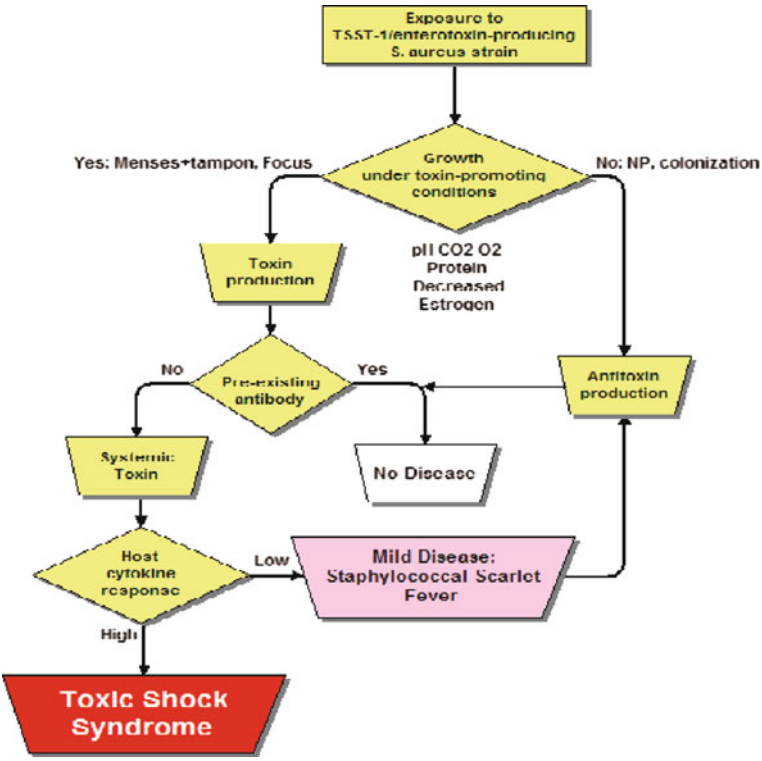

Fig. 1 Pathogenesis of staphylococcal toxic shock syndrome

Toxic shock syndrome should be suspected in febrile patients who have any combination of shock, scarlatiniform rash, strawberry tongue, focus of likely staphylococcal or streptococcal infection, or menstruation with tampon use [21]. Blood and focus should be cultured (preferably with a Gram stain to guide initial therapy). Until a causative organism is identified, initial antibiotic therapy should include an appropriate antistaphylococcal antibiotic [appropriate for methicillin-resistant *S. aureus* (MRSA) if prevalent in the community] and clindamycin. The addition of clindamycin is supported by in vitro, animal, and clinical studies that show that these toxigenic organisms may release more toxin in the presence of a β-lactam antibiotic and release less with better attendant outcomes if a protein synthesis inhibitor like clindamycin is used (the "Eagle" effect) [22, 23].

5 Management

Aggressive fluid management for hypotension is indicated and should account for ongoing second and third space losses due to capillary leakage which often necessitates much higher volumes of fluid than initially might be calculated [24, 25].

Table 1 Clinical case definition of staphylococcal toxic shock syndrome (http://www.cdc.gov/ncphi/disss/nndss/casedef/toxicsscurrent.htm)

Clinical features
- *Fever*: temperature greater than or equal to 102.0°F (38.9°C)
- *Rash*: diffuse macular erythroderma
- *Desquamation*: 1–2 weeks after onset of illness, particularly on the palms and soles
- *Hypotension*: systolic blood pressure less than or equal to 90 mmHg for adults or less than fifth percentile by age for children aged less than 16 years; orthostatic drop in diastolic blood pressure greater than or equal to 15 mmHg from lying to sitting, orthostatic syncope, or orthostatic dizziness
- *Multisystem involvement* (three or more of the following):
  - *Gastrointestinal*: vomiting or diarrhea at onset of illness
  - *Muscular*: severe myalgia or creatine phosphokinase level at least twice the upper limit of normal
  - *Mucous membrane*: vaginal, oropharyngeal, or conjunctival hyperemia
  - *Renal*: blood urea nitrogen or creatinine at least twice the upper limit of normal for laboratory or urinary sediment with pyuria (greater than or equal to five leukocytes per high-power field) in the absence of urinary tract infection
  - *Hepatic*: total bilirubin, alanine aminotransferase enzyme, or asparate aminotransferase enzyme levels at least twice the upper limit of normal for laboratory
  - *Hematologic*: platelets less than 100,000/mm^3
  - *Central nervous system*: disorientation or alterations in consciousness without focal neurologic signs when fever and hypotension are absent

Laboratory criteria
- *Negative results on the following tests, if obtained*:
  - Blood, throat, or cerebrospinal fluid cultures (blood culture may be positive for *S. aureus*)
  - Rise in titer to Rocky Mountain spotted fever, leptospirosis, or measles

Case classification

Probable: a case which meets the laboratory criteria and in which four of the five clinical features are present

Confirmed: a case which meets the laboratory criteria and in which all five of the clinical features are present, including desquamation, unless the patient dies before desquamation occurs

Intravenous immunoglobulin and possibly corticosteroids may be useful in severe cases or those not responding to initial management [24, 26]. Drainage or debridement of a focus of infection (e.g., abscess, necrotizing fasciitis) is essential as a small amount of residual toxin can perpetuate shock [27, 28].

6 Prognosis

Most patients with TSS will survive with early diagnosis and appropriate management. Currently, the fatality rate is less than 5%. Patients in the convalescent phase may experience peeling of the finger and toe tips, hands, and feet with some transient hair loss but generally will return to a normal state of activity and health.

Staphylococcal TSS associated with menstruation and tampon use can recur if the patient is not adequately treated with antistaphylococcal antibiotics and/or tampons are used during the next five menstrual periods [21, 29]. Although the duration

Table 2 Case definition of streptococcal toxic shock syndrome (http://www.cdc.gov/ncphi/disss/nndss/casedef/streptococcalcurrent.htm)

An illness with the following clinical manifestations occurring within the first 48 h of hospitalization or, for a nosocomial case, within the first 48 h of illness:

- *Hypotension* defined by a systolic blood pressure less than or equal to 90 mmHg for adults or less than the fifth percentile by age for children aged less than 16 years
- *Multi-organ involvement characterized by two or more of the following:*
  - *Renal impairment*: creatinine greater than or equal to 2 mg/dL (177 μmol/L) for adults or greater than or equal to twice the upper limit of normal for age. In patients with pre-existing renal disease, a greater than twofold elevation over the baseline level
  - *Coagulopathy*: platelets less than or equal to 100,000/mm^3 (100×10^6/L) or disseminated intravascular coagulation, defined by prolonged clotting times, low fibrinogen level, and the presence of fibrin degradation products
  - *Liver involvement*: alanine aminotransferase, aspartate aminotransferase, or total bilirubin levels greater than or equal to twice the upper limit of normal for the patient's age. In patients with pre-existing liver disease, a greater than twofold increase over the baseline level
  - *Acute respiratory distress syndrome*: defined by acute onset of diffuse pulmonary infiltrates and hypoxemia in the absence of cardiac failure or by evidence of diffuse capillary leak manifested by acute onset of generalized edema, or pleural or peritoneal effusions with hypoalbuminemia
  - A generalized erythematous macular *rash* that may desquamate
  - *Soft-tissue necrosis*, including necrotizing fasciitis or myositis, or gangrene

Laboratory criteria for diagnosis

- Isolation of group *A Streptococcus*

Case classification

- *Probable*: a case that meets the clinical case definition in the absence of another identified etiology for the illness and with isolation of group A *Streptococcus* from a nonsterile site
- *Confirmed*: a case that meets the clinical case definition and with isolation of group A *Streptococcus* from a normally sterile site (e.g., blood or cerebrospinal fluid or, less commonly, joint, pleural, or pericardial fluid)

of tampon usage has not been shown to be causative in menstrual TSS, it may still be related to the severity of symptom, e.g., the longer a sick patient leaves a tampon in place, the more severe are the symptoms. This has resulted in warnings on tampon packages to change tampons frequently and at least every 8 h. Women who are menstruating and using tampons who develop any of the above mentioned symptoms (e.g., fever, nausea, vomiting, rash, muscle-aches, dizziness, or fainting) should remove any tampon and seek medical attention immediately. It would appear that such warnings have resulted in a better informed tampon-using public with a resulting lower severity of illness and fatality rate [5].

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Dissection of B-Cell Development to Unravel Defects in Patients with a Primary Antibody Deficiency

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1 Introduction

The cells of the adaptive immune response (B and T lymphocytes) are powerful players in the immune system. Each lymphocyte creates a unique receptor for recognition of pathogens during precursor differentiation in bone marrow or thymus. Together, this results in a large repertoire of antigen receptors with the potential to recognize many different pathogens specifically. On top of this broad repertoire, the lymphocytes that actually recognize antigen are capable of undergoing enormous clonal proliferation, thereby generating huge numbers of daughter cells with the potential to recognize the same pathogen. This clonal expansion generates effector cells for a strong response and long-term memory in the form of memory B and T cells and immunoglobulin (Ig)-producing plasma cells. The host requires a highly dynamic immune system, which maintains a tight balance between the production of a large repertoire of cells with unique receptors and a strong immune response of groups of cells with a highly specific and thereby a more limited (selected) repertoire.

In this chapter, we provide a brief overview of B-cell differentiation and we place the currently defined antibody deficiency syndromes in this context. Further, we address important issues and considerations in the diagnostic work-up of patients suffering from a primary antibody deficiency.

2 Generation of Naïve Mature B Cells by Stepwise Differentiation in Bone Marrow

Precursor B cells are generated from hematopoietic stem cells in the bone marrow, where they undergo stepwise differentiation independent from antigen (Fig. 1). Their main objective is to create a unique B-cell antigen receptor (BCR), which is

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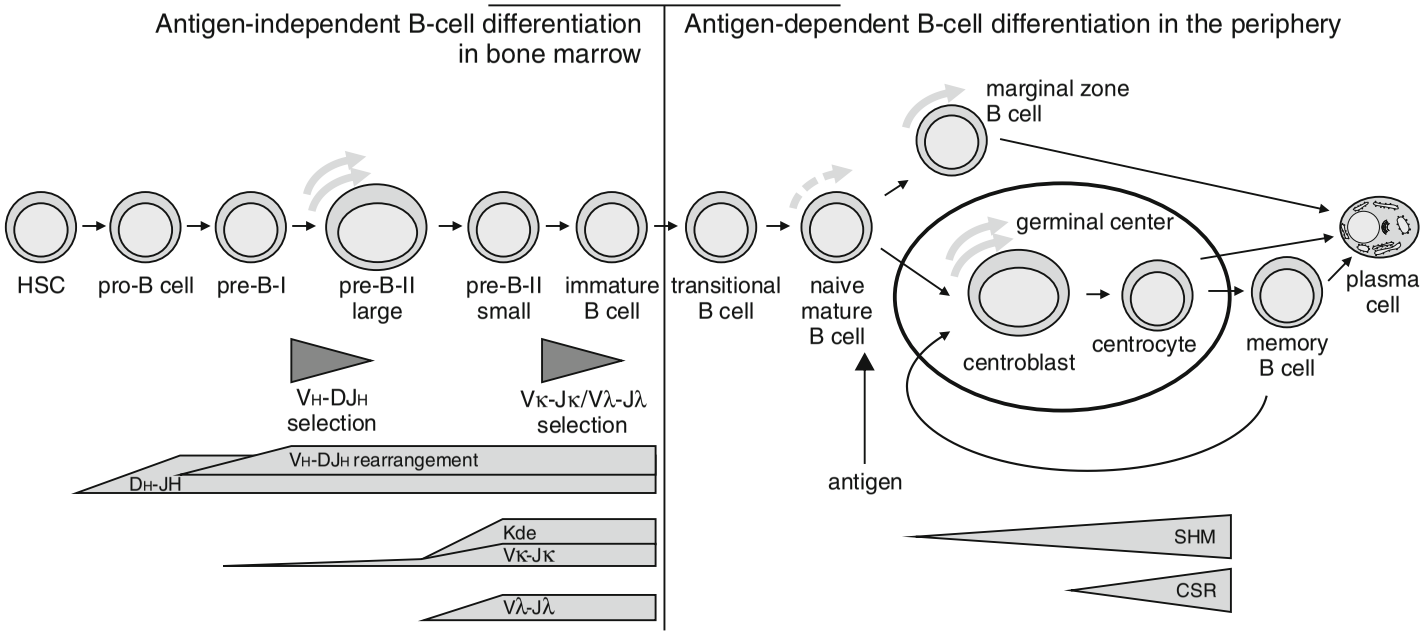

Fig. 1 Molecular processes during the stepwise development of B cells from hematopoietic stem cells (HSC) to memory B cells and plasma cells. The Ig gene rearrangements and the selection of their functionality in the bone marrow compartment, followed by antigen-induced proliferation and selection processes in the periphery represent a highly dynamic cascade of events, which require a tight balance between the antigen-independent and antigen-dependent B-cell differentiation stages. SHM, somatic hypermutation; CSR, class switch recombination; Kde, kappa deleting element

composed of two Ig heavy chains (IgH) and Ig light chains (Igκ or Igλ). The genes encoding these components are subjected to genomic rearrangement, V(D)J recombination, to form functional proteins. In the *IGH* locus, one variable, one diversity, and one joining gene segment are randomly combined to form a functional exon. Similar rearrangements are initiated between one V and one J gene segment in the *IGK* and *IGL* loci. The rearrangement process is accompanied by deletion and random insertion of nucleotides at the ends of V, D, and J gene segments resulting in unique junctions. The combination of V, (D), and J gene segments and the processing of junctional regions contribute enormously to the BCR diversity between precursor B cells.

Ig gene rearrangements are initiated in pro-B cells at the *IGH* locus with D to J rearrangements, followed by V to DJ rearrangements in the pre-B-I cell stage [1]. Upon formation of a functional *IGH* gene rearrangement, an Igμ chain is expressed together with surrogate light chain proteins VpreB and λ14.1 as a pre-BCR (Fig. 2). The cells are now identified as large pre-B-II cells in which proliferation is induced by expression of the pre-BCR, which signals via the CD79 complex and a network of downstream kinases and linker proteins (Fig. 2) [2]. This clonal expansion phase is followed by G1 arrest, during which the surrogate light chain is down-regulated and the rearrangement process is continued at the Ig light chain loci (*IGK* followed by *IGL* in small pre-B-II cells). At the immature B-cell stage, the complete BCR is tested for functionality without high affinity for autoantigens, after which the cell can migrate to the periphery as a transitional B cell (Fig. 1). The continuous production of B cells in bone marrow ensures a high BCR diversity in the naïve B-cell pool.

3 Antigen-Dependent B-Cell Maturation in Secondary Lymphoid Organs

Transitional B cells are immature in their migration capacity and response to antigen, but develop rapidly into naïve mature B cells, which form the bulk of B lymphocytes in peripheral blood. Naïve mature B cells are thought to be short-lived unless they are activated upon antigen encounter with their specific BCR. Upon binding to its cognate antigen, the BCR induces downstream signaling using the same pathways as the pre-BCR, to initiate a Ca^{2+} flux and target gene transcription (Fig. 2). The CD19-complex, consisting of CD19, CD21, CD81, and CD225, is necessary for sufficiently strong signaling of the BCR (Fig. 3) [3–5]. Specifically, signaling molecules are recruited upon phosphorylation of multiple tyrosine residues in the intracellular tail of CD19.

The B-cell antigen response depends greatly on the strength of the BCR–antigen interaction and the presence of costimulatory signals. Cognate CD4+ THELPER(H) cell help results in the strongest humoral response in lymph nodes and other secondary lymphoid organs. Upon CD40L–CD40 interaction (Fig. 4), the activated B cells undergo extensive proliferation and form highly organized structures: germinal centers. In germinal centers, a dark and a light zone can be identified. The

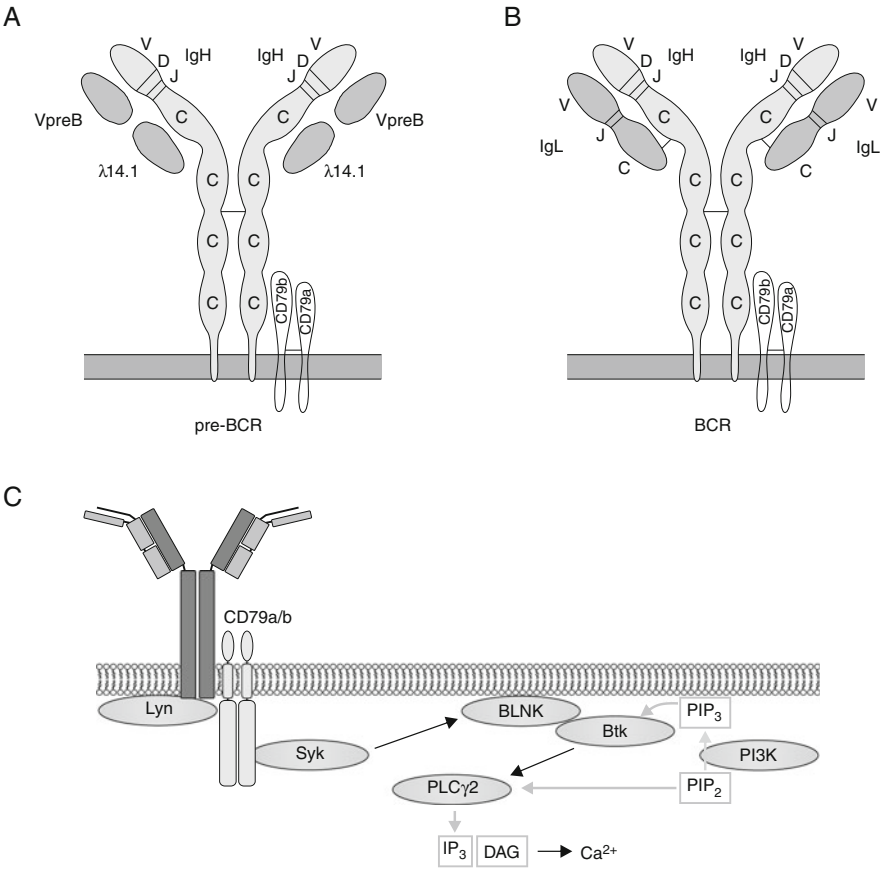

Fig. 2 Pre-BCR and BCR signaling complexes. **a** pre-BCR complex. **b** BCR complex. **c** Schematic representation of important kinases (lyn and syk) and linker proteins involved in downstream signaling from the pre-BCR. BLNK, B-cell linker protein; Btk, B cell or Bruton's tyrosine kinase; PIP_2 and PIP_3, phosphatidylinositol (4,5)-bisphosphate/phosphatidylinositol (3,4,5)-trisphosphate; PI3K, phosphoinositide 3-kinase; IP3, inositol triphosphate; DAG, diacylglycerol; PLCγ2, 1-phosphatidylinositol-4,5-bisphosphate phosphodiesterase gamma-2

dark zone mainly consists of proliferating B cells (centroblasts) in a network of follicular dendritic cells. These cells are from stromal origin and present complete, unprocessed antigen via Fc and complement receptors to stimulate proliferation and survival of antigen-specific B cells. The proliferating centroblasts induce somatic hypermutation (SHM) in their Ig genes, which changes their affinity for antigen. The centroblasts become resting centrocytes and can undergo Ig class switch recombination (CSR) and selection based on high affinity for antigen in the light zone of the germinal center (Fig. 1).

The CD40–CD40L interaction induces translocation of NF-κB to the nucleus, where it activates transcription of target genes, including the gene that encodes activation-induced cytidine deaminase (AID) (Fig. 4). AID deaminates cytidine

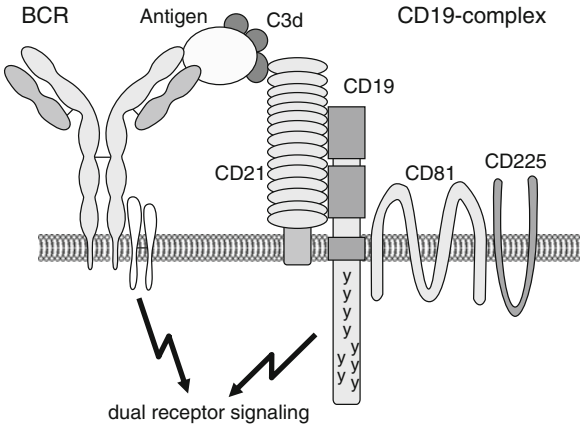

Fig. 3 Schematic representation of "dual receptor signaling" upon binding of antigen to the B-cell receptor (BCR) and the CD19 complexes. In this signaling process, the CD19 complex functions to decrease the threshold for BCR signaling upon antigen binding

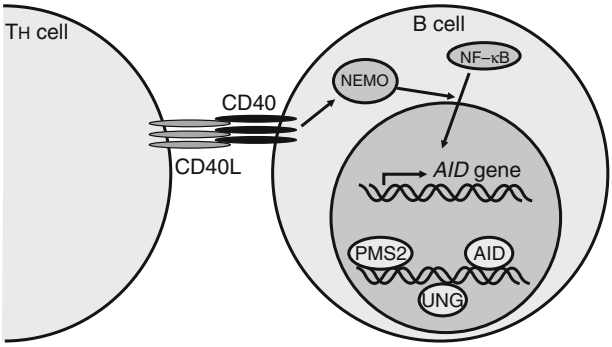

Fig. 4 Induction of class switch recombination (CSR) and somatic hypermutation (SHM) by TH cell–B cell interaction in germinal centers. Upon CD40–CD40Ligand (CD40L) interaction, nuclear factor-κB (NF-κB) essential modulator (NEMO) supports translocation of NF-κB to the nucleus, where it activated activation-induced cytidine deaminase (AID) gene transcription. AID introduces single-strand DNA lesions in immunoglobulin genes, which can result in class switch recombination or somatic hypermutation when repaired by error-prone mechanisms involving uracil-DNA glycosylase (UNG) and postmeiotic segregation increased 2 (PMS2)

residues in Ig genes, which are processed by error-prone DNA repair proteins such as uracil-DNA glycosylase (UNG) and postmeiotic segregation increased 2 (PMS2), that are finally responsible for CSR and SHM [6, 7]. Ultimately, B cells with high-affinity BCRs exit the germinal center and differentiate into antibody-producing plasma cells or long-lived memory cells. Whereas plasma cells generate the antibodies to neutralize antigens, memory B cells are long-lived resting cells that can take part in additional germinal center reactions upon new encounter with the same antigen (Fig. 1).

B-cell responses can also occur independently of T-cell help in the marginal zone of the spleen or in the lamina propria in the gut [8, 9]. These B cells

can be sufficiently activated by the repetitive nature of antigens recognized on blood-borne pathogens [10]. Alternatively, these B cells recognize antigens on pathogens, which also stimulate other receptors of the B cell, such as Toll-like receptors [11]. Marginal zone B cells can be found recirculating in peripheral blood (defined as "natural effector B cells") and have a memory phenotype and carry SHM [12].

4 Dynamics in the Peripheral B-Cell Compartment During Early Childhood

The immune system in young children rapidly produces an antigen-selected B-cell pool in order to acquire sufficiently high levels of antigen-specific Ig molecules. Particularly during the first 2 years (up to the age of 5 years) the absolute B-cell counts in blood and serum Ig levels are different from adults. The absolute B-cell count rapidly increases from 2 months of age to high levels (mean of 1.4×10^6/mL) and remain stable until 2 years of age. These levels subsequently decrease gradually to adult values (mean of 0.2×10^6/mL). This decrease in the absolute B-cell count is accompanied by a shift in the relative distribution toward mature B-cell subsets, i.e., higher frequencies of memory B cells and IgH class switched B cells (Bakker-Jonges and Dik et al. unpublished observations). This shift is paralleled by a moderate increase of serum IgM levels and a strong increase of serum IgG and IgA levels. Thus B-cell dynamics in early childhood should be taken into account when lymphocyte subsets of young children are evaluated. Therefore, we highly value the availability of age-dependent reference values and strongly support their use for correct interpretation of immunodiagnostic results [13].

Besides the kinetics of peripheral B-cell subsets and serum Ig levels, additional immunological parameters are likely to show dynamic age-dependent changes. These could include the replication history and SHM profile of memory B-cell subsets, which are important features of humoral immunity. Therefore, extending insights into B-cell development and Ig repertoire building in healthy individuals with combined flow cytometric, functional, and molecular strategies would provide more parameters to diagnose patients suspected to have an antibody deficiency.

5 Antibody Deficiencies

Antibody deficiencies form the largest category of primary immunodeficiencies (PID) (Fig. 5). Patients with an antibody deficiency can present either in early childhood or in adulthood mainly with increased susceptibility to bacterial infections in combination with hypogammaglobulinemia or agammaglobulinemia [14]. A subdivision into several categories can be made based on the presence of B lymphocytes and serum immunoglobulin (Ig) levels as well as on the type of B-cell defect

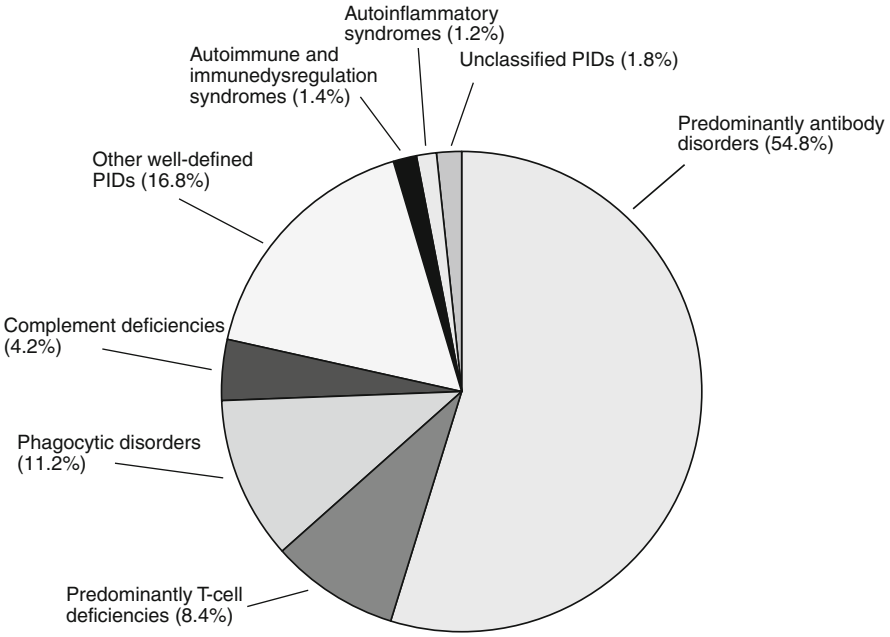

Fig. 5 Distribution of primary immunodeficiencies (PID) per subcategory in Europe (n=10.003). Based on the registry of the European Society for Immunodeficiencies (ESID), September 2009 (www.esid.org)

(Table 1). In fact, these categories affect different stages of B-cell differentiation and maturation as shown in Fig. 6.

5.1 Agammaglobulinemia

Patients with an antibody deficiency who have undetectable or greatly reduced serum Ig levels and peripheral blood B cells are diagnosed with agammaglobulinemia. Approximately 85% of the agammaglobulinemia patients are boys suffering from XLA, (X-linked agammaglobulinemia) due to a mutation in the B cell or Bruton's tyrosine kinase (*BTK*) gene. The remaining 15% of patients (both boys and girls) suffer from an autosomal recessive form. In most of these patients disease-causing mutations have now been identified in *IGHM, L14.1, CD79A, CD79B*, and B-cell linker protein (*BLNK*) (Table 1) [17].

All these genes encode components of the pre-BCR or proteins involved downstream signaling for proliferation and further differentiation (Fig. 2). Consistent with effects on pre-BCR signaling, analysis of the precursor B-cell compartment of patients with a defect in *IGHM* (n=3), *CD79A* (n=1), *BLNK* (n=1), and *BTK* (n=10) showed a block in precursor B-cell differentiation prior to the pre-B-II large cell stage (Fig. 2) [18, 19]. The stringency of the differentiation block seemed to

Table 1 Antibody deficiencies and identified genetic defects

Disease category	Genes	Reported cases in ID bases[a] (unrelated patients)	Cases diagnosed in Rotterdam (unrelated patients)	Percentage of unknown
Agammaglobulinemia	*BTK*	1,112 (974)	93 (83)	5–8%
	IGHM	20 (15)	6 (6)	
	IGL14.1	1 (1)	–	
	CD79A	2 (2)	1 (1)	
	CD79B	1 (1)	–	
	BLNK	1 (1)	1 (1)	
CD19 complex deficiencies	*CD19*	5 (3)	7 (4)	?
	CD81	–	1 (1)	
Ig class switch recombination (CSR) syndromes	*CD40L*	239 (210)	14 (10)	25–35%
	CD40	4 (3)	–	
	AID	78 (59)	3 (3)	
	UNG	3 (3)	2 (2)	
	NEMO	55 (50)	2 (2)	
	PMS2	3 (3)[b]	–	
Common variable immunodeficiency(CVID)	*ICOS*	9 (4)	–	∼90%
	TACI	36 (18)	6 (6)	
	BAFF-R	1 (1)[c]	–	

[a]ID bases: databases for immunodeficiency-causing mutations (http://bioinf.uta.fi/base_root/).
[b]Not in ID bases: Peron et al. [15].
[c]Not in ID bases: Warnatz et al. [16].

be dependent on the genetic defect (Fig. 7). The *IGHM* caused a complete block with the absence of pre-B-II and immature B-cells. A defect in CD79A expression resulted in a severe but incomplete block, referred to as "leaky" based on the presence of a low percentage of pre-B-II cells. In contrast to a published case [20], we identified a mutation in the *BLNK* gene that also resulted in a leaky block with the presence of low percentages of pre-B-II and even immature B cells. The differentiation block in XLA patients appeared to be the least strict with on average 30% pre-B-II and immature B cells. These results show that the differentiation block is more complete if the defect is closer to the pre-BCR (Figs. 2 and 7). This can probably be explained by redundancy in downstream components of the pre-BCR signaling cascade. Despite the variable leakiness of the differentiation block at the pre-B-cell stage, agammaglobulinemia patients have (virtually) no transitional B cells and naïve mature B cells in their blood. This implies that, notwithstanding varying degrees of leakiness, all the identified defects block subsequent BCR signaling in immature B cells.

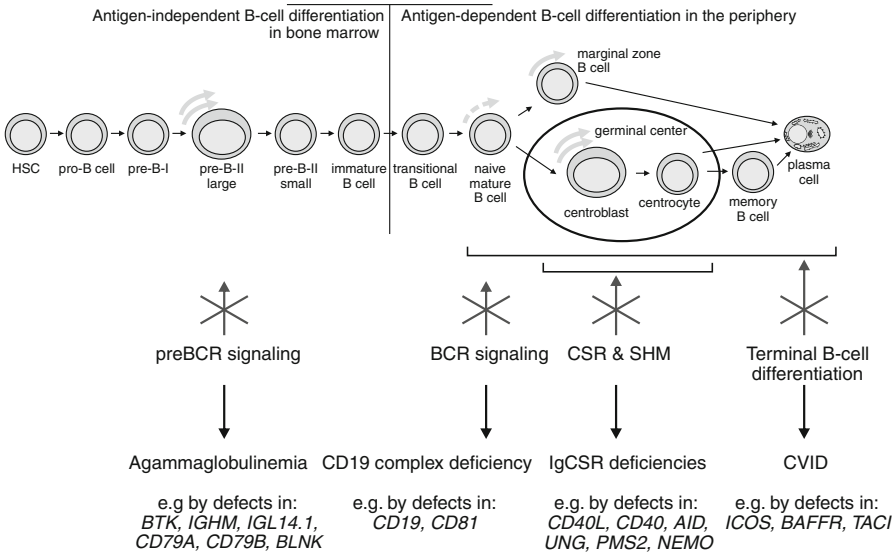

Fig. 6 Complete or incomplete blocks in B-cell development in patients with agammaglobulinemia, CD19 complex deficiency, Ig class switch recombination (CSR) deficiencies, and common variable immunodeficiency (CVID). The known primary immunodeficiency (PID) genes are shown for each PID category (see Table 1 for details). BCR, B-cell antigen receptor; SHM, somatic hypermutation

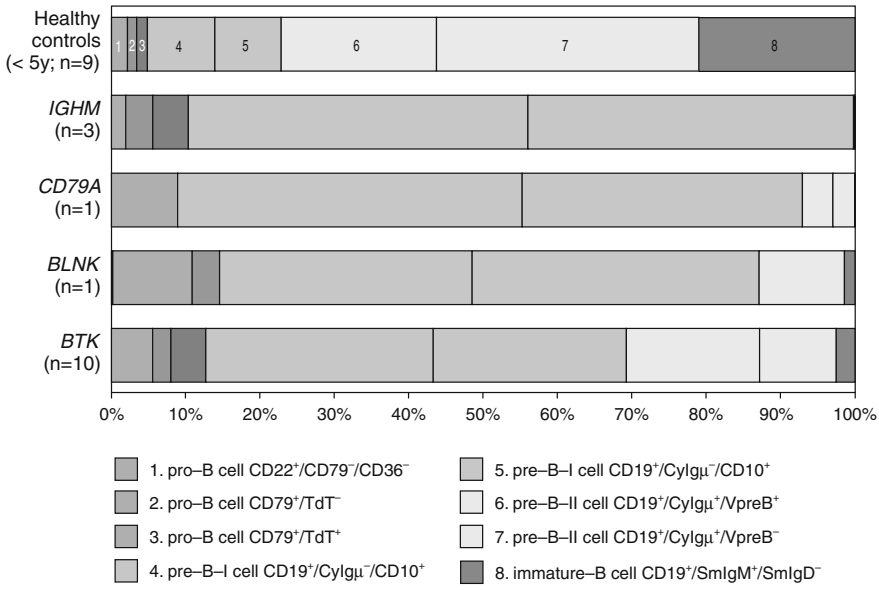

Fig. 7 Composition of the precursor B-cell compartment in healthy children (n=9; <5 year) and in agammaglobulinemic patients with genetic defects in *IGHM* (n=3), *CD79A* (n=1), *BLNK* (n=1), and *BTK* (n=10)

5.2 B-Cell Antigen Receptor Signaling Deficiency

In 2006 and 2007 five antibody-deficient patients were described who lacked CD19 expression on their B lymphocytes [21, 22]. In all patients, mutations were found on both *CD19* alleles that resulted in premature stop codons and non-functional CD19 protein. Consistent with a function in BCR signaling, the CD19-deficient B cells showed impaired Ca^{2+} signaling upon stimulation with anti-IgM [22]. This defect in B-cell activation resulted in reduced memory B-cell formation, greatly reduced serum Ig levels, and impaired Ig responses to vaccination. Although lymphoid tissues of these CD19-deficient patients have normal architecture and contain B-cell follicles, all patients presented with severe infections in early childhood, demonstrating a severe defect in B-cell function.

We recently identified a female CD19-deficient patient, who did not have mutation in her *CD19* genes. The additional absence of CD81 on the patient's leukocytes prompted us to analyze the *CD81* gene, resulting in the identification of a splice site mutation [23]. This mutation resulted in the generation of an alternative splice variant with a premature stop codon prior to the fourth transmembrane domain. Genetic reconstitution of an EBV cell line from the patient with wild-type CD81 resulted in re-expression of CD81 and CD19 on the cell membrane, thus confirming that the *CD81* gene defect caused the CD19 deficiency. Similar to all CD19-deficient patients, B cells from the CD81-deficient patient showed defective Ca^{2+} flux upon BCR stimulation and defective recall to vaccinations.

Thus, CD19 complex deficiencies are a relatively homogeneous group of antibody deficiencies with a severe defect in BCR signaling, resulting in childhood onset of bacterial infections and can be caused by mutations in the *CD19* or *CD81* genes (Table 1 and Fig. 6).

5.3 IgCSR Deficiency

Patients with IgG and IgA deficiencies in the presence of normal B-cell numbers in blood and normal to high IgM serum levels are mostly diagnosed with an IgCSR deficiency. Because many of these patients have increased IgM levels, these were previously termed hyper-IgM syndromes. The common denominator in these patients is the inability of (most of) their B cells to undergo CSR and SHM. Most cases are inherited as X-linked disease due to a mutation in the *CD40L* gene (Table 1) [24]. CD40L is expressed on activated T cells and the interaction with CD40 on activated B cells forms the main stimulus for T cell-dependent B-cell responses. CD40–CD40L interaction is therefore important for the formation of germinal centers by proliferating B cells and follicular T-helper cells (Fig. 4). CD40L deficiency is actually a T-cell defect and as a consequence these patients also have a susceptibility to opportunistic infections, which cannot be controlled by immunoglobulin replacement therapy [25].

In patients with autosomal recessive inheritance, mutations have been identified in *CD40*, *AID*, *UNG*, and *PMS2* (Table 1) [24, 15,26,27]. These are all

intrinsic B-cell defects. In contrast to CD40L and CD40 deficiencies, B cells of patients with defects in AID, UNG, and PMS2 are able to initiate germinal center responses. However, due to a defect in the formation of mutations (AID) or in the error-prone repair of these lesions (UNG, PMS2), they are unable to perform IgH class switching or create mutations in their Ig genes. In contrast, in patients with CD40L or CD40 deficiency, B cells can be found that successfully undergo CSR (IgA-secreting cells in gut lamina propria) or SHM (marginal zone B cells) [28, 29].

Finally, IgCSR deficiency has been identified in patients with anhydrotic ectodermal dysplasia with immunodeficiency (EDA-ID) due to X-linked genetic defects in the nuclear factor-κB (NF-κB) essential modulator (*NEMO*) gene. NEMO is activated via signaling pathways downstream of multiple receptors and functions to activate NF-κB. In activated B cells, NEMO and NF-κB act downstream of CD40–CD40L interactions to upregulate AID (Fig. 4). B cells from EDA-ID patients are therefore unable to express AID and are defective in CSR and SHM (Fig. 4).

5.4 Common Variable Immunodeficiencies (CVID)

CVID are the largest category of symptomatic antibody deficiencies. In contrast to the other categories, in the majority of which a genetic defect can be identified, in >85% of CVID patients the genetic defect is not (yet) identified. Most cases are sporadic and only 10–20% are familial, when defined as having at least one additional family member affected by CVID or selective IgA deficiency. Recently genetic defects have been identified in inducible costimulator (*ICOS*), *TACI*, and *BAFF-R* (Table 1) [16,30–32]. Heterozygous *TACI* mutations increase disease susceptibility but are not likely to be disease causing, because these are also found in healthy individuals [33]. Homozygous *BAFF-R* mutations cause a characteristic immunological phenotype but do not always result in a clinically manifest immunodeficiency [16]. The clinical heterogeneity of the immunodeficiency and high frequency of additional symptoms such as autoimmune disease and granulomatous complications in CVID patients emphasize the need for the characterization of immunological and genetic defects, in order to guide treatment and follow-up aiming at the prevention of irreversible organ damage [34–37]. With these considerations in mind, we decided to take a novel approach toward unraveling immunogenetic problems in CVID.

Flow cytometric immunophenotyping is an important and powerful tool for the study of B-cell subsets in peripheral blood and diagnosis of immunodeficiency [38]. However, in CVID patients it is often insufficient to identify the immunological defect. CVID is a heterogeneous disease entity and probably consists of several diseases or subgroups with different underlying pathophysiologies. There are additional studies that can be done including quantification of the replication history of naïve and memory B-cell subsets, testing BCR signaling capacity,

quantification of SHM in memory B cells, and in vivo antibody responses to vaccination [22, 39, 40]. We have preliminary results on sorted peripheral B-cell subsets of CVID patients which show that their size and replication history can differ significantly between patient groups and may prove useful to discriminate between different underlying immunological defects (Driessen et al. manuscript in preparation) [41].

6 Conclusion

Early recognition of antibody deficiencies and identification of genetic defects form the basis for adequate treatment, monitoring of complications, and estimation of prognosis. Achieving a molecular diagnosis can assist in the development of long-term treatment and follow-up strategies, aiming at prevention of severe complications and irreversible organ damage. It also enhances the quality of information communicated to patients and their families, supporting treatment compliance and permitting genetic counseling. Consequently, fast and precise molecular diagnostics has substantial added value for patient care.

Identification of genetic defects in patients with an antibody deficiency requires thorough understanding of normal B-cell differentiation and its cellular and molecular processes. Therefore, precise clinical evaluation, stepwise immunodiagnostic workup based on such knowledge, and regular exchange of patient information between clinicians and laboratory scientists are crucial for clinical management of patients with B-cell defects.

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Mumps is Back: Why is Mumps Eradication Not Working?

Noni MacDonald, Todd Hatchette, Lotfia Elkout, and Shelly Sarwal

1 Introduction

Mumps has been recognized as a common childhood illness since the time of Hippocrates in the fifth century BC. In 1790, central nervous system involvement was described by Hamilton and, in 1860, sensorineural deafness by Toynebee. By the 1940s, mumps was garnering mounting concern as a cause of painful orchitis, aseptic meningitis, and encephalitis that was substantially affecting troop mobilization. Cultivation of the mumps virus in chick embryos in 1945 led to the development of inactivated vaccine, but killed mumps vaccine only induced short-term memory with low efficacy [1, 2]. Subsequently, effective live-attenuated vaccines have been developed. These were introduced into many industrialized countries heralding a major step forward in prevention with annual rates of mumps cases plummeting by 90%. The effect was so dramatic that by 1992, mumps disease was delineated as one of six potentially eradicable diseases by the International Task Force for Disease Eradication [3]. Despite this optimism, indigenous mumps has not disappeared from all countries that have introduced routine mumps vaccine. Indeed, mumps has made a comeback in many countries in the past decade with numerous outbreaks including a very large one in the United Kingdom (Fig. 1) [4], outbreaks in Sweden [5], the Netherlands [6], Canada [7], Australia [8], the United States [9], Belgium [10], and a number of other countries. In contrast, Finland, which lies close to many of these European countries, has not had any recent outbreaks [11, 12].

What has gone wrong with the mumps vaccine eradication plan? Why have mumps outbreaks occurred in populations with high rates of mumps vaccine coverage? Why might Finland have been spared so far? What are the lessons learned and how might the control of mumps be improved?

The clinical presentation of mumps, the virus and its pathogenesis, mumps epidemiology, diagnostic tools, and vaccines will be reviewed briefly to provide a

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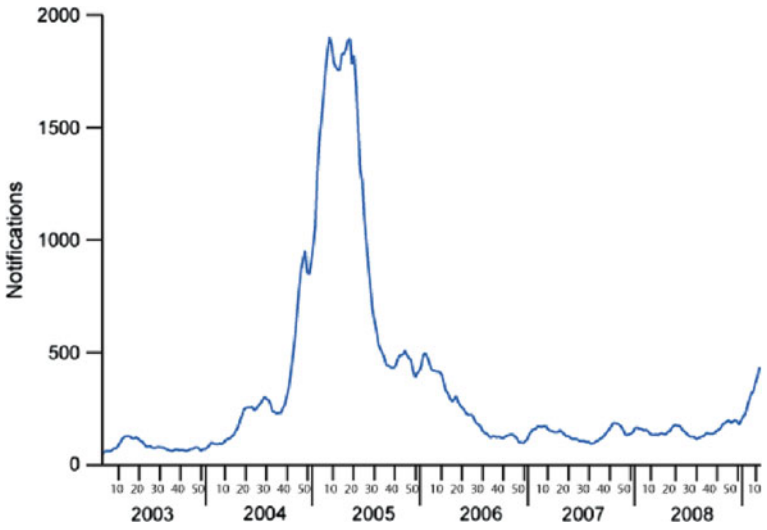

Fig. 1 Mumps notifications in England and Wales: 2004–2009 (Reproduced with permission from Health Protection Report. 2009;3(14) 9 April 2009. Health Protection Agency. http://www.hpa.org.uk.hpr/archives/2009/news1409.htm) [4]

background for discussion of the outbreaks, lessons learned, and questions still arising.

2 Mumps: The Clinical Presentation

Mumps illness is generally mild, typically beginning with a prodrome of nonspecific symptoms including low-grade fever, headache, malaise, and myalgia [13, 14]. However, up to 20% of infections are asymptomatic. The well-recognized parotid gland swelling (either unilateral or bilateral) occurs in only 30–40% of cases while 40–50% of patients may have respiratory symptoms, particularly children younger than 5 years of age who may develop lower respiratory illnesses. Parotid gland swelling is more common in school-aged children with parotitis occurring in 60–70%, with submandibular swelling in 10% and suprasternal swelling in 6% (See Fig. 2) but can occur, albeit rarely, in infants [15]. As shown in Table 1, mumps infection is often associated with complications [13, 14]. The rates of complications such as orchitis, oophoritis, and encephalitis increase with age and are more prominent in adults [9]. Previous estimates of sensorineural hearing loss may have been on the low side at 0.5–5/100,000 [13] given that a more recent prospective office-based study in Japan, a country where mumps vaccine is not used, found a rate of severe mumps hearing loss of 1/1,000 [16]. Mumps infection in pregnancy appears to increase embryonic and fetal death and spontaneous abortion but the mumps virus does not appear to be teratogenic [19]. There might be a relationship between mumps in pregnancy and endocardial fibroelastosis but data are limited. Little is known about mumps in immunocompromised patients, although mumps

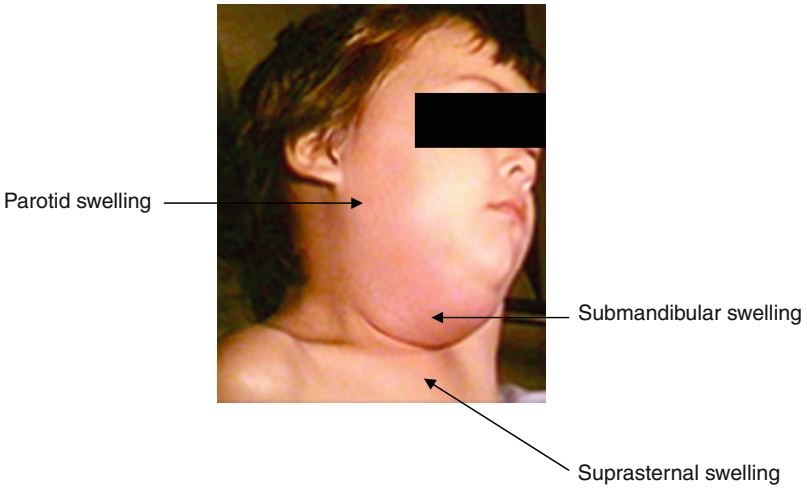

Fig. 2 Child with mumps (Courtesy of CDC/NIP/Barbara Rice. Public Health Image Library. Centers for Disease Control and Prevention. http://www.cdc.gov/vaccines/vpd-vac/mumps/photos.htm)

Table 1 Mumps complications [9, 13, 14, 16, 17, 18]

Mumps complication	Percentage
CNS	
Pleocytosis	40–50%
Clinical meningitis	1–10%
Clinical encephalitis	0.1%
Deafness	
Transient	3–4%
Sensorineural hearing loss	Maybe as high as 0.1%
Orchitis -postpubertal	20–30%
Oophoritis -postpubertal	5%
Pancreatitis	2–5%
Cardiac involvement	
ECG abnormal	Up to 15%
Clinical myocarditis	rare
Renal involvement	
Microscopic hematuria and proteinuria	50–60%
Hospitalization	1–2%
Death	0.1–0.3%

has been recently reported as a cause of kidney rejection in an adult renal transplant patient [17]. Hospitalizations due to mumps are uncommon [9] and death from mumps is rare, occurring in only about one to three cases per 10,000, usually due to encephalitis [13].

3 Mumps: The Virus

Mumps virus is an enveloped, negative-strand RNA virus in the family Paramyxoviridae, genus Rubulavirus (Fig. 3) and is a close cousin of human Parainfluenza virus [18]. The mumps genome encodes for eight proteins including two glycoproteins: hemagglutinin-neuraminidase (HN) and fusion protein (F); five other structural proteins: nucleocapsid (NP), phosphoprotein (P), large polymerase protein (L), matrix protein (M), and small hydrophobic (SH) protein; and two nonstructural proteins V and I encoded within the P protein gene [20]. No single gene mutation appears to be wholly responsible for the overall virulence of a specific mumps strain but rather requires gene mutations leading to changes in several proteins [21]. While monkeys were originally used for testing neurovirulence, a neonatal rat-based assay has now been developed [22].

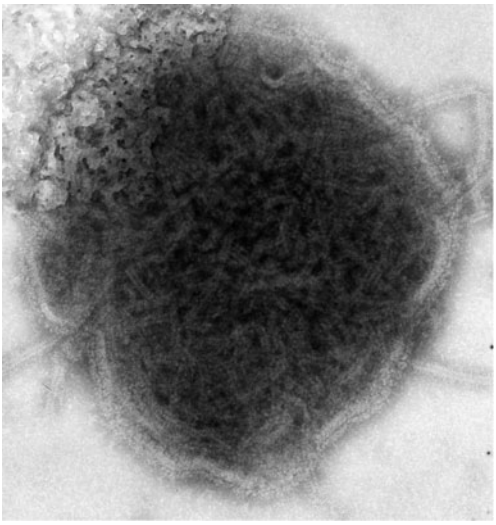

Fig. 3 Negative stained transmission electron micrograph depicting the ultrastructural features displayed by the mumps virus (Courtesy of CDC/ Dr. F. A. Murphy. Public Health Image Library. Centers for Disease Control and Prevention. http://www.cdc.gov/vaccines/vpd-vac/mumps/photos.htm)

Prior to the development of monoclonal antibodies, mumps was declared to be serologically monotypic. However, mumps is now recognized to have multiple genotypes with 12 detected to date based upon variation in the SH gene, the most variable gene in mumps with intra-genomic variation of 2–4% and inter-genomic variation $\geq$ 6% [20, 23]. Although the SH gene does not play a role in protective immunity, sequence variation in this gene reflects the virus' overall genetic and antigenic variability, including the HN gene that codes for the major target of neutralizing antibody [24].

The NP protein is the antigen (S antigen) used in the IgM assays for acute infection while the F and HN proteins are known to be important for inducing protective antibody. To further complicate serological testing, due to some homology between mumps virus F and HN proteins with those of other paramyxoviruses, some serologic cross-reactivity can occur [18].

The mumps virus can replicate in a variety of cell tissue cultures such as primary rhesus monkey kidney cells and HeLa cells as well as in embryonated hens' eggs. In tissue culture, the cytopathic effect is indistinguishable from that of respiratory syncytial virus. Given that mumps has an envelop, it is sensitive to ether. Mumps virus is stable at 4°C for several days, and can be kept at –65°C for years but is sensitive to repeated thawing and freezing.

4 Mumps: The Pathogenesis and Transmission

Mumps is a respiratory infection transmitted through close contact by inhalation of infectious droplet nuclei, by direct contact, or by autoinoculation after hands contact virus-contaminated fomites and touch the nose or mouth. Infection can remain localized in the respiratory tract but viremia frequently occurs, usually late in the incubation period [25, 26]. Spread within the body can also occur through infected mononuclear cells. The virus has a predilection for glandular (e.g., parotid glands, ovaries, testes, and pancreas) and nerve tissue [14]. The virus enters the central nervous system through the vasculature of the choroid plexus [18]. The incubation period for mumps is 14–18 days but maybe as long as 25 days. Based upon studies of household contacts, mumps is less infectious than measles or varicella [27]. The period of peak contagion is from 3 days prior to the onset of symptoms until 5 days afterwards [20]. However, the mumps virus can be isolated from saliva from as early as 7 days before to 9 days after onset of clinical symptoms [14]. Those with subclinical infection can spread the virus while the respiratory tract and salivary glands are involved. Salivary secretion of virus correlates inversely with local virus-specific IgA secretory antibody levels [25, 26]. With respect to prevention, mumps virus is most accessible and susceptible to immune attack during the period of viremia when it is cell free [18]. Humoral antibodies appear to restrict plasma viremia. The role that cell-mediated immunity plays is not well understood.

5 Mumps: The Diagnosis

The clinical diagnosis of mumps rests upon the standard case definition: acute onset of unilateral or bilateral swelling of the parotid or other salivary glands lasting 2 or more days without any other apparent cause [28]. This will miss cases where parotid gland swelling either is not prominent or does not occur. Use of the clinical diagnosis is most effective in countries without routine mumps immunization programs where outbreaks regularly occur but is more problematic for sporadic cases or in countries where mumps vaccine is widely used. Similar presentations (parotid swelling and low-grade fever) can occur in infections with other viruses such as Epstein-Barr, parainfluenza types 1, 2, or 3, adenovirus, Human herpesvirus 6, enteroviruses, influenza A, and lymphocytic choriomeningitis virus [29–31]. In a study in Alberta, Canada, before the introduction of mumps vaccine, about one-third

of cases of sporadic mumps reported by family physicians could not be confirmed by serology suggesting that causes other than mumps to be the etiology [32]. A further problem in countries with routine mumps immunization programs is that mumps may be misdiagnosed or missed completely due to lack of clinical familiarity with the presentation of mumps in different age groups and the expectation that all cases have parotitis.

5.1 Mumps: Laboratory Diagnosis for Mumps Infection

Laboratory-confirmed mumps is defined by the United States Centers for Disease Control and Prevention and the Public Health Agency of Canada as a patient with clinical symptoms compatible with mumps who has positive mumps IgM antibody (without previous immunization in the last 6 weeks), or seroconversion as defined by a fourfold rise in IgG titer to mumps, or isolation of mumps virus, or detection of mumps RNA in saliva, urine, or CSF [33]. However, laboratory confirmation of mumps infection can be difficult, particularly in recent outbreaks that have affected partially immune populations.

5.1.1 Specimen Collection

As with any other diagnostic test, the accuracy of the results will be influenced by the type of specimen collected, the timing of specimen collection, and the conditions under which the specimen is transported to the laboratory. As indicated previously, mumps virus can be detected for 5 days after the onset of symptoms in buccal specimens and up to 13 days in urine specimens in non-vaccinated individuals [34]. Buccal swabs are the ideal specimen for the diagnosis of acute mumps infection and generally have a higher sensitivity than urine [35, 36]. To collect a buccal sample, the patient should first have their parotid gland massaged to express infected saliva. A swab should be rubbed along the buccal mucosa near stenson's duct, placed in appropriate viral transport media and shipped to the laboratory at 4°C [37] (Fig. 4). Due to the labile nature of the virus, the specimen should not be frozen at –20°C.

5.1.2 Tissue Culture

The mumps virus can replicate in a variety of cell tissue cultures such as primary rhesus monkey kidney cells, HeLa cells, Vero cells, human neonatal kidney cells, as well as in embryonated hens' eggs. In tissue culture, the cytopathic effect usually occurs 6–8 days post-inoculation and is characterized by the formation of syncycia which may be indistinguishable from that of respiratory syncytial virus. Confirmation of a positive culture is done using mumps-specific immunofluorescence. Methods using centrifugation enhanced inoculation of shell vial tissue cultures offer more rapid results (2–5 days) than traditional culture [38]. Mumps virus is stable at 4°C for several days and can be kept at –70°C for years but is sensitive to repeated thawing and freezing [34, 39].

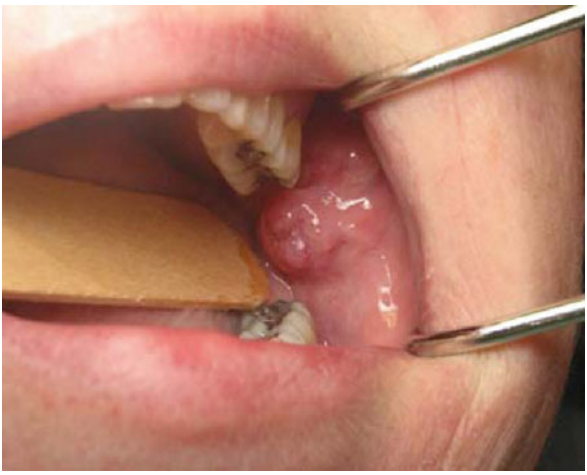

Fig. 4 Collection of buccal specimen for detection of mumps virus by culture and polymerase chain reaction (PCR). Swab the buccal cavity, i.e, the space near the upper rear molars between the cheek and the teeth. Swab the area between the cheek and gum by sweeping the swab near the upper molar to the lower molar area (adapted from the Illinois Dept. of Public Health – Div. of Laboratories (Chicago Virology Section) Courtesy of CDC http://www.cdc.gov/vaccines/vpd-vac/mumps/downloads/detection_IL.pdf)

5.1.3 Molecular Testing

Nucleic acid-based tests (NAT), such as reverse transcriptase PCR, have been developed to detect mumps virus in clinical samples. Both conventional hemi-nested RT-PCR and real-time RT-PCR methods directed at the SH and F genes have been validated for clinical use [39–42]. NAT methods are more rapid and can generate a result in 4–8 h compared to 5 days for tissue culture. In addition, NAT has superior sensitivity compared to culture. The sensitivity of culture for identification of mumps from oral specimens collected from patients with suspect mumps is 73% compared to real-time RT-PCR methods [35, 39]. NAT methods have the ability to detect as little as 0.01 $TCID_{50}$ of virus or ten copies of RNA [34, 41, 43]. Faster turnaround time to results (often in as little as 4 h), reduced potential for amplicon contamination, and in some cases further enhancement of sensitivity, are all advantages to real-time methods compared to the conventional nested RT-PCR. As these molecular methods become more common, and protocols become more standardized, NAT methods will likely become the new standard in the diagnosis of acute mumps infection.

5.1.4 IgM Serology

The identification of an IgM response to a specific pathogen is often used as a way to diagnose acute infection. However, experience with the use of IgM-specific serology in the most recent mumps outbreaks in North America and the United

Kingdom suggest that IgM serology is a very insensitive test. Studies from Canada and Europe demonstrated that the sensitivity of different commercially available kits for the detection of IgM antibodies to the mumps virus ranged from 24 to 51% [36, 44]. Why these assays perform poorly is likely a reflection of the population being tested. These assays were originally developed to test the primary immune response individuals without pre-existing immunity to mumps. In the recent North American outbreaks many of the cases had only received a single dose of mumps-containing vaccine or had two doses with waning immunity [24]. This partial immunity did not prevent re-infection but could alter the natural history of the immune response where the IgM response is blunted and below the limit of detection of the commercially available assays.

5.1.5 IgG Serology

Many commercial assays are available to document the presence of anti-mumps IgG in patients' specimens. A positive result is often used as a surrogate marker of immunity. However, data from the United States have shown that many of those infected with mumps in the recent outbreak had two doses of MMR and IgG antibodies at the time of presentation, which questions whether these assays are a reliable measure of immunity [45].

The serologic response to mumps can be measured using neutralization assays, hemagglutination inhibition (HI) assays, and enzyme immunoassays (EIA); however, there is poor correlation between these different methods [46]. When a person is exposed to a live virus (wild or attenuated vaccine strains), both neutralizing and non-neutralizing antibodies are generated. Antibody titers are commonly measured using commercial EIAs which are simple to perform compared to the more technically demanding neutralization assays. However, these EIA methods do not distinguish between neutralizing and non-neutralizing antibodies. The gold standard method of testing for protective immunity is to measure neutralizing antibodies by a neutralization assay. In this assay a patient's serum is mixed with a known concentration mumps virus and put on a cell line that is permissive to infection with the mumps virus. If there are antibodies in the patients serum that are effective at preventing infection, they will bind to the mumps virus and "neutralize" it so that the cell line does not get infected [47].

Unlike measles and rubella in which a protective titer has been established using an internationally recognized reference standard, no such benchmark exists for mumps, making determination of who is truly immune difficult.

Documentation of a fourfold rise in IgG titer is also a criterion for laboratory confirmation. However, this rise may be difficult to document in infected individuals who have had previous vaccination. The purpose of vaccination is to prime the immune response to respond rapidly to a pathogen. The IgG response in these individuals may be so rapid that a fourfold rise could be missed.

The laboratory confirmation of mumps infection can be challenging, particularly in a partially immunized population. Although newer molecular detection methods such as real-time reverse transcriptase PCR are becoming the standard in mumps diagnostics, no diagnostic testing is 100% sensitive, and as such mumps infection

cannot be absolutely ruled out by a negative diagnostic test, particularly in a partially immunized population [48].

6 Mumps: The Epidemiology

Mumps occurs worldwide but only in humans; there are no natural animal reservoirs. A carrier state is not known to exist in humans and mumps infection is thought to confer lifelong immunity. In the pre-vaccine era, more than 50% of reported cases of mumps were in children aged 5–9 years, and over 90% of reported cases were in those under 14 years of age. This may have been related both to the ease of spread in this age group and to the high frequency of clinically apparent parotitis, and thus increased probability of considering the diagnosis. Within a household, the secondary attack rate is estimated to be 80–90%. Mumps is uncommon in infants under one year of age likely due to the presence of maternal antibodies as well as limited exposure if both parents are immune either through natural infection or through childhood immunization.

Mumps commonly occurs in the winter and spring in temperate climates but does not show seasonality in tropical climes [18]. In countries without routine mumps immunization programs, mumps epidemics occur approximately every 3–4 years. Mumps rates vary by country with an average of 300/100,000 in countries without immunization programs [18]; however, the rates have dropped dramatically in countries where routine mumps immunization has been introduced as shown in Table 2 [13, 18]. While single-dose routine programs led to a marked decrease in cases, two-dose programs were even more effective (Table 2).

By 2000, mumps appeared to be well controlled in many countries with routine mumps immunization programs such as the United Kingdom, the United States, Canada, and Australia. However, in the past 5 years, each of these countries has

Table 2 Impact of introduction of mumps vaccine on annual rates of reported mumps in selected countries in Europe

	Pre-routine vaccine Year	Rate/100,000	Post-routine vaccine Year	Rate/100,000	Percent decrease in mumps
Two dose					
Denmark	1977–1979	726	1993–1995	1	>99%
Finland	1977–1979	223	1993–1995	>1	>99%
One dose					
Armenia	1983–1985	280	1993–1995	16	94%
England	1983–1985	40	1993–1995	12	88%
No vaccine					
Poland	1983–1985	415	1993–1995	361	–
Romania	1983–1985	242	1993–1995	217	–

Adapted from [13]. Adapted with permission from the World Health Organization.

experienced major outbreaks predominately in older adolescents and young adults but not in young age children as occurs in countries without immunization programs [7–9, 49]. These outbreaks were unexpected and have raised concerns about the effectiveness of mumps vaccine and mumps vaccine programs.

Mumps virus, based upon SH region variation, shows distinct geographic clustering by genotype and redistribution may occur over time [50]. More than one genotype may circulate simultaneously in a geographic region. In the Western Hemispheres, genotypes C, D, E, G, and H predominate while in Asia, genotypes B, F, and I are more common [14]. The mumps strains that caused the outbreaks in the United Kingdom, United States, and Canada were G and all related genetically [7] and different from the genotype A found in Jeryl Lynn vaccine.

7 Mumps: The Vaccines

Although effective live-attenuated mumps vaccines have been available for more than 30 years [18], use has not been widespread until recently. By 2007, 114 countries, plus parts of China, had introduced mumps vaccine. All commercially available mumps vaccines contain live-attenuated mumps virus that is lyophilized and must be reconstituted before use. Mumps vaccines may be monovalent but more often are given in combination with measles and rubella vaccine (MMR) or, more recently, with added varicella vaccine (MMR-V).

At present, there are at least 11 different attenuated mumps vaccine strains in use throughout the world, but only Jeryl Lynn (developed in the United States), UrabeAm9 (developed in Japan), and Leningrad-3 (developed in the former Soviet Union) and their derivatives (RIT 4385: viral clone of Jeryl Lynn; Leningrad–Zabreg: further attenuated Leningrad-3) are used widely [14, 51]. Vaccine preparations using the attenuated parent vaccine strain may differ by manufacturer because of differences in passage history, cell substrates, or manufacturing processes [52]. The currently used attenuated vaccine strains belong to different genotypes, i.e., Jeryl Lynn genotype A and Urabe Am9 genotype B [50]. Each of the vaccines also differs in their immunogenicity, efficacy, effectiveness, and associated adverse events. For example, Urabe Am9 has been associated with enhanced neurovirulence compared to Jeryl Lynn, while Jeryl Lynn (genotype A) has been shown to have reduced cross-neutralization capacity with genotype D [24, 50].

Mumps vaccines are considered very safe. In large field trials before licensure, no serious adverse events were reported with the Jeryl Lynn vaccine [53]. Overall, adverse reactions to mumps vaccination are uncommon and usually mild, i.e., slight injection site local soreness and swelling; occasionally mild parotitis and low-grade fever may occur. More serious adverse events such as sensorineural deafness have been reported albeit at a reporting rate of one case per 6–8 million doses of mumps vaccine with causality not fully established [54]. The more common serious adverse event attributable to mumps vaccine is aseptic meningitis reported at widely varying frequencies as high as one case per 1,000 vaccinations (Leningrad-3 and Urabe Am-9) to as low as 1/1,800,000 vaccinations (Jeryl Lynn) [51, 52]. Causality has

been proven through isolation of the vaccine mumps strain from the cerebrospinal fluid. The difference in frequency of vaccine-associated aseptic meningitis reflects both differences in vaccine strains and vaccine preparation, as well as variation in study design, diagnostic criteria, and clinical practice [51, 52]. As with wild mumps, asymptomatic pleocytosis in the cerebrospinal fluid may occur. Of note, there are reports of horizontal transmission of mumps vaccine virus (Leningrad-Zagreb and Leningrad-3) to family contacts [55–57].

Mumps vaccines are immunogenic but less so than natural disease; with Jeryl Lynn, the mean neutralizing titers in children are 1:9 vs. 1:60 with natural disease [58]. There is variation in immunogenicity by strain. Urabe not only has a higher rate of aseptic meningitis than does Jeryl Lynn [59], but also has consistently reported higher seroconversion rates although both are very immunogenic [51]. Antibodies persist for at least 10 years after immunization with two doses being more effective than one, but immunity does wane significantly over time [18, 60]. Based upon titers, there does not seem to be any advantage to delaying the second dose from 4–6 to 9–11 years [60]. Antibodies appear to decline more quickly with Jeryl Lynn than with Urabe. In a study in the United Kingdom, 4 years after receipt of MMR, the rate of seronegativity was 19% with Jeryl Lynn compared to 15% with Urabe [61]. In a study from Finland, 21 years after last dose of MMR, 24% had no measurable mumps antibody using enzymoimmunoassay [62]; however, given that there are no accepted surrogate serological markers for protection, extrapolation of these immunogenicity studies to the real world is difficult. Cellular immunity may also be important. Of note, all (n=14) of the seronegative group noted above in the Finnish study had evidence of cell-mediated immunity (mumps antigen-specific lymphoproliferative responses) and only one of the seropositive vaccinees (n=36) had none after 20 years. This suggests that cell-mediated immunity may persist for a very long time but the clinical importance of this is still unclear [63].

With respect to efficacy, most trials done in the 1960s and 1970s were short-term only and showed efficacy for Jeryl Lynn of 95–96% and for Leningrad-3 of 91–99% [18]. However, studies of effectiveness in outbreaks have noted consistently lower ranges (61–91% for Jeryl Lynn and Urabe) [18, 64, 65]. Of note, Leningrad–Zagreb vaccine, at only a fraction of the cost of Jeryl Lynn vaccine, has been shown to be very effective – 95% in one study [66]. Single-dose vaccine is only effective in decreasing mumps by 80–90%: for elimination, two doses are a must [13]. Furthermore, very high coverage rates are needed (first dose >95% and second dose $\geq$80%) to interrupt indigenous mumps transmission in a country [67]. Low to moderate levels of mumps vaccine coverage may actually increase the number of susceptibles and the number of cases in older age groups.

8 Mumps: Recent Resurgence

Mumps has made a resurgence in a number of countries in the past 5–10 years. Outbreaks in countries that previously had reported good mumps control, such as the United States, Australia, and Canada, are especially concerning. In each of

the outbreaks, the clinical presentation of mumps whether in an immunized or a non-immunized cohort has not differed from that described earlier [7, 65]. Parotitis, orchitis, aseptic meningitis, pancreatitis, and encephalitis have all occurred [68]. The hospitalization rates have remained low with only 85 patients out of 6584 (<2%) hospitalized in the 2006 outbreak in the United States [9]. Deaths have not been reported [7–9, 69]. The epidemiology and underlying contributing factors differ in these outbreaks with some due to vaccine program problems while others suggest vaccine problems. Five patterns are seen (Table 3).

Table 3 Mumps resurgence: underlying factors

1. Vaccine program problem: refusal to accept immunization
2. Vaccine program problem: failure to immunize an age group: lost cohort
3. Vaccine program problem: failure of single dose: forgotten cohort
4. Vaccine failure: primary vaccine failure: ineffective vaccine
5. Vaccine failure: two-dose vaccine failure: waning immunity

8.1 Vaccine Program: Refusal to Accept Immunization: 2007–2008 Outbreak in the Netherlands

In the Netherlands, mumps vaccination using Jeryl Lynn was introduced in 1987. The routine childhood immunization program includes mumps vaccine as part of MMR at 14 months and a second dose at 9 years. This regimen appeared to be bringing mumps under good control leading to fewer than 50 cases reported each year and dropping to less than 10 cases per year by 2005 [8], although there was an outbreak in an international school in 2004 [6] with a 12% attack rate among students immunized according to the Dutch schedule. In 2007, 87 cases of mumps were reported in less than a year; median age 13 years (range 2–56 years) [6]. The geographic distribution coincided with areas with low immunization rates (Fig. 5a, b). Of the 87 cases, only 29 were vaccinated. Mumps may have occurred due to the reduced cross-neutralization capacity of Jeryl Lynn for Group D [8, 70], the most frequently isolated genotype in this outbreak. In the 58 who were unvaccinated, the main reason given in 36 was religion – Orthodox Reformed Christians. The geographic distribution of cases coincided with the "Bible belt." Even with high rates of immunization in surrounding areas (90–95% vaccine coverage) [70], mumps still readily infected the non-immunized population with some spread to the immunized. Herd immunity did not protect the non-immune population. Similar outbreaks in non-immunized populations within well-immunized communities have occurred elsewhere [71].

8.2 Vaccine Program: Failure to Immunize an Age Group: "Lost Cohort": 2004–2006 Outbreak in United Kingdom

Routine immunization for mumps was introduced into the United Kingdom in 1988 as MMR vaccine. The vaccine was recommended for children 12–15 months of

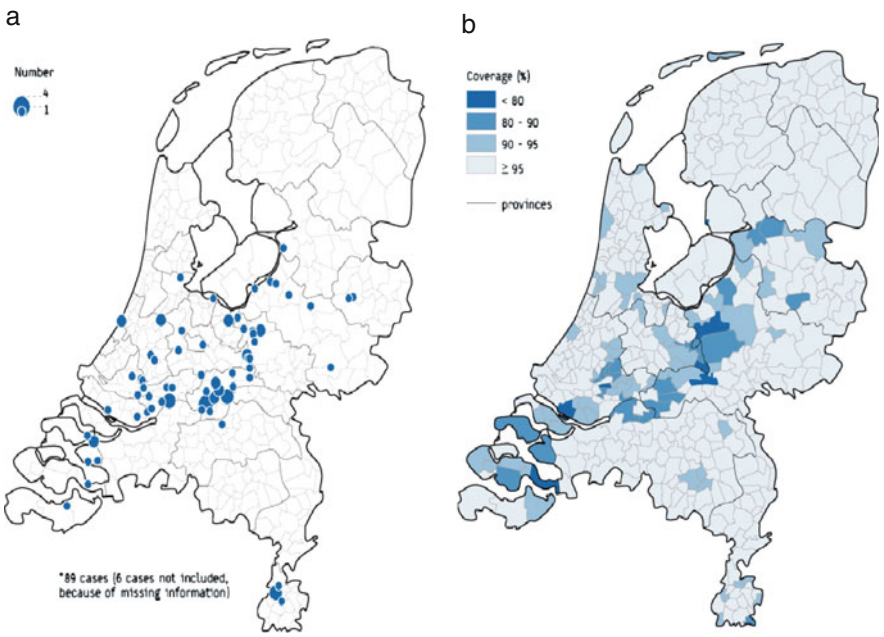

Fig. 5 **a** Geographical distribution of notified mumps cases in the Netherlands from August 1, 2007 to May 15, 2008 [6]. **b** Geographical distribution of measles–mumps–rubella vaccination coverage by municipality at the age of 2 years in the Netherlands, 2008 [6]. *Source*: Centre for Infectious Disease Control, Rijkinstituut voor Volksgenzonheid en Milieu (RIVM), The Netherlands. With permission Eurosurveillance

age. Cases of mumps declined as did mumps hospitalizations [72]. Initial modeling done at that time suggested that the critical level of mumps vaccine uptake needed to eliminate transmission of mumps was approximately 85% of each cohort by the age of 2 years [73]. Both Jeryl Lynn and Urabe Am-9 vaccine strains were used until the early 1990s when the decision was made to no longer purchase Urabe Am-9 [74] due to concerns about the associated high rates of aseptic meningitis [59]. In 1994, an outbreak of measles prompted a national catch-up campaign for 5- to 16-year olds, but due to a world shortage of MMR, measles–rubella vaccine was used [75]. Modeling of efficacy data for mumps vaccine at that time indicated that mumps was unlikely to be eliminated with a single-dose program [72] so this led to the introduction of a two-dose MMR childhood regimen in 1996. Of note, by 1999, cases of mumps in adolescents began to rise [76] although major outbreaks were not occurring. A catch-up MMR campaign was started in early 2004 primarily aimed at those older than 19 years [49], but this is a difficult age group to reach [49, 77].

During 2004–2006, the United Kingdom experienced a major nationwide outbreak of mumps with over 50,000 cases reported in 2005. As shown in Fig. 6, the majority of cases represented a “lost cohort” – too young to have had natural disease at school when mumps was spreading widely, too old when routine MMR vaccine

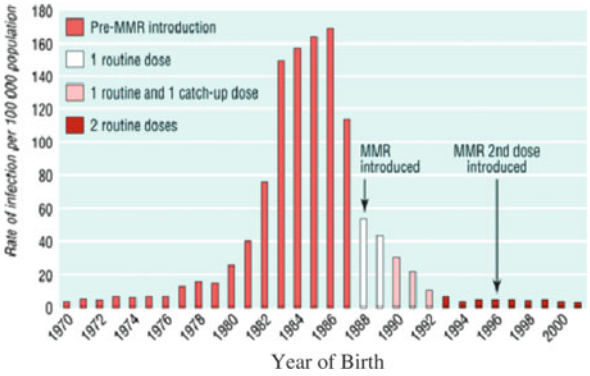

Fig. 6 For England and Wales, confirmed mumps cases in 2004 by year of birth (1970–2001) per 100,000 population and opportunity for MMR vaccination [76]. Reproduced from (Mumps outbreaks across England and Wales in 2004: observational study. Savage E, Ramsay M, White J, Beard S, Lawson H, Hunjan R, et al. BMJ 330(7500):1119–20 copyright notice 2005) with permission from BMJ Publishing Group

was introduced in 1988 (no catch-up), no mumps vaccine in the MR catch-up program in 1994, and too old for the two-dose program that started in 1996 [76]. In 2004, almost 79% of the confirmed cases were persons 15–24 years of age and only 3.3% reported having received two doses of vaccine and another 30% had received one dose only [75]. By 2005, most of the cases were 19- to 23-year olds in college or universities [75]. Although mumps vaccine (MMR) uptake had been declining in the England since 2000 following adverse publicity about possible (since discredited) links to autism and Crohn's disease [78], this was not the cause of this large outbreak as only 2.4% of the confirmed mumps cases in 2004 were in persons who would have been eligible for two doses of MMR [75]. The vaccine effectiveness in this large outbreak was estimated to be 87.8% for one dose and 94.6% for two doses of vaccine [79].

This was not an outbreak primarily due to vaccine failure but rather an outbreak due to vaccine program failure to fully immunize a cohort resulting in a "lost cohort." Furthermore, given that not all members of this lost cohort had mumps during this outbreak, the potential continues for more mumps cases to occur in adults as this "lost cohort" ages. Given that mumps continues to be a problem in some European Union countries where endemo-epidemics continue [68, 80], the potential for further mumps outbreaks in the United Kingdom remains. This "lost cohort" shows that a susceptible group who are not fully immunized nor exposed to mumps as children can fuel a huge outbreak when mumps is introduced.

Besides the "lost cohort" in these large United Kingdom outbreaks, there was also evidence that waning immunity may have played a role [79]. Of the over 300 cases reported in children in 2004–2005, close to 17% had received one dose of MMR and 31% two doses. Vaccine effectiveness was 88% for one dose and 95% for two doses but the effectiveness of one dose declined from 96% in 2-year olds

to 66% in 11- to 12-year olds while the effectiveness of two doses declined from 99% in 5- to 6-year olds to 86% in 11- to 12-year olds [79]. This illustrates that these outbreaks have multi-factorial underpinnings albeit with one major factor – the "lost cohort" – predominating.

8.3 *Vaccine Program: Single Dose: Forgotten Cohort 2004–2007 Outbreaks in Canada, 2005–2007 in Australia*

In both Canada and Australia, mumps vaccine (Jeryl Lynn or a derivative) has been given routinely (MMR) for 30 years or so, with two-dose MMR regimens introduced in Canada in the mid 1990s (second dose at age 18 months or at 4–5 years of age depending upon the province) and in Australia in 1994 for children aged 10–16 years [7, 8]. In both countries, mumps outbreaks occurred in the past 5 years (2004–2007 in Canada; 2005–2007 in Australia). In Canada, the major outbreaks, involving over 1,200 cases, occurred in 2007 initially in Nova Scotia and then in Alberta (Fig. 7) [81]. Young adults were especially prominent with 58% of the cases occurring in those 20–29 years of age and 5% in those 30–39 years of age [81]. This age group represents a "forgotten cohort" in Canada. Those over 40 years of age at the time were likely immune to mumps due to natural exposure and infection as children. Those aged 12–17 years of age and younger in 2007 had received two doses of MMR vaccine due to the introduction of a second dose of measles–mumps–rubella (MMR) vaccine for measles control in 1996–1997 in most provinces and territories. This left the "forgotten" cohort – those born predominately between 1970

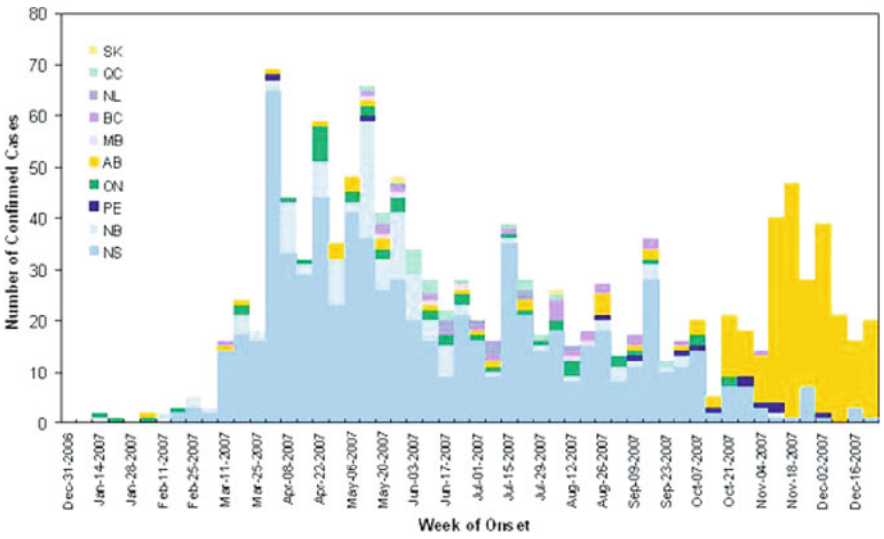

Fig. 7 Mumps outbreaks in Canada by province in 2007 [81] (Courtesy of Public Health Agency of Canada. © Public Health Agency of Canada, 2009 http://www.phac-aspc.gc.ca/mumps-oreillons/prof-eng.php)

and 1990 who had only received one dose of MMR vaccine [81]. The outbreak in Australia followed an eerily similar pattern – 41% of cases in young adults aged 20–29 years of age with cases concentrated in the birth cohort 1978–1982 [8]. This age group was too old for the two-dose program and too young for natural immunity through mumps infection as a child – a "forgotten cohort" again. Of note, in Canada the genotype of the mumps virus was G, similar to the wild mumps virus causing the major outbreak in the United Kingdom, suggesting spread from there [7]. The outbreaks in the "forgotten – only one dose – cohorts" in both Canada and Australia emphasize the earlier observations that a single dose of mumps vaccine in early childhood is not effective in eliminating endemic mumps in a country [13] as some remain susceptible due to waning immunity, as has been noted in the United Kingdom outbreaks discussed above [79].

The young adults in these forgotten cohorts form an especially vulnerable group for mumps due to their very social and mobile lifestyles [77, 81]. They have limited interest in adhering to isolation procedures or participating in immunization catch-up programs, frequently share living accommodations, have large social networks (bars/pubs/nightclubs and sports) where secretions can easily be spread, as well as having a penchant for travel during college holidays and breaks where mumps can be picked up and brought back to campus or their workplace. The fairly long infectious period (up to 16 days), long incubation period (14–25 days), and high rate of asymptomatic illness make mumps an ideal microbe to spread in this age group if they are not well protected by immunization. Additional cases in this demographic group and possibly in other jurisdictions where forgotten cohorts exist would not be unexpected.

8.4 Vaccine Failure: Primary Vaccine Failure: Ineffective Vaccine – Rubini

Outbreaks of mumps in countries where Rubini vaccine was used provide excellent examples of primary mumps vaccine failure. Although a very safe vaccine, it has very low or no clinical efficacy. In Singapore, three different MMR vaccines were used since 1990 – Jeryl Lynn, Urabe, and Rubini. Disease surveillance showed that despite a robust two-dose MMR program, mumps cases continued to occur, mainly in children under 15 years of age (61% of the cases) [82]. Where known, almost 74% had received Rubini vaccine, 21% Jeryl Lynn, and 5% Urabe. The vaccine efficacy was –55.3% (yes minus) for Rubini [83]. Similar poor outcomes with Rubini vaccine have been shown in Switzerland with no evidence of protection compared to 70% effectiveness of Jeryl Lynn [84]. A review of the literature by Dayan and Rubin noted vaccine effectiveness for Rubini of 0–33% [24]. The outbreaks in these two countries were noticeably in younger populations with clear evidence of primary vaccine failure. Given that not every child who has received Rubini has now received two doses of a more effective vaccine, countries where Rubini has been used have an aging cohort where further outbreaks of mumps may occur.

8.5 Vaccine Failure: Two-dose Vaccine Failure: Waning Immunity: 2006 Outbreaks in the United States

The large mumps outbreak in the United States provides yet another pattern. In the 2006 outbreaks, 85% of the mumps cases lived in eight contiguous states in the Midwest and over 80% were enrolled in college [9]. Overall, 63 and 84% of those between 18 and 24 years of age had received two doses of mumps vaccine. These were neither "lost cohort" (no vaccine) nor "forgotten cohort" (one dose only) outbreaks as had occurred in the United Kingdom and in Canada and Australia. The high two-dose mumps vaccine coverage did protect many students in these Midwest outbreaks, as shown by the relatively low secondary attack rate for clinical mumps in roommates of only 2.2–7.7% [85]. As in the Canadian and Australian outbreaks, the age and college lifestyle of those most affected may have contributed to fueling the outbreaks [20].

The waning immunity noted to have played a factor in contributing to the rise of mumps in children during the United Kingdom outbreak [79] may also have been at play in these Midwest outbreaks [20, 65]. Support for this hypothesis comes from the observation that, compared to roommates without mumps, those with mumps were more likely to have received their second dose of MMR more than 10 years earlier [85]. Antibody induced by the Jeryl Lynn mumps vaccine was able to effectively neutralize this wild genotype G strain of mumps [86], albeit the geometric mean titers were only about one-half of those against the vaccine strain. While the G genotype did have antigenic differences, these were not so great as to make the antibody response from Jeryl Lynn ineffective. Earlier studies in Japan have shown that secondary vaccine failures in school children with exposure to wild mumps may be due to lower avidity antibody [87].

9 Mumps Elimination in Finland

While many countries have experienced a recrudescence of mumps in the past decade after periods of good control with two-dose regimens, Finland has a more than 25 year history of nationwide elimination of mumps [11] with the most recent outbreak occurring in 1987–1988 [88]. While incidental cases have occurred in the past 10 years (two to eight cases per year in 2003–2007), these have been predominately in non-immunized people and have not led to outbreaks [11]. Many cases had a connection to a foreign country often where epidemics were occurring. Only limited secondary vaccine failures were seen: of seven of 22 mumps cases, three cases had had one dose while four cases had had two doses, all with Jeryl Lynn [11]. Of note, when Finland undertook its program to eliminate indigenous mumps, it ensured that not only did children receive vaccine, but also young adults in schools and in the military. The attention to this age group and long-standing programs that ensure very high rates of MMR uptake (over 95%) means that neither a lost nor a forgotten cohort is present in Finland. This rate of uptake also far exceeds the uptake

rates in the United States. However, Finland may be at risk for outbreaks given the potential for importation of mumps from countries where mumps is endemic combined with evidence of waning antibodies and no endemic mumps to boost response [11]. The clinical relevance of the demonstrated continued cell-mediated immunity in those who are antibody negative remains to be seen. Perhaps this will be protective.

10 Mumps: Public Health Control Strategies in Outbreaks

Public health units have employed a variety of strategies to limit the spread of mumps once an outbreak is occurring. Table 4 summarizes similarities and differences in isolation strategies used in Canada, the United Kingdom, and the United States [9, 75, 81, 89, 90]. Of note, the data to support these differences are limited and suggest that more work needs to be done to evaluate the effectiveness and cost of these strategies.

Table 4 Differing public health mumps isolation strategies used in outbreaks [9, 75, 81, 89, 90]

	Canada	United Kingdom	United States
Number of isolation days recommended	9*days (now 5)	None	5 or 9*days (varied by state) (now 5 days)
Individual follow-up of cases	Initially only – due to workload	No	Yes
Quarantine of contacts	No	No	No
Contact tracing for cases with air travel	No	No	Yes if travel >5 h
Immunization programs	Differed by province NS – school leaving – college/university students, AB – anyone up to age 25 years	School leaving, 16- to 23-year olds, university students	Differed by state Iowa – 18–46 years
Vaccine uptake in college/university programs	25% – not fixed forgotten cohort	20–30% – not fixed lost cohort	Not reported

*Originally 9 days, now 5

11 Mumps Outbreaks: Lessons Learned

There are many important lessons to be learned from these mumps outbreaks. In countries where mumps is uncommon, health-care providers may need reminders of how mumps can present in different age groups. These outbreaks have shown that single-dose mumps vaccine programs are inadequate for the control of endemic mumps. Even with two-dose programs, very high rates of uptake are required (95%

first dose; >80% second dose) if control is to be established. Herd immunity will not protect non-immunized pockets. Incomplete and low immunization rates among older adolescents and young adults can lead to large outbreaks. This is an age group where mumps can be easily spread and can be hard to control. Waning immunity, as measured by antibody titers, may be an underlying factor in mumps outbreaks in fairly well-immunized groups. The role of persistent cell-mediated immunity in long-term protection from mumps is unknown. Mumps vaccines vary in terms of adverse event rates and effectiveness. Leningrad-3, which is only a fraction of the cost of Jeryl Lynn [3, 91], is not only associated with more aseptic meningitis but also has higher effectiveness. Reliable laboratory diagnostic tests for mumps in a highly immunized population beyond detection by culture or PCR are needed. Lastly, these outbreaks suggest that long-term control of mumps with two doses of vaccine given in early childhood may not lead to control even with very high uptake rates if cell-mediated immunity and not antibody is the key to long-term protection. Additional booster doses may need to be considered in the future. This leads to the conclusion that ongoing surveillance for mumps is crucial as is further research into control.

12 Mumps Control: Unanswered Questions

A number of unanswered questions arise from observation of these outbreaks.

- How does mumps behave in a population with very high immunization rates over the long term? What will happen over time in Finland?
- Does eliminating cases of mumps in childhood through infant and preschool immunization lead to an increased number of older susceptible adults due to waning immunity and missed immunizations?
- Will booster doses of mumps vaccine be needed in older youth or young adults immunized in early childhood? If so, what vaccine would be best?
- Given the effectiveness and cost of Leningrad-3, is it time to reassess its use against Jeryl Lynn despite its rate of aseptic meningitis?
- Why has mumps now disappeared in Canada, Australia, and the United States?
- Does enzyme immunoassay correlate well with measured neutralizing antibodies?
- How do we measure protection against mumps? What level of antibody is protective? Is testing for cell-mediated immunity required to determine protection?
- What are the best strategies for controlling mumps in developing and developed countries that have no or poor mumps immunization programs? How can barriers to mumps vaccine be overcome?
- As the mumps viruses causing outbreaks change, do the vaccines need to change?
- Are catch-up programs needed for the lost and forgotten cohorts as well as those who have been immunized with ineffective vaccines such as Rubini?
- What are effective public health strategies for dealing with outbreaks of vaccine preventable disease like mumps in older youths and young adults?

13 Summary

This decade has seen an unprecedented resurgence of mumps in countries where mumps had previously been well controlled. The factors contributing to these mumps outbreaks have included vaccine program failures including failure to accept immunization by a select group, failure to immunize a cohort, and failure to provide a second dose to a cohort, as well as examples of primary and secondary vaccine failures. The Finnish data suggesting that for good control first dose uptake rates of 95% and second dose uptake rates of over 80% are required are sobering. Many industrialized countries have past uptake rates below this and in some, recent MMR uptake has fallen [78, 92] leaving a wider swath at risk for mumps. Mumps outbreaks are highly likely to re-occur. Mumps is indeed back.

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Neonatal Herpes Simplex Virus Infections: Where Are We Now?

Clara Thompson and Richard Whitley

Abstract Neonatal herpes simplex virus (HSV) infection continues to cause significant morbidity and mortality despite advances in diagnosis and treatment. Prior to antiviral therapy, 85% of patients with disseminated HSV disease and 50% of patients with central nervous system disease died within 1 year. The advent of antiviral therapy has dramatically improved the prognosis of neonatal HSV with initially vidarabine and subsequently acyclovir increasing the survival rate of infected neonates and improving long-term developmental outcomes. More recently, polymerase chain reaction has allowed earlier identification of HSV infection and provided a quantitative guide to treatment. Current advances in the treatment of neonatal HSV infections are looking toward the role of prolonged oral suppression therapy in reducing the incidence of recurrent disease. Of concern, however, are increasing reports of acyclovir-resistant HSV isolates in patients following prolonged therapy.

1 Background

Neonatal herpes simplex virus (HSV) infection continues to cause significant morbidity and mortality despite significant advances in treatment [1]. The current estimated rate of occurrence of neonatal HSV disease in the United States is approximately 1 in 3,200 deliveries and an estimated 1,500 cases of neonatal HSV infection per year [2]. The frequency of neonatal HSV disease varies from country to country; most nations have significantly lower incidences than the United States for reasons that are not clearly understood [3].

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2 Epidemiology of Neonatal HSV

HSV disease of the newborn can be acquired during one of three time periods: in utero, perinatally, or postnatally. The most common mode of transmission is via direct contact of the baby with infected vaginal secretions during delivery [2]. The risk of transmission is influenced by several factors. The risk is greater with primary HSV infection acquired during pregnancy compared to reactivation of previous infection [2, 4, 5]. Among mothers with primary infection, acquisition near the time of labor is the major risk factor for transmission to the neonate [6]. The risk of transmission increases with the length of time the membranes are ruptured [7] and is increased if there is disruption of the mucocutaneous barriers (e.g., through the use of fetal scalp electrodes) [2, 8]. Transmission of infections is substantially reduced by caesarean section [2]. Maternal seroconversion prior to delivery is also associated with a decreased risk of neonatal HSV. This observation is probably related to a protective role of HSV-specific maternal antibodies [9].

3 Clinical Manifestations of Neonatal HSV

HSV infections in newborns can be classified into three patterns, which occur with roughly equal frequency [10]. These comprise *disseminated disease* involving multiple visceral organs, including lungs, liver, adrenal glands, skin, eyes, and the brain; *central nervous system* (CNS) *disease*, with or without skin lesions; and disease limited to the skin, eyes, and/or mouth (*SEM disease*). Patients with disseminated disease and SEM disease present earliest, generally at 10–12 days of life, whereas CNS disease presents during the second or third week of life [10].

The initial manifestations of CNS disease are frequently non-specific and include temperature instability, respiratory distress, poor feeding, and lethargy, which can then progress to hypotension, disseminated intravascular coagulation (DIC), apnoea, and shock. Between 60 and 70% of babies with CNS disease have associated skin vesicles [11]. In disseminated disease, involvement of the CNS is a common feature occurring in approximately 60–75% of infants. Severe coagulopathy, liver dysfunction, and pulmonary involvement are further serious complications. Twenty percent of neonates with disseminated HSV disease will not develop cutaneous vesicles during the course of infection [10].

4 Mortality and Morbidity

Since the advent of antiviral therapy the prognosis of neonatal HSV has improved [11]. Prior to antiviral therapy, 85% of patients with disseminated HSV disease and 50% of patients with CNS disease died within 1 year [12]. With the use of high-dose acyclovir (60 mg/kg/day for 21 days), 12-month mortality has reduced to 29% for disseminated neonatal HSV disease and to 4% for CNS HSV disease [13].

Improvements in morbidity rates in these disease categories have not been so dramatic with the advent of antiviral therapy. Morbidity figures show that in survivors with neonatal disseminated HSV disease, normal neurological development occurs in 83% [11], an increase from 50% in the pre-antiviral era [12]. In the case of CNS neonatal HSV disease, little change has occurred with 31% of patients today having normal neurological development [12, 13]. In contrast, the morbidity from SEM disease has improved dramatically with the advent of antivirals with fewer than 2% of patients today having developmental delay after SEM disease compared with 25% historically [11, 12].

5 Antiviral Therapy

In 1980, the National Institute of Allergy and Infectious Diseases (NIAID) Collaborative Antiviral Study Group (CASG) reported the first successful trial of vidarabine for treatment of neonatal HSV infections [12]. Fifty-six infants were enrolled in the trial of which 31 were randomized to receive vidarabine at a dose of 15 mg/kg/day for 10 days. This study showed a significant reduction in mortality at 6 months, irrespective of gestational age at delivery, in babies with CNS and disseminated disease from 74 to 38% with vidarabine therapy (P=0.014). In addition, morbidity was improved threefold, as 29% of treated infants compared to 11% of placebo recipients developed normally at 1 year of life.

Although treatment with vidarabine was significantly better than placebo, mortality and morbidity remained high, irrespective of the dose of vidarabine used [14]. Also of concern was the fact that disease progression, from a lesser to more severe form, occurred in 8 out of 29 patients (21%) treated with vidarabine, and in infants with SEM disease who survived, 86% had recurrent skin lesions during the first year of life after treatment [14].

In the 1980s acyclovir was developed as a selective and specific inhibitor of viral replication. Initial reports suggested that acyclovir was superior to vidarabine in treatment of biopsy-proven HSV encephalitis [15]. Whitley et al. [16] conducted a randomized controlled trial comparing vidarabine and acyclovir and their effect on treatment outcomes of neonatal HSV infection in 210 infants with virologically proven neonatal HSV infection [16]. Ninety-five infants received intravenous vidarabine (30 mg/kg/day) and 107 infants received acyclovir (30 mg/kg/day) for 10 days. These results were disappointing, showing no significant differences between the two agents in either morbidity (P=0.83) or mortality (P=0.27) [16]. Despite adjusting for the extent of disease, the statistical power was insufficient to determine whether sizeable differences existed within disease categories. However, the number of patients that continued to shed virus during treatment declined more rapidly in the acyclovir group (P<0.001). The disease progressed in four infants during therapy, specifically disease advanced from being localized to the skin to involve the central nervous system or from brain involvement to multiorgan disease [16]. In six infants with disseminated infection, four treated with vidarabine and two receiving acyclovir, the disease spread to an additional organ after the beginning of therapy

[16]. Eight percent of babies relapsed after treatment, suggesting that not only a higher dosage but also a more prolonged course of intravenous therapy may be more effective.

6 Determining Dosage and Duration of Treatment

Kimberlin et al. [10] investigated the therapeutic efficacy and safety of high-dose acyclovir in the treatment of neonatal HSV disease [11]. In this study a more prolonged treatment course of 21 days was used. Sixteen patients received intermediate-dose acyclovir (45 mg/kg/day) and 72 patients received high-dose acyclovir (60 mg/kg/day). Data were also compared with those of a previous NIAID CASG trial in which 15 patients received standard-dose acyclovir (15 mg/kg/day) for 10 days [16]. Recipients of high-dose acyclovir had an increased survival rate compared to patients treated with standard-dose acyclovir (P=0.0035; OR=3.3; 95% CI: 1.5–7.3) (Figs. 1 and 2). A higher percentage of patients treated with high-dose acyclovir were developmentally normal at 12 months, but the difference was not significant (P=0.539; OR=1.5; 95% CI: 0.4–5.7). However, when controlled for the confounding factor of HSV type (HSV-1 vs. HSV-2) a borderline significant difference in the number of children returning to normal between high-dose and standard-dose acyclovir groups was found (P=0.051).

As a result of these studies [11, 16] the current recommended antiviral regimen for neonatal HSV infection is intravenous acyclovir 60 mg/kg/day divided into three doses (i.e., every 8 h) for 21 days in patients with CNS or disseminated disease [11] and for 14 days in patients with SEM disease [11].

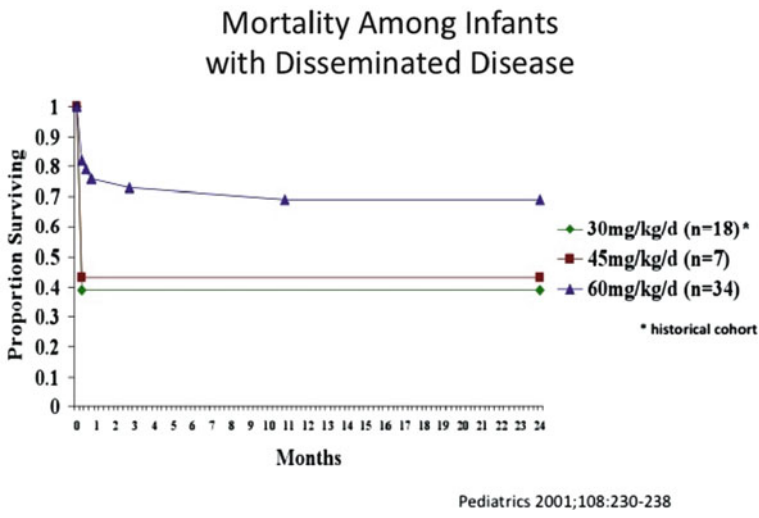

Fig. 1 Reduction in mortality from disseminated HSV disease with 60 mg/kg/day acyclovir

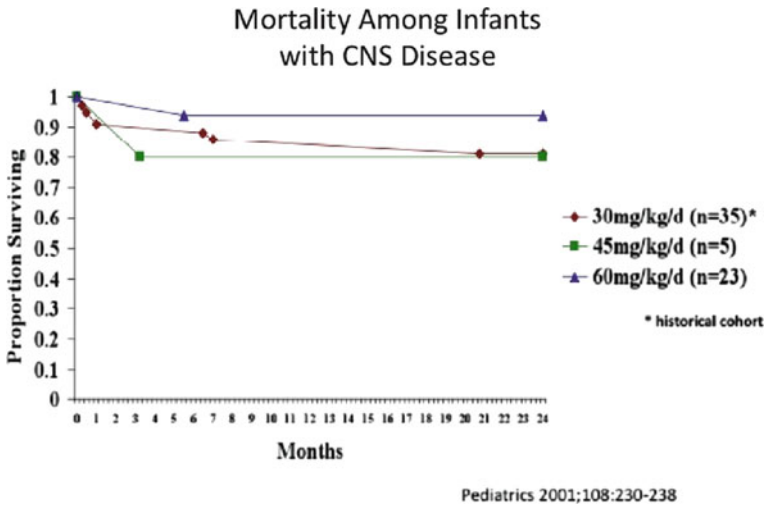

Fig. 2 Reduction in mortality from CNS HSV disease with 60 mg/kg/day acyclovir

7 Polymerase Chain Reaction (PCR)-Guided Therapy

Despite these achievements the morbidity and the mortality from neonatal HSV infection remain unacceptably high [11]. The diagnosis of neonatal HSV infections has been revolutionized by the application of PCR to clinical specimens including CSF [17] and blood [18]. The PCR method has enabled the detection and implementation of treatment earlier in the illness, before widespread viral dissemination throughout the body or significant replication within the nervous system, and it has also allowed clinicians to study the response to treatment.

Serial examinations of CSF for viral DNA have shown that HSV DNA is present for a mean of 10.1 days after onset of neurological disease and has been shown to temporarily recur in the CSF in patients with recurrent illness [19]. Kimberlin et al. retrospectively evaluated 77 neonates with culture-proven HSV disease by PCR [17]. They found that HSV DNA was detected by PCR in the CSF of 7 of the 29 infants who had been categorized previously as having SEM disease, 13 of the 14 infants previously classified as having disseminated disease, and 26 of the 34 infants previously categorized as having CNS disease [20]. This gave an overall specificity of 71% and sensitivity of 80%. In comparison, other retrospective studies have reported higher specificities of 100% [21, 22] and greater sensitivities of 100 [22] and 75% [23].

In addition to diagnosis, PCR has been used to guide treatment by establishing quantitative measures of assessing HSV DNA in the CSF of patients with HSV encephalitis to determine the effect of antiviral therapy on the clearance of viral DNA from the CSF [24]. This study suggested that patients with less than 100 copies of HSV DNA per microliter of CSF prior or within 4 days of initiation of treatment had more favorable outcomes (Fig. 3). Moreover, a retrospective study performed

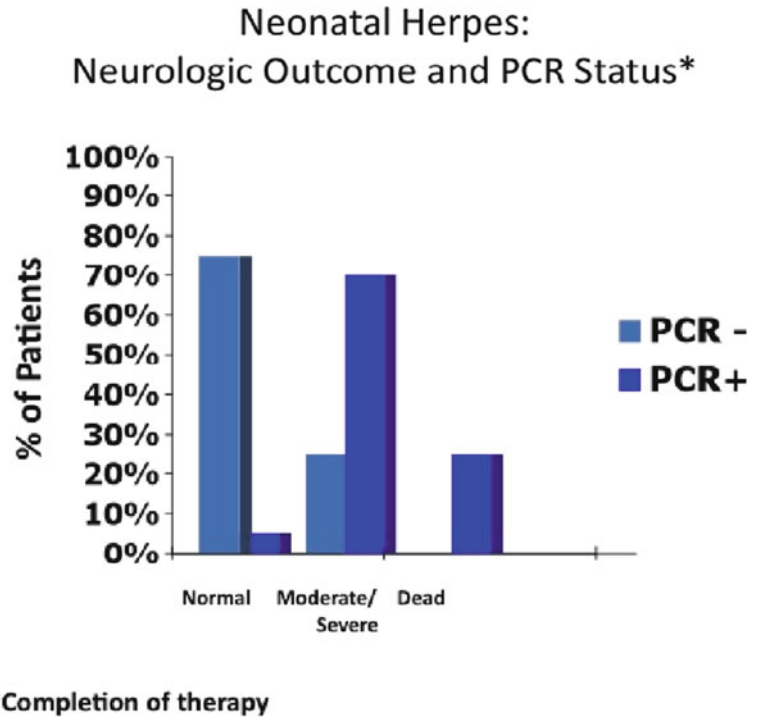

Fig. 3 Neurological outcome following completion of intravenous acyclovir therapy depending on the status polymerase chain reaction result in the CSF. PCR positive was defined as greater than 50 copies of HSV DNA per 200 μL

by Kimberlin et al. demonstrated that infants who had HSV DNA detected in the CSF by PCR after completing intravenous antiviral therapy had poorer outcomes compared to infants who were CSF PCR negative at the end of therapy [20]. The use of quantitative PCR measures of HSV DNA in combination with clinical observations and neuroimaging may help guide in defining the duration of therapy of CNS disease. Current recommendations are that all patients with CNS HSV disease should have a repeat lumbar puncture performed at the end of intravenous acyclovir treatment to ensure that the CSF PCR is negative [11]. Those patients who remain PCR positive continue to receive antiviral therapy until PCR negativity is achieved as a component of ongoing NIH studies in the United States [10].

PCR evaluation of serum from HSV-infected neonates is a promising diagnostic modality, since serum viral load correlates with classification of disease [25]. Neonates with disseminated disease have a statistically higher viral load than infants with CNS disease ($P<0.0001$) or infants with SEM disease ($P<0.001$) [25]. Serum viral load is also significantly higher in patients who die from neonatal HSV disease compared to those who survive and are neurologically normal ($P=0.005$) or who survive with neurological sequelae ($P=0.0008$). However, data on using

serum PCR in HSV disease are insufficient to use this modality to determine the effectiveness of antiviral therapy or guide the appropriate time to discontinue therapy [26].

8 Oral Suppression Therapy

Current advances in the treatment of neonatal HSV have looked toward the role of oral suppression therapy. The studies performed by the NIAID CASG demonstrated that there was a direct correlation between the frequency of recurrent skin lesions after HSV-2 SEM disease and the development of adverse neurological complications [27]. Normal development was seen in all babies with SEM disease who had fewer than three episodes of recurrent skin lesions over a 6-month period compared to only 79% of babies with three or more recurrences of skin lesions [16] (Fig. 4). As a result of these observations, the CASG conducted a Phase I/II trial of oral acyclovir therapy for the suppression of cutaneous recurrences after SEM disease in 26 infants [28]. Suppressive oral acyclovir therapy (300 mg/m^2 given either twice daily or three times per day) was administered for 6 months. Thirteen of the 16 infants (81%) who received acyclovir three times a day had no recurrent lesions while on therapy. However, in the 6 months after cessation of oral prophylaxis, 7 of the 16 patients on the three times a day regime experienced cutaneous recurrences of HSV disease. Interestingly, of those available for evaluation at 1 year of life (13 out of 18 on suppressive therapy), all were developing normally (Fig. 5).

Twelve of the 26 patients developed neutropenia during treatment. Ten of the 12 infants continued taking acyclovir without interruption throughout the period of neutropenia and had spontaneous recovery in their absolute neutrophil counts. The other two infants' neutropenia resolved after a dose reduction of 50% (Fig. 6).

The results of this study suggest that oral acyclovir could be given safely during the first 6 months of life if monitored closely for neutropenia. However, the study

Prognostic Factors
SEM Disease

Dominant Factors	Relative Risk Morbidity
# of cutaneous recurrences	
<3	**1**
≥3	**21**
Virus Type	
HSV-1	**1**
HSV-2	**14**

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Fig. 4 Factors affecting outcome in SEM HSV disease

Fig. 5 Cutaneous recurrences of HSV disease with twice daily vs. three times a day oral suppression therapy in 18 patients evaluated for safety, efficacy, and PK data

Cutaneous Recurrences of HSV During Suppressive Acyclovir Therapy

	300mg/m²/dose	
Number of Skin Recurrences	TID N-16	BID n-=2
0	13 (81%)	0 (0%)
1	1 (6%)	1 (50%)
2	1 (6%)	1 (50%)
≥3	0 (0%)	0 (0%)
Unknown	1 (6%)	0 (0%)

Pediatr Infect Dis J 1996,15.247-54

Fig. 6 Abnormal absolute neutrophil counts during twice daily vs. three times a day oral suppression therapy in 26 patients evaluated for safety and PK data

Abnormal Laboratory Values During Suppressive Oral Acyclovir Therapy

	Dosage (300mg/m²/dose)		
	TID	BID	Total
Abnormal Laboratory Value	N-21	N=5	N=26
Absolute Neutrophil Count			
<50/mm³	3 (14%)	1 (20%)	4 (15%)
500-1000/mm³	6 (29%)	2 (40%)	8 (31%)

Pediatr Infect Dis J 1996;15:247-54

lacked adequate power to assess the effect of suppressive therapy on neurological outcome.

9 Antiviral Resistance

A concerning finding from this study was the identification of an isolate of HSV that was highly resistant to acyclovir [28]. This was identified by culture in a patient with a cutaneous recurrence that occurred 36 h after cessation of the 6-month suppressive course of acyclovir.

There have been two further case reports in the literature that have documented acyclovir-resistant HSV in treated infants. One was in a premature infant with

SEM disease who, after completing 21 days of acyclovir, developed disseminated HSV with an acyclovir-resistant isolate and subsequently died [29]. Another identified an acyclovir-resistant HSV isolate from clinical specimens from a case of fatal disseminated neonatal HSV during the first 7 days of acyclovir therapy [30]. These case reports and the Phase I/II trial of suppression therapy illustrate that prolonged administration of oral acyclovir after neonatal HSV disease may have adverse virological consequences.

10 Conclusion

High-dose acyclovir (60 mg/kg/day) given in three divided doses over 21 days has been shown to improve both mortality and morbidity from neonatal HSV disease and is the current recommended treatment regime [11]. PCR has dramatically revolutionized the early diagnosis and implementation of treatment of neonatal HSV disease and is important in guiding the duration of treatment.

In the future the role of oral suppression therapy and its effect on neurological outcome needs to be assessed, as does the role of antiviral prophylaxis during pregnancy. Ultimately, elimination of neonatal HSV requires the development of an effective HSV vaccine that will protect against genital HSV-1 and HSV-2 infection and disease.

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Rational Approach to Pediatric Antifungal Therapy

William J. Steinbach

1 Introduction

To begin a rational approach to the treatment of pediatric invasive fungal infections, one has to first correctly diagnose the infections. While this is not the focus of this review, the critical nature of the correct diagnosis cannot be overemphasized. For instance, medically important fungi can be generally divided into yeasts and molds, and often that simplistic delineation is the only result the clinician will have for many days while the microbiology laboratory is fully identifying the pathogen. However, even this initial distinction can be crucial to beginning empiric antifungal therapy in the highest risk patients. While a yeast infection can largely include *Candida*, *Cryptococcus*, or *Trichosporon* species, a mold infection is more complicated as they can be further subdivided into septate and aseptate (without hyphal septa). Without listing all available nuances, the most clinically relevant divide is that Zygomycetes contain non-septate hyphae, while important septate hyphal infections include *Aspergillus*, *Fusarium*, *Scedosporium*, and *Paecilomyces* species. This distinction between septate and aseptate is not purely academic, as the treatment options differ vastly. A more complete synopsis on the currently available diagnostic strategies for the two most common invasive fungal infections, infection with *Candida* and *Aspergillus* species, can be found in the recent *Infectious Diseases Society of America* (IDSA) guidelines [1, 2].

Since the initial approval of amphotericin B deoxycholate by the United States (US) Food and Drug Administration in 1958, there have been a total of 14 systemic antifungals approved for clinical use. While this number seems daunting, it is all the more complex when one considers that different agents possess vastly different properties and indications. For instance, the antifungal sites of action differ among the classes of antifungals. While the polyene class (largely amphotericin B products) functions by cell membrane osmotic lysis, the azole class functions by inhibiting cell

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membrane ergosterol production and the allyamine class (e.g., terbinafine) works by inhibiting squalene epoxidase also in the cell membrane. This is in contrast to the echinocandin class which uniquely inhibits fungal cell wall β-1,3-glucan synthesis. Additionally, some agents act in a fungicidal fashion to kill the pathogen, while others function fungistatically. One important distinction lies in the azoles, which are fungistatic against *Candida* infections, but fungicidal against mold infections. This is in contrast to the echinocandin class, which acts as a fungicide against *Candida* infections and only in a fungistatic manner against mold infections. This point may be crucial for a neutropenic patient who would rely on a fungicidal agent for efficacy.

2 Treatment of Pediatric Invasive Aspergillosis

Amphotericin B deoxycholate has been the first-line therapy of invasive aspergillosis (IA) and the gold standard against which other therapies have been measured. However, with overall survival rates of ~34% among patients treated with amphotericin B, in addition to limited tolerance as a result of acute and chronic toxicities, there was a clear need to develop a better antifungal. While itraconazole became available in 1990 for the treatment of *Aspergillus* infection, it was not without flaws; most prominent among these were decreased fungicidal activity compared to amphotericin B, unpredictable bioavailability in high-risk patients, and significant drug interactions [3]. The next step taken in the search for a better antifungal was the development of three different lipid formulations of amphotericin B whose advantages included an increase in the daily dose, high tissue concentrations with better delivery to reticuloendothelial organs, decrease in infusion-related side effects, and decrease in renal toxicity [4]. These three lipid formulations, amphotericin B lipid complex, amphotericin B colloidal dispersion, and liposomal amphotericin B, are currently indicated for patients with systemic mycoses, primarily IA, who cannot tolerate or are refractory to conventional amphotericin B [3].

Voriconazole is a second-generation triazole and a synthetic derivative of fluconazole that is now recommended in the IDSA guidelines for primary therapy of IA [2]. A multicenter, randomized, open trial compared the outcome of patients receiving either intravenous voriconazole or amphotericin B deoxycholate for primary therapy of invasive aspergillosis [5]. In the voriconazole arm a total of 144 patients (13–79 years, mean 48.5 years) received a loading dose of 6 mg/kg IV BID on day 1, followed by 4 mg/kg IV BID for at least 7 days, and 200 mg BID orally for a total of 12 weeks. In the amphotericin B treatment group, 133 patients (12–75 years, mean 50.5 years) were administered 1.0–1.5 mg/kg IV once daily. The overall response rate at 12 weeks showed statistically significantly more complete and partial responses in patients treated with voriconazole (52.8%) compared to patients treated with amphotericin B (31.6%). Voriconazole-treated patients also had a higher survival rate than amphotericin B-treated patients (70.8 vs. 57.9%; $p = 0.02$) and showed significantly fewer drug-related severe adverse side affects. While this study revolutionized the landscape of invasive aspergillosis treatment, the study did not include children younger than 12 years and enrolled very few under 18 years of age.

A minority of patients in the voriconazole group (52/144 (36%)) and a majority of patients in the amphotericin B group (107/133 (80%)) received another licensed antifungal therapy in addition to their primary study drug, resulting in a smaller final group of patients receiving only voriconazole or only amphotericin B. Patients who received voriconazole as initial therapy and then switching to another antifungal drug still had a higher rate of complete and partial responses than patients who received amphotericin B as initial therapy followed by another antifungal agent (48 vs. 38%) [6], suggesting that the initial therapy with the triazole was more effective.

The largest pediatric voriconazole study reported on 58 children (ages 9 months to 15 years, mean 8.2 years) with invasive fungal infections, generally IA, that were refractory to or intolerant of conventional antifungal therapy [7]. Voriconazole was administered as a loading dose of 6 mg/kg IV BID on the first day of therapy, followed by 4 mg/kg IV BID. If feasible, the IV dose was then later switched to an oral dose of 100 or 200 mg BID for children weighing < 40 or ≥ 40 kg, respectively. Responses to the therapy with voriconazole were complete or partial in 26 (45%), stable in 4 (7%), and failed in 25 (43%) patients. In a subsequent case series, voriconazole was used as salvage therapy in seven children (age range 2–13 years, median 5 years) with invasive aspergillosis [8]. Complete and partial response was observed in each of two patients, stable response in one patient, and failure of the voriconazole treatment in two patients.

One large remaining clinical question is the issue of salvage therapy for IA after primary therapy fails. The IDSA guideline recommendations state that options include adding an additional antifungal in combination therapy, or switching antifungals to a different class of drugs, such as an amphotericin B product or an echinocandin. The echinocandins are an entirely new class of antifungals which interfere with cell wall biosynthesis. This is a distinct mechanism of action compared to the second-generation triazoles which work by inhibiting cytochrome P450 enzymes responsible for conversion of lanosterol to sterol and thus compromising the fungal cell membrane [9]. Three compounds are currently approved in the United States: caspofungin, micafungin, and anidulafungin.

The first clinical trial with an echinocandin against IA [10] was an open-label, non-comparative salvage study of caspofungin in 90 immunocompromised patients with definite or probable IA. Caspofungin produced a complete or partial response in 45% of patients, including 50% of 64 patients with pulmonary IA and 23% of 13 patients with disseminated aspergillosis. This was validated by two subsequent clinical trials. Caspofungin resulted in complete resolution or stabilization of disease in 74% patients (n=31) in one of these trials. In the other trial, caspofungin had an overall response rate of 56% (n=32) [11, 12]. Micafungin is another echinocandin with median minimum inhibitory concentration (MIC) values for *Aspergillus* species substantially lower than those of other non-echinocandins [13]. A non-comparative, open-label, multicenter study in adult and pediatric patients to examine the safety and efficacy of micafungin in the treatment of IA that had failed to respond to prior therapy or could not tolerate other therapy was conducted during 1998–2002. Of the 225 patients who met diagnostic criteria, a favorable response rate was seen in 35.6% (80/225), and of those treated with only micafungin, a favorable response was seen in 6/12 (50%) of the primary and 9/22 (40.9%) of the

salvage therapy group [14]. Anidulafungin is the third echinocandin which has been approved in the United States for treatment of candidemia and esophageal candidiasis and in an in vitro study looking at its efficacy against *Aspergillus* species isolates, it showed it to be the most active antifungal agent of those tested, with an MIC90 of <0.03 mg/L [15]. Most clinicians use the echinocandins in salvage therapy. The major difficulty with the echinocandins in IA therapy is that they are fungistatic against *Aspergillus* species, acting specifically on the growing hyphal tip only [16].

Another new triazole is the recently approved posaconazole, a derivative of itraconazole. The bioavailability of posaconazole increases significantly when administered in divided doses. One study showed the bioavailability of posaconazole in fasting healthy volunteers was higher in those receiving 800 mg/day divided into four doses than those receiving 800 mg/day divided into either one or two doses [17]. When administered in the fed (non-fasting) state, 800 mg/day divided into two doses gave similar serum levels to dosing four times daily and is more practical for compliance. Another study reported on posaconazole in 98 adult patients with febrile neutropenia or refractory fungal infections and found that a daily dose of 800 mg/day given as 400 mg BID provided the greatest posaconazole exposure [18].

There are currently very limited pediatric pharmacokinetic data for posaconazole. Serum samples obtained on 12 pediatric (aged 8–17 years) and 194 adult (aged 18–64 years) patients from a multicenter, phase 3, open-label study of patients with invasive fungal infections refractory to standard antifungal therapies were analyzed as preliminary comparisons of adult and child pharmacokinetics [19]. All patients received a maintenance dose of 800 mg/day in divided doses except one pediatric patient who obtained 400 mg/day as a divided dose on the day of sample collection. The mean plasma concentrations of posaconazole in children and adults were 776 ng/mL (median 579 ng/mL; range 85.3–2,891 ng/mL) and 817 ng/mL (median 626 ng/mL; range 0–3,710 ng/mL), respectively, suggesting similar plasma concentrations in pediatric and adult patients. One limitation of this study is that while the age range of the 12 pediatric patients was 8–17 years, it consisted of a single 8-year-old patient, a single 10-year-old patient, and all other patients were older than 12 years of age. This skew toward teenagers could explain the results being similar to adult dosage findings.

2.1 Voriconazole Dosing in Children

While the antifungal choice is important in treating IA, the dose selection is paramount since many antifungals are not dosed correctly in children following adult parameters. An open, multicenter study investigated the safety, tolerability, and pharmacokinetics of intravenous voriconazole in immunocompromised pediatric patients [20]. This study included a single-dose study of 11 patients aged 2–11 years (mean age 5.9 years) receiving 3 or 4 mg/kg and a multiple-dose study of 24 patients aged 2–11 years (mean age 6.4 years) receiving a loading dose of 6 mg/kg BID on day 1, followed by 3 mg/kg BID on day 2 to day 4, and 4 mg/kg BID

on day 4 to day 8. In contrast to the previously established nonlinear elimination of voriconazole in healthy adult volunteers [21] and adult patients [22], this study found linear pharmacokinetics of voriconazole in children over a dosage range of 3–4 mg/kg BID. Therefore, children require higher dosages of voriconazole than adults to achieve comparable serum levels and a maintenance dose of 4 mg/kg BID in children approximates to only 3 mg/kg BID in adults [20]. Extrapolation of area under the curve (AUC) and maximum serum pharmacokinetic levels in children revealed an estimated 11 mg/kg/dose would be required to achieve adult values obtained with only 4 mg/kg/dose if linear pharmacokinetics was maintained throughout the full dosage interval.

A subsequent pharmacokinetic study evaluated higher voriconazole doses and studied 82 children separated into two age groups (2–6 years, 6–12 years) [23]. Each child received either 4 mg/kg/dose followed by 6 mg/kg/dose or 6 mg/kg/dose followed by 8 mg/kg/dose. Each child also received at least two different doses in escalating order and then was switched to oral voriconazole. The intravenous doses had similar pharmacokinetic parameters within each age cohort, except a greater AUC level in the 8 mg/kg/dose range in the older age cohort. After oral dosing, the older age group had higher AUC and maximum serum levels compared to the younger children. However, even at the 8 mg/kg/dose, the AUC in children was still less than that seen with adult patients at the 4 mg/kg/dose level. Interestingly, the bioavailability of oral voriconazole was lower (44.6%) than seen in adult patients (96%) [24]. This study also demonstrated a high interpatient pharmacokinetic variability, as has been reported for studies concerning voriconazole drug level monitoring, but no significant differences were seen in the 2- to 6-year compared to the 6- to 12-year age groups except at the 8 mg/kg/dose.

The European Medicines Agency (EMEA)-approved dosing for voriconazole in children is to load with 7 mg/kg/dose for 1 day and then continue with the same 7 mg/kg/dose as a maintenance dose. This dosing recommendation is a clear step forward in the correct use of voriconazole in children compared to earlier uses when adult dosages were followed and infected children were greatly underdosed. However, this second pharmacokinetic study raised several important points that remain inconclusively investigated. The higher dose of 8 mg/kg/dose still yielded an AUC level lower than in adult patients received a standard dosing, suggesting that the optimal voriconazole dose in children is likely even higher. With linear pharmacokinetics in children, clinicians have a wider therapeutic window with which to safely increase the voriconazole dose. These dosing concerns are especially problematic with the known differences in metabolism across patient subpopulations and the concerns of a lack of standard effective voriconazole level goals.

For children, voriconazole is also available as an orange-flavored suspension (40 mg/mL). A common practice in pediatrics is to crush tablets and administer them to pediatric patients through a gastric or duodenal tube. A recent study examined the bioavailability of crushed or whole voriconazole tablets in an open-label, randomized, two-way crossover study in 20 health volunteers [25]. While there was

a slightly faster time to maximum serum concentration with the crushed tablets (0.5 vs. 1.5 h), the bioavailability was equivalent in crushed and whole tablets.

Voriconazole is known to be mostly metabolized by the hepatic enzyme CYP2C19, a subfamily of the cytochrome P450 (CYP) [26]. The genotype of CYP2C19 plays a crucial role in the pharmacokinetics of voriconazole. Due to a point mutation in the gene encoding CYP2C19, some individuals are poor metabolizers with fourfold higher levels of voriconazole compared to extensive metabolizers. Additionally, patients can be either homozygous or heterozygous for this metabolization phenotype. Poor metabolizers compose about 3–5% of the Caucasian and African population, but up to 15–20% of the Asian population [20]. This suggests that optimal dosing of voriconazole may be related to pharmacogenomics, which could loosely be assisted by using ethnicity as an indirect marker.

Measuring voriconazole therapeutic levels is complicated due to the large interpatient variability seen among patients. It is generally believed that it is best to have a voriconazole level above the MIC of the organism (typically > 0.5 μg/mL) and below the levels of clear toxicity. However, there is no clear extrapolation between higher levels equating to better clinical efficacy. While some studies have suggested that a cutoff of 2.0 μg/mL is appropriate [27], many other studies have shown no clear relationship [28]. Additionally, most of the voriconazole levels reported in these studies are random levels, when a consistent trough or peak is required to make pharmacokinetic conclusions.

2.2 Combination Therapy for Invasive Aspergillosis

Although there are several reviews of combination therapy in systemic mycoses [29, 30], only a few retrospective clinical reviews of combination IA therapy exist [31, 32]. There has only been a single published combination antifungal clinical trial for IA [33] and it was never completed. That study enrolled 18 patients with documented pulmonary aspergillosis; only one of nine patients receiving amphotericin B monotherapy survived and two of nine treated with amphotericin B plus 5-fluorocytosine (5-FC) survived. The study was terminated because of poor outcome in both arms, but outcome might have been poor because a lower dose of amphotericin B (0.5 mg/kg/day) was used and a definite diagnosis was required. Therefore, in view of the low number of patients and the poor outcome in both arms, no firm conclusion could be drawn about the possible superiority of that combination therapy.

Although no controlled clinical trial supports its use and the efficacy of combination therapy for IA has not been conclusively established [34], clinicians are desperately seeking new strategies for improved outcome. However, the experimental and clinical data do not reveal proven benefits with combinations, nor do they fully define the preferred combination for further exploration in a large clinical trial. The range of data from synergy to antagonism actually parallels the wide range of

unproven treatment practices used by clinicians today searching for the best care for their patients.

The largest analysis of combination therapy for IA reviewed 6,281 total cases of IA management and added an additional 12 years and 386 clinical cases of combination antifungal therapy in 236 articles [35] to an earlier 1990 study [32]. In that analysis of a total of 249 clinical combination cases in 128 articles, three combination regimens comprised the majority of reported clinical experience. Many (49%) involved amphotericin B plus 5-FC, while amphotericin B plus itraconazole (17%) and amphotericin B plus rifampin (11%) were less common. While these data document the use of combination therapy in the past, the combinations used historically are not necessarily the combinations employed by today's clinician.

One study to help address this issue of combination therapy analyzed voriconazole plus caspofungin in a retrospective review of 47 patients with proven or probable IA from 1997 to 2001 who experienced failure of primary therapy with amphotericin B formulations [36]. Patients initially received therapy with amphotericin B ($\geq$ 1 mg/kg/day) or a lipid formulation (ABLC or liposomal amphotericin B, both at 5 mg/kg/day). Salvage therapy was begun with either voriconazole (n=31) or with voriconazole plus caspofungin (n=16) after 7 days or more of amphotericin B therapy. The change to the combination antifungal therapy was a change made in the institution's protocol in February 2001 in the middle of the retrospective window analyzed. In early 2001 the protocol was altered so that caspofungin (70 mg load, then 50 mg daily) was used in combination with voriconazole therapy, thus creating the combination therapy arm of this retrospective analysis. Importantly, the majority of patients received salvage therapy due to clinical failure, not antifungal intolerance, yet no patients received their voriconazole or voriconazole plus caspofungin as primary therapy for IA.

The overall survival rate of 3 months after the day of diagnosis of IA was higher among those who received combination therapy (p=0.048). Similarly, 3-month survival after the start of salvage therapy was greatest among combination therapy patients (hazard ratio (HR) 0.43; 95% CI 0.17–1.1, p=0.050). Finally, the probability of death was also lower in those combination patients (p=0.024). In the bivariable analysis [controlling for antifungal therapy and receipt of hematopoietic stem cell transplantation (HSCT)], the combination showed an improved 3-month overall survival rate (HR 0.27; 95% CI 0.09–0.78; p=0.008).

Others have suggested that the preclinical data with combination therapy seem promising, yet inconsistent [37]. The current clinical use of combination therapy for IA is rampant, even in the absence of a definitive clinical trial advocating its benefit. There are many editorials on combination therapy design questions, calls to arms to begin a combination antifungal clinical trial, standardization attempts for animal models, and opinions on the value of in vitro checkerboard testing. The data continue to mount with no clear indication. Perhaps we will never achieve the same improvement in mortality seen with changing from amphotericin B to voriconazole, but will only be able to obtain a slight increase in benefit with combination strategies. Maybe a decrease in fungal burden is all we can hope for and this will translate into clinical benefit.

3 Treatment of Pediatric Invasive Candidiasis

New IDSA guidelines on the treatment of invasive candidiasis [1] divide therapeutic choices based on neutropenic or non-neutropenic patients. In non-neutropenic patients, fluconazole or an echinocandin is recommended as initial therapy, and an echinocandin is preferred in those patients who have had recent azole exposure. In those patients who have *Candida* isolates likely susceptible to fluconazole, then a transition from an echinocandin to fluconazole is recommended once the patient is stable. However, for infection due to *Candida krusei* or *Candida glabrata*, echinocandin therapy is recommended due to the history of fluconazole resistance in these species. Fluconazole is the preferred agent for infection with *Candida parapsilosis*, due to the concerns about lower in vitro and possibly clinical efficacy with an echinocandin against this species. In neutropenic patients, an echinocandin is recommended for most patients except those with known infection with *C. parapsilosis* where fluconazole or a lipid amphotericin B product is recommended. However, if the patient with *C. parapsilosis* infection is clinically stable, then there is no need to change the echinocandin. In neonatal candidiasis, amphotericin B is recommended and flucytosine is not routinely recommended. Importantly, a lumbar puncture is crucial due to the high incidence of neonatal meningitis in neonates with candidemia [38], and intravascular catheter removal is critical for successful treatment.

It is clear that simple conversion of the corresponding adult dosage of fluconazole on a weight basis is inappropriate for pediatric patients. Fluconazole clearance is generally more rapid in children than adults, with a mean plasma half-life of approximately 20 h compared to approximately 30 h in adult patients. Therefore, to achieve comparable exposure in pediatric patients, essentially the daily fluconazole dose needs to be doubled. Correct pediatric fluconazole doses should be proportionately higher than adult doses, generally 12 mg/kg/day. In neonates, the volume of distribution is significantly greater and more variable than in infants and children, and doubling the dose for neonatal patients is necessary to achieve comparable plasma concentrations. The increased volume of distribution is thought to be due to the larger amount of body water found in the total body volume of neonates. A recent population pharmacokinetic study in premature infants suggests that maintenance of fluconazole doses of 12 mg/kg/day is necessary to achieve exposures similar to older children and adults [39]. In addition, a loading dose of 25 mg/kg would achieve steady-state concentrations sooner than the traditional dosing scheme. Side effects of fluconazole are uncommon, but generally include gastrointestinal upset (vomiting, diarrhea, and nausea) or a skin rash.

Prophylaxis with fluconazole to prevent neonatal candidiasis remains a controversial topic. In a prospective, randomized double-blind trial over a 30-month period of 100 infants with birthweights less than 1,000 g, those infants who received fluconazole for 6 weeks had a decrease in fungal colonization (22 vs. 60%) as well as a decrease in the development of invasive fungal infection compared to placebo (0 vs. 20%) [40]. Other studies have yielded similarly encouraging results and demonstrated that the use of fluconazole prophylaxis for 4–6 weeks in high-risk infants did not increase the incidence of fungal colonization and infections caused by natively

fluconazole-resistant *Candida* species. However, the universal implementation of such strategy across nurseries is discouraged because the rate of *Candida* infections varies greatly among centers and there is insufficient neurodevelopmental follow-up data in these infants to justify prophylaxis.

The pharmacokinetics of caspofungin is slightly different in children compared to adults, with caspofungin levels lower in smaller children and with a reduced half-life. A study evaluated the pharmacokinetics of caspofungin in children with neutropenia and showed that in patients receiving 50 mg/m^2/day (maximum 70 mg/day), the levels were similar to those for the exposure in adults receiving 50 mg/day and were consistent across age ranges [41]. In this study, weight-based dosing (1 mg/kg/day) was suboptimal when compared to body surface area regimens, so caspofungin should be appropriately dosed in children as a loading dose of 70 mg/m^2/day, followed by daily maintenance dosing of 50 mg/m^2/day.

Caspofungin in children has been reported as safe and yields similarly good efficacy as seen in adult patients. Caspofungin pharmacokinetics was evaluated in older infants and toddlers at 50 mg/m^2/day and found to be similar to adults receiving the standard 50 mg daily dose [42]. Caspofungin in newborns has been used off-label as single or adjuvant therapy for refractory cases of disseminated candidiasis. Neonates with invasive candidiasis are at high risk of central nervous system involvement; it is not known if the dosages of caspofungin studied provide sufficient exposure to penetrate the central nervous system at levels necessary to cure infection. Therefore, caspofungin is not recommended as monotherapy in neonatal candidiasis.

The pharmacokinetics of micafungin has been evaluated in children and young infants. An inverse relation between age and clearance was observed, with mean systemic clearance significantly greater and mean half-life significantly shorter in patients 2–8 years of age compared to those 9–17 years of age. Therefore, dosing of micafungin in children is age related and needs to be higher in children under the age of 8 years [43]. To achieve micafungin exposures equivalent to adults receiving 100, 150, and 200 mg daily, as evidenced by simulation profiles, children require dosages higher than 3 mg/kg. A pediatric substudy as part of a double-blind, randomized, multinational trial comparing micafungin (2 mg/kg/day) with liposomal amphotericin B (3 mg/kg/day) as first-line treatment for invasive candidiasis [44] showed similar success for micafungin or liposomal amphotericin B. In general, micafungin was better tolerated than liposomal amphotericin B as evidenced by the fewer adverse events that led to discontinuation of therapy.

Several pharmacokinetic studies of micafungin in term and preterm infants [45] have shown that infants have a shorter half-life and a more rapid rate of clearance compared with published data in older children and adults. These results suggest that young infants should receive 10 mg/kg daily of micafungin if used to treat invasive candidiasis.

Anidulafungin has the longest half-life of all the echinocandins (approximately 18 h). In a study of 25 neutropenic children receiving anidulafungin as empirical therapy, four patients in the group receiving 0.75 mg/kg/day experienced adverse events such as facial erythema and rash, elevation in serum blood urea nitrogen, and fever and hypotension. The concentration profiles at those maintenance doses

of anidulafungin in pediatric patients 2–17 years of age were similar to those of adult patients receiving 50 or 100 mg/day. The current anidulafungin formulation requires reconstitution with 20% dehydrated alcohol, therefore, its safety and pharmacokinetic profile in infants younger than 2 years is being evaluated.

A randomized, double-blind study in adult patients without neutropenia showed that anidulafungin was not inferior to fluconazole in the treatment of invasive candidiasis [46]. In this study, the frequency and types of adverse events were similar in the two groups, and all-cause mortality was 31% in the fluconazole group and 23% in the anidulafungin group. No clinical studies of anidulafungin in pediatric patients are currently available.

4 Conclusion

The rational approach to antifungal therapy for pediatric invasive fungal infections begins with the correct diagnosis, as many infections require different approaches. Established guidelines for some of the more common infections are helpful for the clinician. Selection of the correct antifungal has many nuances, but especially important in pediatrics is the use of the correct pharmacokinetically proven dose in children. The future of the field lies in dedicated investigations into both pediatric disease epidemiology and diagnosis, as well as focused pediatric therapeutic trials through international consortiums such as the International Pediatric Fungal Network (http://pfn.pediatrics.duke.edu).

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Antiviral Therapy of CMV Disease in Children

Mike Sharland, Suzanne Luck, Paul Griffiths, and Mark Cotton

Abstract Cytomegalovirus (CMV) remains an important cause of morbidity and mortality in infants and children. The main burden of disease occurs in congenital infection, postnatal infection in premature infants and in older immunocompromised children (now predominantly following transplantation) in developed countries. In lower income countries, CMV is a major co-pathogen in human immunodeficiency virus [HIV]-infected infants. Antiviral treatment options remain very limited. The guanosine analogue ganciclovir (GCV) was first used in children over 20 years ago, but the optimal dose, duration and route of administration remain poorly evidence based. In particular there are very limited data in premature infants and older children. Direct comparison studies between the intravenous ganciclovir and the oral valyl-ester valganciclovir (VGCV) have not been performed. CMV disease is important, but not very common and there remains a need to identify useful surrogate markers of successful antiviral therapy to facilitate clinical trials. Cidofovir and foscarnet have very significant toxicity. No other anti-CMV agent has successfully completed phase III studies. There remain few other antiviral agents effective against CMV on the horizon. This chapter reviews the current clinical spectrum of CMV disease in childhood and the evidence base for both GCV and VGCV use in clinical practice. It also discusses the antiviral studies currently being performed and those that need to be performed.

1 Spectrum of Clinical Disease

1.1 Congenital CMV

Congenital CMV (cCMV) is the most common congenital infection in developed countries, causing very significant long-term disability due to deafness and mental handicap [1, 2]. Birth prevalence varies widely both between and within different

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countries and has been reported to occur in around 0.18–6.2% of live births [2]. A study based in London reported CMV infection in 0.33% of live births [3]. Similar prevalence has been reported in Austria and Italy [2]. Historically the diagnosis has been made by isolation of CMV from a body fluid within the first 21 days of an infant's life. The development of methods for detecting CMV DNA in blood captured on Guthrie cards (dried heel-prick blood spots) taken after birth in most developed countries has enabled the retrospective diagnosis of congenital CMV in older babies, although not all infected babies have detectable viraemia at birth. CMV disease also occurred commonly in infants born to mothers with HIV and CMV coinfection prior to the use of perinatal antiretroviral therapy and the subsequent marked reduction in mother to child transmission of HIV. This remains a major problem in resource-poor countries.

1.1.1 Clinical Features

Congenital CMV infection is usually asymptomatic. A recent meta-analysis reported that symptomatic infection (historically referred to as CMV inclusion disease or CID) is seen in around 12.7% (0.0–25%) of babies with cCMV [2]. The classical features of CID are thrombocytopenia, blueberry muffin rash, petechiae, intrauterine growth retardation (IUGR), microcephaly, hepatosplenomegaly and jaundice (Table 1). Long-term sequelae are mainly in the form of sensorineural hearing loss, neurodevelopmental delay and, rarely, visual loss.

Table 1 Definition of symptomatic disease

One or more of the following
a. Thrombocytopenia
b. Petechiae
c. Hepatomegaly
d. Splenomegaly
e. Intrauterine growth restriction
f. Hepatitis (elevated transaminases and/or bilirubin)
g. Central nervous system involvement of the CMV disease [including microcephaly, radiographic abnormalities indicative of CMV CNS disease, abnormal CSF indices for age, chorioretinitis, hearing deficits as detected by formal brainstem-evoked response (not a screening auditory brainstem response ABR) and/or positive CMV PCR from CSF]

Studies have reported that 40–58% of infants who are symptomatic at birth develop permanent sequelae compared to 13.5% of those without symptoms [1]; around 18% of all congenitally infected infants will be left with some long-term impairment. Characteristic of CMV has been the discovery that hearing loss may be progressive, fluctuate and have a late onset. The pathological processes underlying this delayed presentation of symptoms are currently uncertain and the true incidence is probably underestimated due to inadequate follow-up periods [1]. Overall congenital CMV is reported to cause around 20% of all cases of sensorineural hearing loss and up to 40% of profound hearing loss cases in childhood [4, 5]. CMV

therefore has very significant cost implications for the wider community relating to the healthcare needs, education and support of these children.

1.1.2 Predicting Long-Term Neurological Impairment

Clinical Associations

IUGR, petechiae, hepatosplenomegaly, hepatitis, thrombocytopenia and intracerebral calcifications have been established as risk factors for subsequent hearing impairment in one cohort study, although only IUGR and petechiae remained independent risk factors following multiple regression analysis [6]. Another study has reported microcephaly (adjusting head (occipito-frontal) circumference for weight) as being the most specific predictor for poor neurological outcome whereas a normal CT scan and head circumference were associated with a good outcome [7]. However, clinical features alone are of limited use in predicting those destined for subsequent sensorineural hearing loss, particularly if babies are only mildly symptomatic or asymptomatic at birth. There remains a real need to develop surrogate markers for prediction of long-term outcomes in clinical trials.

Viral Load

Studies in adult transplant recipients have correlated systemic viral load with the risk of clinical disease [8, 9]. An early culture-based study of serial urine specimens taken from both congenitally infected and postnatally infected infants conducted over 30 years ago showed that viral load in the early neonatal period was higher in those babies with symptomatic congenital infection when compared to those with asymptomatic or postnatal infection [10]. The presence of viraemia was first reported to be associated with a poor hearing outcome in neonates with CMV disease in 2005 [11], and subsequently an association has been reported between quantitative CMV viral load at birth in urine, blood and in dried blood spots and symptomatic infection later in childhood [6, 12–14]. Recent data from one of the groups that initially described an association between viral load and hearing outcome do not demonstrate blood viral load to be different between those with and without subsequent hearing loss among either symptomatic or asymptomatic babies. Despite this, among asymptomatic babies, a low blood viral load continued to have a good negative predictive value for an abnormal hearing outcome [15].

A few small studies have reported on virus detection in CSF [16, 17]. In one of these studies only babies with symptomatic CMV had virus detectable, although only at low levels (<400 copies/mL). Based on experience gained with neonatal herpes simplex virus infection, the measurement of CMV in CSF is being carried out by some clinicians as a marker for disease progression and a guide for commencing treatment. Formal studies regarding the utility of carrying out such procedures diagnostically are therefore needed and may help to inform treatment in asymptomatic or mildly symptomatic babies.

1.2 PostNatal CMV

Infection in the neonatal period is most commonly acquired from ingestion of CMV-infected maternal breast milk. The risk of CMV transmission via breast milk was first identified in the late 1960s and in term babies, infection was nearly always found to be asymptomatic [18]. However, using polymerase chain reaction (PCR) to detect CMV in breast milk, up to 96% of seropositive women have been found to have CMV DNA detectable in their breast milk, termed DNAlactia. Some authors have reported transmission in up to 37% of premature babies of whom a significant proportion then develops symptoms [19, 20]. Not all authors have found such high rates of transmission, and a summary of studies addressing the issue of transmission via breast milk can be found in a recent review [21]. The different rates of transmission and symptomatic postnatal disease reported across Europe in different studies may well be in part due to variation in feeding practices in different centres.

1.2.1 Clinical Features

A number of clinical symptoms and signs have been described in association with symptomatic postnatal CMV infection including pneumonitis, hepatosplenomegaly, lymphadenopathy, enteritis and aseptic meningitis [22, 23]. Hamprecht et al. described a sepsis-like syndrome in 4/16 infants with symptoms associated closely with the onset of CMV detection in blood and urine [20]. Other studies have since confirmed this finding, a summary of which can be found in a recent review [21].

Data are very limited on long-term follow-up of infants with postnatal acquisition of CMV. Initial studies of term infants showed no obvious long-term sequelae. Some concerns were raised when a study in preterm infants indicated a trend towards neurological impairment in infants <2,000 g who were infected postnatally [24]. More recent studies, including a prospective, matched, case–control study, do not support this finding [25].

Data reporting CMV viral load for babies treated for postnatally acquired CMV disease are confined to single case reports. How viral load correlates with symptoms and response to treatment remains completely undefined in this age group. The current standard of care for the treatment of premature infants with symptomatic CMV disease is 2 weeks of IV GCV at 12 mg/kg/day.

1.3 CMV Disease in Older Children

CMV in older children is predominantly a disease of the immunocompromised. The main burden of CMV disease in Europe is currently in children undergoing either haemotopoietic stem cell transplantation (HSCT) or solid organ transplantation (SOT), predominantly renal, liver, lung or heart. Using quantitative PCR, it can be shown that CMV replicates rapidly in vivo. The replication dynamics of primary CMV infection in adult allograft recipients are very similar to those of primary HIV infection [26]. The dynamics of CMV replication is very rapid in these

patients [8] and multivariable statistical analysis shows that measures of viral load explain all of the previously recognised risk factors for CMV end-organ disease [9, 27, 28]. The serial measurement of viral load in post-transplant patients can be used to guide initiation and cessation of antiviral therapy and to monitor for the emergence of antiviral resistance. Such pre-emptive therapy is now widely used for the management of both adults and children undergoing transplantation across Europe, although paediatric clinical practice is largely based on adult data.

CMV infection has also long been recognised as a cause of serious pneumonitis, retinitis and organ disease in children with HIV in Europe and the USA. Since the introduction of combination antiretroviral therapy, CMV disease is now rare in this setting. However, CMV continues to be a very serious cause of disease in children born with HIV in the resource-poor setting. CMV has clearly been linked to rapidly progressive HIV in young South African infants, often associated with *Pneumocystis* pneumonitis [29]. In 2003, 24 of 47 infants admitted with pneumonitis had evidence of CMV infection [30]. Similarly, CMV was implicated in 50% of HIV-infected infants < 1 year of age not responding to antibiotics within 48 hours in Kwazulu-Natal in the pre-antiretroviral era [31]. CMV retinitis has also been described in HIV-infected infants and children in Cape Town. O'Connell and colleagues described six children (mean age 6.7 months) with CMV retinitis [32]. Currently as antenatal testing for HIV is not universal in high-prevalence African countries, many children are being born with both HIV and CMV disease. However, there is now widespread implementation of combination antiretroviral therapy and in some centres children are also being treated for CMV disease. The relative risks and benefits of IV GCV and oral VGCV in this setting also remain important questions.

2 Ganciclovir and Valganciclovir Use in Children

2.1 Pharmacokinetics

2.1.1 Ganciclovir

GCV is a deoxyguanosine analogue that is triphosphorylated intracellularly to its active metabolite. The CMV encoded UL97 protein kinase is necessary for the first phosphorylation step. The triphosphate then acts as an alternate substrate with the other deoxynucleoside triphosphates, ultimately leading to viral DNA chain termination. The drug is excreted virtually unchanged by the kidneys, with minimal protein binding, but oral absorption is poor. The use of GCV in children was first reported in the late 1980s with most early reports involving children coinfected with HIV. Over the subsequent two decades there have been very few further studies reporting pharmacokinetic data in the paediatric age group and even fewer involving neonates who are well known to have distinct pharmacokinetics. There is a dearth of knowledge from controlled or randomised clinical trials, and interpretation of studies conducted so far is hampered by the difficulty in comparing results

using different drug doses, duration of therapy or follow-up period, as well as different study end points. In neonates, plasma levels comparable to those found in adults can be achieved with intravenous (IV) dosing of 6 mg/kg/dose twice daily (Table 2).

Table 2 Pharmacokinetic parameters of ganciclovir in neonates

Age	Single dose[a]	Cl (L/h/kg)	Vd (L/kg)	C_{max} (μg/mL)	$t_{1/2}$ (h)	References	Number of cases
4–49 days	4 mg/kg	0.19 ± 0.03	0.67 ± 0.07	5.5 ± 0.4	2.4 ± 0.4	Trang et al. [33]	14
2–30 days	6 mg/kg	0.21 ± 0.02	0.75 ± 0.07	7.0 ± 0.5	2.4 ± 0.4	Trang et al. [33]	13
NA	4–6 mg/kg	0.42 ± 0.08	1.8 ± 0.32	NA	NA	Zhou et al. [34]	27
Adult	NA	0.28 ± 0.11	1.1 ± 0.30	6.6 ± 0.8b	4.3 ± 1.6	Pacifici et al. [35]	NA

Adapted from Pacifici et al. [35]. The figures are the mean ± SEM.
[a]Ganciclovir was administered by an intravenous infusion over 1 h.
[b]Following a single 6 mg/kg dose over 1 h of infusion.
Reproduced from Expert Rev. Anti-infect. Ther. 4(6), 1005–1020 (2006) with permission of Expert Reviews Ltd. [36].

Changes in body weight and age have been correlated with changes in the volume of distribution and creatinine clearance of GCV, respectively [33, 34]. A recent pharmacokinetic study in a group of congenitally infected infants aged 30 days or less at the start of treatment showed an increase of 73% in renal excretion of GCV from 3.8 mL/min/kg on day 4 to 6.8 mL/min/kg on day 34 following a dose of IV GCV; a corresponding decrease in the area under the curve (AUC) of 41% over the same time period was observed [37]. This has potential implications for dosing regimens during prolonged periods of GCV treatment used in the period of rapid postnatal growth seen in neonates.

Even in older children, very limited GCV pharmacokinetic (PK) studies have been performed, usually in children treated for CMV after renal transplantation. After a first report in three children [38], the main study from the same group reported on a further 11 children with a mean age of 11 years [39] and the dose of 5 mg/kg used in this study has now been widely adopted, based on very limited data. Another study has subsequently reported sub-therapeutic trough levels (<0.5 mg/L) in 8/9 children treated with this dose of IV GCV, as well as wide inter- and intra-patient variability [40].

2.1.2 Valganciclovir

VGCV is the L-valyl-ester pro-drug of GCV and after oral administration it is rapidly converted to GCV by hepatic and gastrointestinal esterases. Unlike GCV, VGCV is a substrate for peptide transporters in the intestinal wall, explaining its enhanced bioavailability when given orally [41]. In adult studies oral bioavailability has been reported to be around 60% when taken with food (compared to 6%

for orally administered GCV). In adults GCV exposure, expressed as mean AUC_{24}, following 900 mg oral VGCV administration is comparable to that achieved with 5 mg/kg IV GCV [42].

Population pharmacokinetics in a phase II evaluation of VGCV solution conducted in congenitally infected neonates suggested a dose of 16 mg/kg twice daily as giving equivalent drug exposure, measured using AUC, to the 6 mg/kg IV GCV used in earlier studies conducted by this same group. Very wide variation in AUC was noted [43]. One case report has commented on the need to adjust dosing of VGCV frequently to maintain adequate plasma levels [44].

The phase II study described above reported oral bioavailability of only 48% on day 6 which increased to 64% at day 36. It has been hypothesised that this is due to maturation of the GI and hepatic esterases involved with de-esterification of valGCV to active GCV with increasing post-conceptional age [37]. Due to the concurrent increase in renal clearance of GCV described above, overall AUC only decreased by 15% following a dose of oral VGCV, compared to the 41% decrease seen with administration of IV GCV. More recent data on the use of VGCV in a paediatric renal transplant population of 22 children identified creatinine clearance and bodyweight as individual factors influencing the apparent oral clearance [45].

2.1.3 CSF and CNS Penetration

The parameters affecting penetration of drug into the CSF and brain parenchyma are complex and are not well described for GCV [46]. Cerebrospinal fluid concentrations of GCV in adults indicate a penetration of 24–67% [47]. Data are very limited and there are no studies in the paediatric age group or neonates in whom differences in the blood–brain barrier or the transport systems involved may alter drug availability to this compartment. There are no existing data relating to penetration of GCV into CSF or brain parenchyma following oral VGCV administration. Data obtained for aciclovir and valaciclovir have shown stable levels in the CSF when compared to serum implying that the CSF may act as a reservoir for drug. This may imply that absolute peak serum levels of drug are not important and equivalent drug exposure measured by AUC may be sufficient [48]. These studies have also found that timing of sampling of CSF may also not be important once steady state has been achieved, as shown by similar CSF concentrations found after 6 days and after 6 months of oral treatment with valaciclovir. One of the key questions raised by the use of oral VGCV, particularly in unwell infants with CNS disease, is whether the same CSF levels are obtained using VGCV compared to IV GCV.

2.2 Pharmacodynamics

Two early studies found better virological responses with a higher dose of GCV, and/or increased therapy duration (one study compared 6 mg/kg twice daily dose of GCV to 4 mg/kg twice daily; the other compared 5 mg/kg twice daily for 2 weeks with 7.5 mg/kg twice daily for 2 weeks followed by 10 mg/kg three times weekly for 3 months): those babies achieving viral suppression in the urine at the end of the

treatment course in both these studies had a better neurological outcome [49, 50]. Other authors have reported on the increase in symptoms from CMV following termination of treatment associated with a corresponding increase in viral load [44].

In a recent study treating congenitally infected babies with VGCV only 6/18 viraemic babies were CMV PCR negative at the end of 6 weeks treatment [37]. In this study viral suppression correlated neither with clinical resolution nor with any of the pharmacokinetic parameters measured (C_{max}, AUC_{12}, C_{last}, T_{last}). However, C_{max} and AUC were correlated with decreased white cell count and absolute neutrophil count.

Cinque et al. reported that CMV DNA in CSF of seven adult HIV patients with CNS disease was undetectable by PCR at the end of 3 weeks GCV treatment in three patients with low-level virus detected at start of treatment and was decreased, but still detectable, in four patients with a higher starting value. At autopsy, virus was detectable in CNS by PCR in 4/4 patients, including three of those who had continued to have detectable CMV by PCR [51].

Preliminary data from our group have shown that during a standard course of antiviral treatment, viral load decreases but is rarely fully suppressed in blood, urine or saliva. Indeed in 15 babies treated with 6 weeks' GCV, virus was still detectable at the end of treatment in 8/12 (67%), 8/10 (80%) and 2/5 (40%) of those with blood, urine and saliva samples available (Luck, personal communication). Following treatment cessation a rebound in both urine and salivary viral load is observed, with an increase in viraemia; this has not been widely reported in the adult literature.

Viral half-life in the first week of treatment has been estimated to be around 2.37 days using data derived from a study by Kimberlin et al. ([37] and unpublished observations). This is similar to rates calculated in patients with HIV infection, or following liver transplant and bone marrow transplant (2.56, 2.36 and 1.52 days, respectively) [52]. With more frequent sampling, the half-life of decline in CMV in HIV-positive patients was actually closer to 0.9 days. This observation has also been replicated in preliminary results of our studies with the half-life of decline in CMV DNA in both urine and blood in the first 3 days of treatment being 0.56 and 1.81 days, respectively. The reason for the failure for virus to be fully suppressed in blood does not therefore seem explicable by a difference in viral half-life, certainly during this first phase of viral decline. Given the preliminary nature of these data, correlation with clinical outcome is not yet possible.

2.3 Resistance

Resistance in CMV is usually due to mutations in pUL97, which encodes the viral protein kinase, or in the viral polymerase, UL54, gene and is associated with low plasma levels of GCV [53–55]. The use of oral GCV, and the hypothesised subsequent exposure to low levels of drug for long periods, has been associated with increased emergence of resistance mutations and clinical treatment failure [53, 55]. Sub-optimal dosing of GCV in children may likewise select for viral resistance and subsequent treatment failure.

2.4 Drug Levels

Routine therapeutic drug monitoring of GCV is not performed as part of standard clinical care in adults as there has been no clear correlation demonstrated between drug levels and clinical efficacy. There remains a real paucity of data on drug levels following routine treatment in infants and children and the relationship between drug levels and clinical outcome. Pilot data from our group, in collaboration with the Bristol Antimicrobial Reference lab (BCARE), have shown that in 339 trough samples GCV levels are significantly lower in younger children and those aged 5–18 years when compared to adults (Fig. 1).

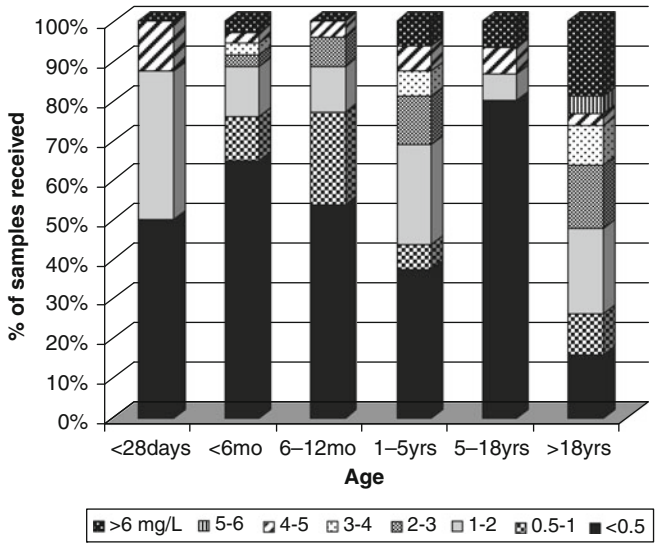

Fig. 1 Trough levels of ganciclovir (mg/L) in 192 children aged under 18 years compared to 147 adult GCV levels

As discussed above, there is a real concern that in older children sub-therapeutic levels may be common, leading to treatment failure and the development of resistance, particularly if longer treatment courses are being considered. In the congenitally infected group, monitoring treatment according to clinical and virological response poses a further challenge whereby the outcomes of interest are not immediately evident during treatment. The need for further data in this area is apparent.

2.5 Safety

Laboratory abnormalities reported during the Collaborative Antiviral Study Group (CASG) randomised study of IV GCV [56] were assessed utilizing NIAID Division of AIDS (DAIDS) toxicity tables. The most frequent adverse event was neutropenia,

which was experienced by a higher proportion of patients on the GCV arm of the study. Twenty-nine (63%) of 46 GCV-treated patients developed Grade 3 or 4 neutropenia during the 6 weeks of study drug administration, compared with 9 (21%) of 43 patients in the no-treatment group over the same period of time ($P < 0.01$). Dose adjustments were required for neutropenia in 14 (48%) subjects, although only four patients had the drug permanently discontinued. In all affected patients, the neutropenia resolved.

Anaemia was also more common in patients who received GCV (8 vs. 3% untreated patients), although the incidence of thrombocytopenia appeared comparable in the two treatment groups (2% GCV-treated patients vs. 3% untreated patients). The incidence of bloodstream infections, haematochezia and diarrhoea was also higher in patients who received GCV. Neurotoxicity, although very rare, has been documented in adults and is generally associated with poor renal function as GCV is almost completely renally excreted. Long-term side effects of treatment are also of potential concern in the paediatric age group. In animal models decreased spermatogenesis has been observed causing infertility. Increased tumours, lymphoma cell mutagenesis and DNA damage at doses lower than the anticipated human dose exposure have also been reported in animal models and in vitro (http://www.rocheusa.com/products/valcyte/pi.pdf; [57]).

In view of the concerns relating to carcinogenesis and gonadal toxicity, long-term follow-up of 47 children enrolled into a phase II trial of GCV as neonates between 1986 and 2001 has been conducted. Data relating to cancer incidence, sexual development and pubertal development in this small group of children is awaiting publication (http://clinicaltrials.gov/ct2/show/NCT00031421?term=ganciclovir+and+puberty&rank=1).

There are no other initiatives in place, to our knowledge, to conduct long-term monitoring of the subsequent development of neonates or older children treated with GCV or VGCV. We have recently developed a novel web-based pilot register for CMV-infected infants treated with GCV and VGCV. This is part of a pan-European treatment initiative to facilitate long-term post-treatment surveillance and linkage where possible of treated infants to cancer registries (www.ecci.ac.uk).

3 Clinical Studies of GCV in Childhood CMV Infection

3.1 Congenital CMV

Reports of treating congenitally infected neonates with severe CMV inclusion disease started to emerge in the mid-1990s. Case-based reports predominated and initially showed a trend towards improved outcome with treatment courses of IV GCV with publication bias being of possible concern (see Table 3).

Table 3 Summary of studies using ganciclovir to treat symptomatic congenital cytomegalovirus infection

Study	Patients	Dose GCV	Duration	Outcome	Side effects
Nigro [49]	12 infants	Gp 1 (*N*=6) 10 mg/kg/day	2 weeks	0/4 with persistent CMV DNA in urine had improved or normal outcome. Two babies in whom virus undetectable had clinical improvement and normal outcome	One patient in Gp 2 had decreased neutrophil count
		Gp 2 (*N*=6) 15 mg/kg/day IV then 10 mg/kg 3× per week	2 weeks then 3 months	All five children had clinical improvement	
Whitley et al. [50]	14 babies with symptomatic CMV involving CNS. <1-month old.	8 mg/kg/day IV	6 weeks	Mortality 4/47 (9%). 8/13 with retinitis resolved. No difference in neurological outcome. 12 mg/kg significantly improved viral suppression. Pharmacokinetics correlated with stabilised or improved hearing	9/47 (19% of children in each dose group) drug stopped due to toxicity. Dose modified in 6 (3 in each dose group). Neutropenia in 63% 8 mg/kg and 19% 12 mg/kg
	28 babies with CNS disease. <1 month	12 mg/kg/day IV			
Halwachs-Baumann et al. [16]	30 congenitally infected neonates: 21 treated (48% symptomatic). Nine no treatment (44% symptomatic)	10 mg/kg/day IV decreased to 5 mg/kg/day if side effects	Minimum 3 weeks	1/10 symptomatic children treated with antivirals symptomatic at 1 year compared to 2/3 symptomatic babies not treated. Large loss to follow-up	Three children drug dose decreased due to toxicity – not specified what

Table 3 (continued)

Study	Patients	Dose GCV	Duration	Outcome	Side effects
Michaels et al. [58]	9 children less than 1 year (5/9 started less than 10 days old).	10 mg/kg/day IV	2–4 weeks	No progression of hearing loss, 3/5 children improved hearing. Improvement in tone in three children	One child dose-dependent neutropenia, six children catheter-related problems
		5 mg/kg/day IV 550 mg/m^2/dose po GCV	5.8–18 months 8 months to 3 years		
Kimberlin et al. [56] (only randomised trial to date)	100 babies all with CNS disease. Enrolled less than 30 days old. 47 received drug, controls no treatment	12 mg/kg/day IV	6 weeks	At 1 year, 21% of treated babies worsening hearing compared to 68% untreated on best ear assessment	63% grade 3 or 4 neutropenia. 3 catheter infections
Rojo and Ramos [59]	Five babies less than 3 months of age all with neurological symptoms	8–12 mg/kg/day IV	6 weeks	No progression of symptoms	Neutropenia in child receiving 90 mg/kg/day
		30–90 mg/kg/day po GCV	6–12 months		
Tanaka-Kitajima [60]	Six cases of symptomatic congenital CMV (life-threatening or CNS involvement) D0–D45 (median D14)	5–12 mg/kg/day IV	2–7 weeks	All babies neurological deficits. Two babies hearing loss improved. Two patients with chorioretinitis improved, no visual loss at follow-up	Not reported

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In 1997 Whitley et al. first reported an improved virological outcome with a dose of 6 mg/kg IV GCV twice daily when compared to 4 mg/kg twice daily, with a trend towards better neurological outcome in those receiving the higher dose [50]. The dose chosen in this initial study was based on pharmacokinetic data targeting a similar area under curve to that achieved in treatment regimes in adults [33]. Six weeks was chosen as the duration based purely on this being the considered upper limit of acceptability for intravenous therapy.

A randomised, although not placebo-controlled, study was subsequently conducted by the US CASG (CASG 106) in which 100 babies less than 1 month of age and of more than 32 weeks' gestation with evidence of CNS signs/symptoms of CMV disease were enrolled. These babies were randomised to receive either 6 weeks IV GCV or no treatment. Six weeks was chosen to be compatible with the previous randomised-controlled trial. In this study, which took 10 years to complete, none (0%) of 25 GCV recipients had worsening of hearing in their best ear between baseline and 6 months, compared with 7 (41%) of 17 patients in the no-treatment group [adjusted P-value < 0.001; OR 21.11 (95% CI: 2.84, ∞)]. Five (21%) of 24 GCV recipients had worsening of hearing in their best ear between baseline and $\geq$ 1 year, compared with 13 (68%) of 19 patients in the no-treatment group [adjusted P-value = 0.002; OR 10.26 (95% CI: 1.79, 81.92)]. GCV-treated patients also had a more rapid median time to normalisation of ALT (19 days) compared with patients in the no-treatment group (66 days) ($P = 0.03$) and a greater degree of weight gain ($P = 0.02$) and growth in head circumference ($P < 0.01$) at the end of 6 weeks treatment [56]. Importantly, subsequent analysis of neurological outcome has also shown, in the 74 infants with data available, that those who received antiviral treatment had fewer delays in the acquisition of expected developmental milestones (assessed using the Denver developmental assessment tool) compared to those in the untreated group [9.78 vs. 17.14 (P=0.007)] [61]. There are theoretical benefits in treating all symptomatic babies (not just those with CNS symptoms at birth), based on earlier cohort studies finding petechiae and IUGR to be independent risk factors for subsequent hearing loss [6]. In addition, the observation that some of the favourable outcomes seen in the earlier randomised-controlled study only reached significance in assessments close to the time of drug termination and the knowledge that neurological development continues for some years postnatally suggest that longer treatment courses are rational; such studies are now feasible with the availability of an oral preparation. A placebo-controlled, double-blind, randomised study (CASG 112) comparing 6 weeks vs. 6 months of oral VGCV has commenced recruitment in the USA (and will shortly start recruiting in Europe) aiming to address some of these issues.

Oral VGCV solution is now commercially available from Roche and licensed in Europe for treatment of CMV disease in patients aged over 18 years. The solution has good stability data and has a reasonable concentration of 50 mg/mL. This makes treatment outside formal studies possible and appealing to many clinicians. There is already increasing use of this drug in an off-label, unlicensed, manner in children in Europe including the prolonged treatment of babies infected with congenital CMV (personal communications). The use of oral VGCV allows earlier

discharge with treatment in the ambulatory setting, reducing costs and decreasing the complications associated with long-term venous access. The latter has special relevance for lower resource settings. However, recruitment into the CASG 112 study should be encouraged wherever possible (the USA and the UK) to provide the evidence for or against using a longer duration of treatment than 6 weeks.

3.2 Clinical Use of GCV in Postnatal CMV Infection

The evidence for efficacy of treating premature infants with CMV is very sparse and is based purely on a few case reports [62, 63]. There are few data on treating postnatally infected, premature infants and pharmacokinetic data only exist for those treated for congenitally acquired infection aged more than 32/40. A number of authors have described benefit in using GCV for treatment of neonatal hepatitis and cholestasis and gastrointestinal manifestations [64, 65]. The studies already discussed above found a more rapid normalisation of AST in congenitally infected babies, although all subjects (including those not receiving treatment) had eventual resolution of liver enzyme abnormalities [56]. Other authors have observed that babies either controlled virus themselves, had spontaneous resolution of their symptoms or that GCV treatment had no obvious benefit [23]. If treatment is commenced, GCV is the standard of care. The optimal dose, duration and clinical efficacy are largely unknown. GCV remains the drug of choice on neonatal units, despite this almost complete lack of data.

3.3 CMV Disease and GCV Treatment in Older Immunocompromised Children

The strategy of pre-emptive therapy based on regular screening by CMV PCR and treatment of positive viraemia with GCV has been widely adopted and taken up by paediatric transplant units. Again there are very limited data at all on the efficacy of this approach and the optimal dose of GCV to use. Although a 12 mg/kg/day dose is used now for congenital CMV, in older children and adolescents the standard dose is 10 mg/kg/day.

Local transplantation treatment protocols vary, but in Europe include

- In HSCT
  a 2-week IV GCV period followed by oral VGCV (VGCV stopped when level of immunosuppression is decreased)
- In SOT
  prophylaxis in high-risk patients: oral VGCV (VGCV stopping when level of immunosuppression is decreased)
  pre-emptive treatment: a 2-week IV GCV period followed by oral VGCV based on detection of CMV PCR or antigenaemia.

There is a need for more detailed PK studies to provide improved dosing recommendations, monitoring and dose adjustment schedules in this age group.

4 Summary

Both GCV and VGCV are increasingly being used in babies and children. Despite this, there are major limitations in the evidence base underlying their clinical use. The recent failure of Maribavir in its pivotal phase III trial means that there is unlikely to be any new anti-viral treatment for CMV disease entering clinical practice for many years. The therapeutic market for CMV infection is small and GCV is now off patent. VGCV is only licensed in adults. Current agents have major toxicities. There is a need to develop long-term treatment registries to follow-up children treated in early childhood. These registers have major ethical, financial and regulatory difficulties. This review has highlighted the main areas of clinical disease and indications for treatment. The lack of key data in specific areas, especially premature infants, CSF penetration, surrogate markers of efficacy and data in older children has been outlined along with suggestions for future trials that need to be conducted to improve the evidence-based prescribing of antiviral therapy in children with CMV disease.

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Infectious Hazards from Pets and Domestic Animals

Mona Al-Dabbagh and Simon Dobson

1 Introduction

Most pet owners consider their pets to be family members. Approximately 63% of US households own at least one pet [1], and statistical analysis done in the United States in 2006 showed that there are more than 72 million pet dogs and nearly 82 million pet cats, with an average veterinary expenditure per household for all pets of around $366/year [2]. According to a survey conducted by the American Animal Hospital Association (2002), 94% of pet owners consider their pet to have human personality traits, 93% say that they would risk their own life for their pet, and half said that they would choose their dog as their sole companion if stranded on a Desert Island [3]. As a consequence, people tend to treat the health of their pets as they would with their own children and spend more money at the veterinary clinic. This also involves purchasing treatment with broad-spectrum antibiotics.

Pets serve valuable social roles in society, and studies have shown that pet owners had significantly lower systolic blood pressure, reduced cholesterol and triglyceride levels than non-pet owners, and had less feelings of loneliness, while increasing opportunities for exercise, outdoor activities, and socialization [4]. Despite these benefits, pets present zoonotic risks, especially for immunocompromised hosts [5].

Animal to human transmission of infections has been documented to occur through direct contact with an infected animal or through contact with infectious saliva that contaminate bite wounds, skin abrasions, or mucous membranes and through hand-to-mouth transfer of microorganisms, cysts, or oocysts from feces of an infected animal [6].

Other ways of transmission of animal to human infections include aerosol transmission from body fluids (e.g., Q fever) and vector-borne diseases (e.g., Lyme disease, Babesiosis, and Ehrlichiosis). Children are considered at highest risk for infection from pets because they are more likely to have close contact with pets

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and thus more vulnerable to bites and scratches from them. Children are more likely to put objects in their mouth that provides closer contact with the household environment contaminated by pets.

New pets are considered of more health risk than established pets, especially imported ones, since their health history and vaccination records may not be known, while older pets are generally safer than younger ones because they are less likely to be involved in playful activities and thus less biting and scratching [6].

Over and beyond the usual ways of looking at zoonotic infectious hazards, pets and domestic animals afford opportunities for emerging infectious disease threats and a vehicle for the spread of antibiotic-resistant organisms.

2 Global Trends in Emerging Infectious Diseases (EID)

The past two decades have seen the emergence of pathogenic infectious diseases that are associated with a range of underlying causal factors [7]. These include interactions with zoonotic pathogens within the continuum between wildlife, domestic animal, and human populations [8] (Fig. 1).

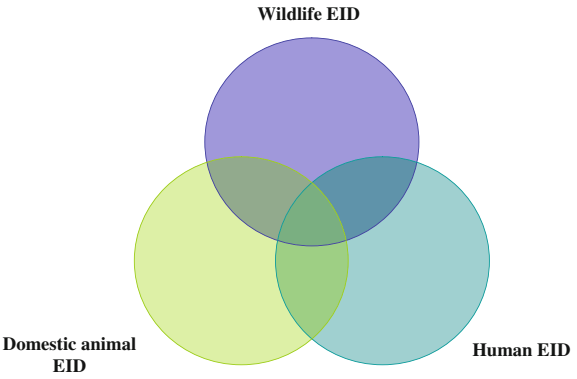

Fig. 1 Most emerging infectious diseases (EID) exist within a host and an infectious agent (parasite) continuum between wildlife, domestic animal, and human populations

In a dramatic study, Jones et al. analyzed the emergence of 335 infectious diseases between 1940 and 2004. A majority (60.3%) of these emerging infectious diseases were zoonoses [9] and 72% of these originated in wildlife (Fig. 2). Data show that those pathogens regarded as emerging and reemerging were more likely to be zoonotic than those that are not [10]. And zoonotic species are overall twice as likely to be associated with emerging disease than non-zoonotic species [11].

About 54.3% of pathogens involved in EID were bacteria and rickettsia (with drug-resistant strains), 25.4% were viral pathogens, 10% were protozoa, 6.3% were fungi, and 3.3% were helminths [9] (Fig. 3). Antimicrobial-resistant microbes constituted 20.9% of these EID and have significantly increased with time

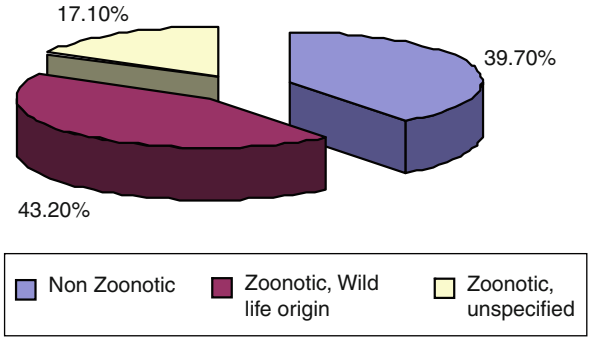

Fig. 2 A pie presentation showing that 60.3% of EID are transmitted by zoonotic pathogens and that wildlife constitutes 71.8% of zoonotic infections (43.20% of overall EID)

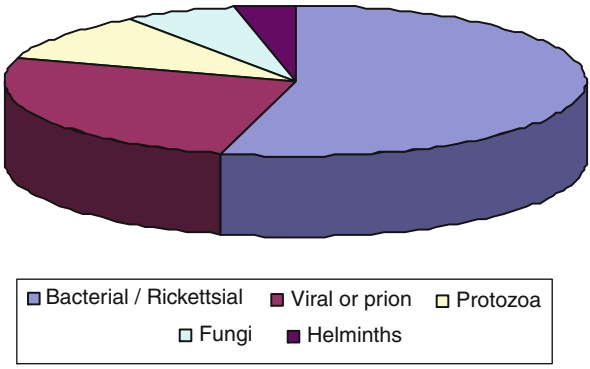

Fig. 3 The EID plotted by different pathogen types

($P < 0.05$). This is thought to be related to the rise in antimicrobial drug use, especially in developed countries [9].

Jones et al. point out that surveillance and reporting of these EIDs are biased. The risk "hot spots" of emergence are in lower latitude developing countries whereas the surveillance resources are in more well-off countries where emergence of such infections is less likely [9]. They advocate for re-allocation of these resources so that they are available in areas where zoonoses will emerge. Wildlife zoonotic EIDs relate to species richness in such parts of the world. EIDs that emerge in the developed world such as antibiotic-resistant organisms tend to be non-wildlife related and are partially driven by population density.

Although zoonotic pathogens do represent the most likely source of emerging and reemerging infectious disease, only small minorities have proved capable of causing major epidemics in the human population [10], and closer collaboration between medical and veterinary researchers is needed for the management of these pathogens.

3 Pets and Domestic Animals as Reservoirs of Antimicrobial Resistance (AMR)

The widespread use of antibiotics in human medicine and agriculture and the related growing problem of bacterial resistance to antibiotics provide a rich area for study and debate. Most of the rising antimicrobial resistance problems in human medicine are due to the overuse and misuse of antimicrobials [12].

Use of antimicrobial agents for nonhumans includes application for a variety of purposes: in production of food animals (livestock, poultry), aquaculture, plant and crop protection, food production, and industrial use, such as the cleaning of oil pipes. At sub-therapeutic levels, antimicrobials are used for growth promotion in livestock and poultry, at varying levels for prophylaxis and at therapeutic levels for treatment.

In addition there has been an increased attention devoted to small animal welfare, with increased expenditure on veterinary care, and prevention and therapy of infectious diseases resulting in the frequent use of antimicrobial agents in pet animals [13], and in particular, broad-spectrum agents such as clavulante-potentiated aminopenicillins, cephalosporins, and fluoroquinolones [14]. All of these ways of exposure, either directly or indirectly, might position pets and domestic animals to develop an inevitable and irreversible antimicrobial resistance, which is a natural consequence of bacterial cell adaptation to exposure to antimicrobial agents.

Bacterial resistance can result from changes in the antibacterial's target or from bypassing of that target, or it can occur as a result of impermeability, efflux pumping, or enzymatic deactivation. Some bacteria are inherently resistant. In others, resistance may arise via hypermutation or horizontal gene transfer by plasmids and transposons [15].

Another mechanism of bacterial resistance is biofilm formation, especially in cases of implant infections, urinary tract infections, and cystic fibrosis [16].

Rapid spread of genes resistant to antimicrobial agents can occur in a bacterial population and from one ecosystem to another. Particular antibiotic resistance genes first described in human-specific bacteria have been found in animal-specific species of microorganisms and vice versa, suggesting that bacterial populations can share and exchange these genes [13].The development of resistance in one bacterial population can spread to other populations over time (Fig. 4).

A recent review suggested that <4% of antimicrobial resistance problems in humans could be associated with animal sources and that this resistance is largely related to zoonotic organisms [17].

Several longitudinal studies conducted at veterinary hospitals have indicated antimicrobial resistance to various agents has emerged among pet animal isolates of *Staphylococcus intermedius*, *Escherichia coli*, and other bacteria with potential for zoonotic transmission and resistance phenotypes, such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), and multidrug-resistant *Salmonella typhimurium* DT104 [13].

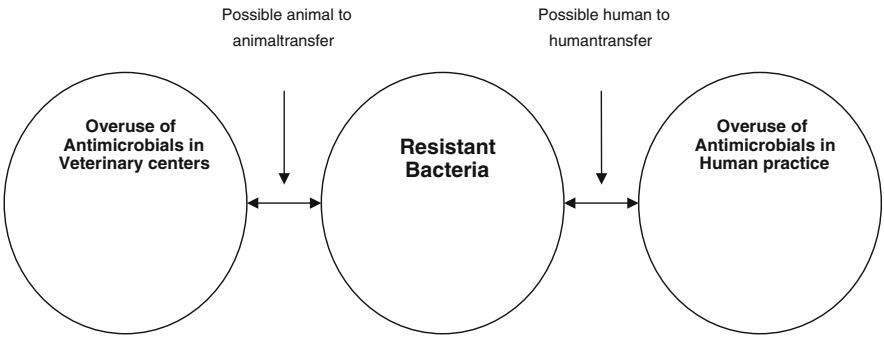

Fig. 4 Schematic representation of the consequences of antimicrobial overuse in animal veterinary practice and human medicine on exchange of resistant bacteria and resistance genes between pet animals and humans

The same applies to food animals, where food production and distribution may represent a dynamic environment for the continuing transfer of antibiotic resistance between bacteria. The widespread use of antibiotics in food animal production has resulted in the emergence of antibiotic-resistant bacteria that can be transmitted to humans through the food chain [18, 19]. In such animals, antimicrobials are used for treatment and illness prevention as well as for growth promotion purposes.

Antimicrobials used for food animal treatments include amoxicillin, erythromycin, gentamicin, tylosin, fluoroquinolones, and sulfonamides [20]. An example of the food-borne transmission of resistant strains is zoonotic apramycin and gentamicin cross-resistance, when apramycin (an aminoglycoside) was introduced as a veterinary antimicrobial in the 1980s to France. A few years later, cross-resistance to both apramycin and gentamicin was detected in *E. coli* and *S. typhimurium* strains isolated from cattle and sheep flocks, which was found to be a plasmid-mediated mechanism of resistance. Between 1985 and 1988, nosocomial human isolates of *E. coli*, *S. typhimurium*, and *Klebsiella pneumoniae* infections were recovered from hospitals that shared the same resistant strains [21].

Some of the growth promoters used belong to the glycopeptides and streptogramins groups, which are essential drugs in the treatment of invasive staphylococcal or enterococcal infections in humans. Research has shown that resistance of these bacteria to classic treatment in humans is often a consequence of the use of antimicrobials in food promotion. The exposure of humans to bacteria resistant to antimicrobials and to resistant genes through food can be reduced effectively by intervention [22].

4 *Staphylococcus intermedius* in Pets

Staphylococcal infections in pets are predominantly caused by *S. intermedius*, with *S. aureus* representing <10% of clinical staphylococcal isolates from dogs and cats. [23, 24].

S. intermedius has been considered as one of the major coagulase-positive species of staphylococci commencing on the skin and mucosal surfaces of canines and is considered the principal cause of canine pyoderma and may cause sepsis and shock in dogs [25].

In humans, *S. intermedius* is rarely associated with severe clinical disease and transmission from dogs does not appear to cause a serious risk for human health. The reported prevalence of *S. intermedius* infection among hospitalized patients is only 6×10^{-4} [26]. However, *S. intermedius* can be a responsible pathogen for some canine-inflicted human wound infections, thus representing a zoonotic pathogen [27]. The possible transmission of *S. intermedius* from dogs to humans was previously reported [28], and there is good evidence from molecular typing studies that it can be transferred the other way between humans and animals also [29]. Man and canine carry the same strains of *S. intermedius* and probably act as a reservoir for these strains. Indeed, the domestication of the dog and the increasing physical relationships between man and dogs may have led to the original zoonotic spread of the organism.

There has been increased prevalence of multidrug-resistant *S. intermedius* strains in pet animals from 11 to 28% between 1986–1987 and 1995–1996 in France [30], with reported resistance to penicillin, neomycin, sulfonamides, and erythromycin between 1980 and 1999–2000 in Switzerland [31]. Guardabassi et al. reported the carriage of *S. intermedius* by 7 out of 13 owners of dogs with pyoderma, where six of the isolated strains from the dog owners were identical to the strains in their dogs and all strains were resistant to up to five antimicrobial drugs, and he also reported that the occurrence of *S. intermedius* in dog owners was significantly higher compared with the non-animal owner control group ($P = 0.03$), which supports the association and possible transfer of *S. intermedius* from dogs to human [29].

In 2007, Loeffler et al. reported the first multiresistant, mecA-positive *S. intermedius* in Europe, where mecA-positive methicillin-resistant *S. intermedius* (MRSI) was isolated from 11 dogs and a cat in Europe. The presence of the gene conferring resistance to all β-lactam antibiotics (mecA) was demonstrated and confirmed by Pulsed Field Gel Electrophoresis (PFGE) and Polymerase Chain Reaction (PCR) testing [32].

Recently, mecA-positive MRSI transmission between animal and human has been reported, where multiresistant MRSI was isolated from infected surgical wounds of five dogs and a cat at the Veterinary Microbiological Diagnostic centre in Netherlands. MRSI with the same resistance pattern was cultured from the nose of a surgeon, three technicians, four environmental samples, and a healthy dog of a staff member in the same clinic. All isolates were proved to be mecA positive by PCR, and PFGE profile done on 13 out of the15 isolates showed that the isolates were indistinguishable [33].

One implication of the transfer of coagulase-positive staphylococci between owners and pet dogs is of the potential transfer of antibacterial resistance between *S. intermedius* and *S. aureus*; as it has been reported previously that canine *S. intermedius* and human *S. aureus* strains share the same tertracyclin resistance genes and

structurally related plasmids which can be mobilized or transduced between members of the same or related staphylococcal species and possibly between animals and humans without knowing the exact direction of transmission [34].

Since antimicrobial resistance is one of the most important emerging public health problems for the future, the veterinary profession needs to monitor trends in antimicrobial use in pets and avoid overuse in order to limit the emergence of multiple antimicrobial resistance in *S. intermedius* and the potential transfer of resistance genes to human staphylococci.

5 MRSA in Pets and Domestic Animals

Methicillin-resistant *S. aureus* (MRSA) is an important worldwide cause of human infections both community acquired and nosocomial. It can cause a wide range of conditions from mild skin and soft tissue infections to life-threatening bacteremias. Recently, infections with MRSA have been increasingly reported as an emerging problem in veterinary medicine, particularly in small animal and equine practices. It has been reported in horses [35], dogs [36], cats [37], chickens [38], cows [39], and pigs [40].

Several reports have presented information suggesting that animals may serve as reservoirs for MRSA infection of humans. In 2005, Loeffler et al. demonstrated that 18% of 78 staff members and 9% of 45 hospitalized dogs in a small animal veterinary referral hospital were carriers of MRSA. After PFGE typing, around 82% of the isolates recovered were indistinguishable or closely related to EMRSA-15 (the predominant strain responsible for human nosocomial infections in the UK) [41].

Manian et al. reported recurrent MRSA infection in a patient with diabetes and in his wife. Decolonization was unsuccessful despite repeated attempts. Sampling of the nares of the family dog revealed MRSA colonization with an indistinguishable PFGE type of MRSA. Further recurrence of MRSA infection and colonization in the couple was only halted after successful eradication of MRSA from the dog [42].

Equine to human transmission of MRSA infection has also been reported in a 24-h-old foal that was admitted to the Ontario Veterinary College Veterinary Teaching Hospital (OVC-VTH) neonatal intensive care unit for treatment of acute renal failure and septicemia. MRSA had been isolated from the admission nasal swabs of both the foal and its mare. MRSA arthritis and omphalophlebitis had developed in the foal during its course of illness. Seven days after admission, three cases of MRSA skin infections of the foal watch personnel were reported, and MRSA colonization was identified in 10 of 103 (9.7%) screened personnel (four foal watch personnel and six veterinary personnel). All isolates were indistinguishable via PFGE and classified as CMRSA-5 [43].

Recent reports suggest that pig farmers are at increased risk of nasal *S. aureus* (including MRSA) colonization. In 2003 Voss et al. demonstrated transmission of MRSA between pigs and pig farmers, pig farmers and their families, and on to a nurse and patient in a nearby hospital where three different MRSA strains were

identified, with an apparent increase in the widespread prevalence of the spa-type t108 strain [44]. In a case–control study done in the Netherlands, 32 out of 35 case patients were carriers of nontypable MRSA (NT-MRSA) and the multilocus sequence typing (MLST) of their isolates was consistent with sequence type (ST) 398. The authors reported that the geographic origin of this NT-MRSA correlated with the density of the pig population, and that the carriage of NT-MRSA was significantly related to contact with pigs and cattle ($P<0.01$) [45]. This study also found that the percentage of NT-MRSA in relation to the overall number of MRSA isolates in the Netherlands had increased from 0% in 2002 to 21% at the end of 2006.

Animal to human transmission of MRSA infection has been reported from major food animals. Lee et al. conducted a study that was designed to determine the prevalence of MRSA isolates among major food animal-related specimens and to determine their genetic relatedness to human MRSA isolates. They found 15 mecA-positive methicillin (oxacillin)-resistant *S. aureus* isolates out of 1913 (3.6%) samples from major food animal-related specimens. Twelve were from dairy cows and three from chicken specimens. Random amplified polymorphic DNA (RAPD) patterns were generated by PCR and showed that six of the isolates from animals were identical to the patterns of the isolates from humans. These data indicated the possibility of transmissions of MRSA infection to humans through contaminated food products made from these animals [38].

On the other hand, there is also evidence that transfer of MRSA strains can occur between humans and animals. Baptiste et al. reported transmission of the predominant human epidemic strain EMRSA-15 between three veterinary staff members and three dogs, suggesting that while interspecies transmission of identical MRSA isolates can happen between pets and humans in contact with them, the original source of the infection and the direction of transmission may be uncertain [46].

6 A Call for an Action

The international public health authorities are urging countries to implement integrated antimicrobial resistance (AMR) surveillance systems. In Canada, The Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) was designed to establish a national surveillance system to monitor AMR and antimicrobial use in the agri-food and agriculture sectors and the impact of resistance on human health. It is designed to generate antimicrobial resistance information from “farm to fork,” as well as from human and animal clinical cases [47]. This program is similar to the Danish Integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP) in Denmark [48], which was established in 1995, and the National Antimicrobial Resistance Monitoring System (NARMS) in the United States [49], which was established in 1996.

All of these programs are designed to monitor the consumption of antimicrobial agents and the occurrence of antimicrobial resistance. They allow for the inves-

tigation of associations between the use of antimicrobial agents in animals and humans and the occurrence of resistance among bacteria from animals, foods, and humans.

On July 3, 2008, the US Food and Drug Administration (FDA) published a final rule that prohibits the extra label use of cephalosporin antimicrobial drugs in food-producing animals, including, but not limited to cattle, swine, chickens, and turkeys [50]. Data provided by the Canadian Animal Health Institute (CAHI) show a dramatic decline of 29% in the amounts of distributed active antimicrobials used for farm and companion animals over a 3-year period, with 1.6 million kg of antimicrobials being distributed in 2001 and 1.1 million in 2003. This decline can be attributed to several factors including the increased awareness of the need for judicious use of medications and improvements in production practices [51].

7 Pets and Immunocompromised Hosts

Immunocompromised patients that are considered at high risk for serious infection from pets include people with waning immunity such as the elderly, children less than 5 years of age, pregnant women, and the clinically immunocompromised. The most recent written evidence-based guidance was produced by the Centers for Disease Control and Prevention (CDC, 2009) for HIV-infected persons and includes the following [6, 52]:

- When obtaining a new pet, HIV-infected persons should avoid animals aged <6 months.
- HIV-infected persons should avoid contact with any animals that have diarrhea.
- HIV-infected pet owners should seek veterinary care for animals with diarrheal illness, and a fecal sample from such animals should be examined for *Cryptosporidium*, *Salmonella*, and *Campylobacter*.
- HIV-infected persons should wash their hands after handling pets, including before eating, and should avoid contact with pets' feces.
- HIV-infected persons should avoid contact with reptiles (e.g., snakes, lizards, iguanas, and turtles) as well as chicks and ducklings because of the risk for salmonellosis.
- Gloves should be used during aquarium cleaning to reduce the risk for infection with *Mycobacterium marinum*.
- Contact with exotic pets (e.g. nonhuman primates) should be avoided.

It may well be prudent to apply such guidelines to other immunocompromised individuals. Pet animal exposure should be avoided in child care facilities and animal exposure should be minimized as much as possible in children less than 5 years of age with the emphasis of proper hand hygiene. The role of pediatricians and veterinarians in pet selection should not be dismissed, and their advice about pet-related hazards and safe pet ownership is important.

8 Conclusion

Humans are usually an accidental host that acquires disease through close contact with an infected animal. Animals are responsible for transmission of an extensive array of zoonotic pathogens that are transmitted by different routes, but this risk of transmission of infections is low and may be further reduced by simple precautions.

Antimicrobial resistance is increasing among organisms causing infections in pets and domestic animals. They are able to acquire and exchange multidrug-resistant pathogens with humans, and this problem has been recognized particularly with respect to MRSA.

Data on pet animals are needed for guiding antimicrobial use policy in small animal veterinary practice and assessing the risk of transmission of antimicrobial resistance to humans. The role of public health in the implementation of quality assurance programs on farm and raising awareness of the need for judicious use of antimicrobial agents will become increasingly important. It is not surprising that since we share our world with animals, we share risks of infections with them too.

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Novel Technology to Study Co-Evolution of Humans and *Staphylococcus aureus*: Consequences for Interpreting the Biology of Colonisation and Infection

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Abstract Human nasal carriage of *Staphylococcus aureus* is a textbook example of an apparently neutral interaction between humans and a bacterial species that can still lead to (severe) opportunistic infections. The co-evolutionary aspects of this interaction are slowly surfacing, facilitated by the emergence of a diversity of diagnostic, epidemiological and molecular research tools. Basic microbiology has helped define persistent vs. non-carriage and the genotype of both host and guest has been explored in search of genetic markers for bacterial persistence. This chapter summarises the current state of affairs relating to artificial human colonisation studies with *S. aureus*, large-scale human cohort studies and innovative assessment of the humoral immune status of (non-)nasal carriers. These experimental approaches have recently assisted in identifying bacterial and human determinants and risk factors for staphylococcal carriage. Further refinement of the model by describing the interactions between *S. aureus* and its human host in molecular detail is important since it may pinpoint novel anti-infectious strategies.

1 Introduction

Staphylococcus aureus is a bacterial species that is capable of colonising a number of mammalian hosts [1, 2]. Among these are several companion animals and animals of veterinary importance [3, 4]. These animals and their human keepers can be colonised by *S. aureus* in an innocent, bystander-type way. However, at least in humans such colonisation can develop into active (auto-)infection [5, 6]. This, obviously, poses an important complication. Little is known on the transition from the colonised into the infected state. Questions on the nature of the most relevant pathogen- and host-defined determinants still remain unanswered in many cases. In

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order to provide answers on these and other questions relating to the nature and consequences of nasal carriage in humans, more detailed studies into the precise co-evolutionary (and molecular) interactions between host and visitor need to be undertaken [7, 8]. This chapter describes three recent large studies that may shed some additional light on the important topic of host–pathogen co-evolution: artificial *S. aureus* inoculation studies in human volunteers; longitudinal follow-up of cohorts of individuals with well-defined bacterial carriage status and assessments of the serological response in people carrying or infected with *S. aureus*. However, first this chapter highlights several of the more basic bacterial and host characteristics that are important in establishing a stable colonised state in a susceptible host.

2 The Microorganism

2.1 Detection and Identification

S. aureus is a coccal-shaped bacterial species that lives in small colonies. These "bunch of grape"-like communities are tight clusters of between five and a few dozen individual cells. Precise quantification of colony forming units of *S. aureus* is hampered by their sticky behaviour and it seems highly likely that there must be some ecological advantage to sticking together [9]. How this works, however, is currently ill defined.

Laboratory diagnosis of *S. aureus* is quite straightforward: Gram staining provides a good clue. Selective culture is possible: *S. aureus* is quite tolerant to table salt, it will even grow in solutions of up to 1 M sodium chloride [10]. In combination with an indicator dye, simple broth media can be used for sensitive and specific enrichment cultures. In addition, a few simple agglutination tests will usually provide definite answers as to species' nature. Finally, molecular testing with ribosomal sequence motifs as targets will provide a watertight diagnostic result [11]. In addition to these clinical diagnostics, suited for the laboratory-based identification of *S. aureus,* there also is a new generation of tests that are suited for the direct detection of *S. aureus* in clinical material. These assays still vary from "old-fashioned" in-house laboratory PCR tests, that sometimes still rely on gel electrophoresis for the visualisation of the amplified product [12], to more innovative tests that employ real-time PCR without subsequent steps. These tests are good, although not all laboratories perform them equally well [12]. Most importantly, these tests can be used to reveal within a working day whether or not a patient is a nasal carrier or not. These data can provide a basis to interfere with carriage (by mupirocin and chlorhexidine treatment, for instance) and in that way generate a blockade to auto-infection.

2.2 Habitat and Genome Complexity

S. aureus has a great capacity to survive and it can do that in both animate and inanimate environments [13]. It will survive for weeks in dry environments but it has also been encountered in and on obligate seawater-dwelling mammals [14].

In the host, physical interactions define the likelihood of developing a successful interaction between man and bacteria. In addition, a variety of bacterial anchors play an important role. The so-called microbial surface components recognising adhesive matrix molecules (MSCRAMMs) form an important category protein facilitating the colonisation of alien niches [15]. MSCRAMMs may recognise host molecules as diverse as collagen, von Willebrand factor and vitronectin. These proteins may also show antigenic variability; simple size variation in regions of repetitive DNA (and, hence, peptides) is also frequently recorded. Overall, *S. aureus* is a bacterial species that is very well equipped for fulfilling its life cycle in a potentially quite hostile *Homo sapiens* environment.

Bacterial versatility is the word that comes to mind and that is also what the staphylococcal genome reflects. Overall, *S. aureus* genomes range between 2 and 3 megabases in length [16]. The genome consists of three major categories of DNA regions. First there is the core genome that is quite well conserved between isolates and encodes all of the requirements needed to fulfil the bacterial life cycle. Second there is core variable DNA. These regions, spread across the entire genome, encode factors that may show genetic variability to a limited degree. This material provides a certain genetic flexibility. Important factors may, for instance, evade host immunity through the Darwinian selection of non-immune-recognised epitopes that are generated through random mutagenesis processes. Last but not least, there are the real variable elements: the so-called mobile genetic elements (MGEs) [17]. These may include transposons, integrated bacteriophages and islands consisting of virulence factors. Such genomic islands play an important role in staphylococcal biology and may be related to host specificity and antimicrobial resistance. The staphylococcal cassette chromosome *mec* (SCC*mec*) is an example of the latter category of elements [18]. On the whole, however, *S. aureus* is a clonal microorganism [19, 20]. This implies that the genome is quite well conserved and that there are significant similarities between many of the *S. aureus* isolates collected in clinical laboratories. However, despite this conservation and the associated genetic flexibility, essentially all strains of *S. aureus* can become invasive to their host given the appropriate conditions [19, 21]. These usually exist in the form of ineffective or waning immunity of the host.

3 The Host

3.1 Human Niches

When living in or on a mammalian host, *S. aureus* interacts with a variety of different molecules and physical conditions. First and foremost, there are the physical constraints. Temperature, salinity, pH, availability of oxygen and hydratation are important determinants of staphylococcal survival. In addition, there are the biological features. In a host, bacteria have to compete with other bacterial species that may be attracted to the same niche [22]. The bacteria have to be able to withstand the innate and acquired immune responses and features such as adherence to extracellular mucus or certain receptor molecules are ultimately of importance

in developing a stable colonised state. Hosts either do or do not provide such conditions. *S. aureus* thrives on human skin, the gastrointestinal tract, the pharyngeal region and, most prominently, the vestibulum nasi of the human nose [1, 23]. The latter ecological niche is considered by far the most important and it is suggested that all colonisation of other anatomical locations derive from nasal carriage.

3.2 Patterns of and Susceptibility to Carriage

Humans can be divided, on the basis of serial cultures, into three phenotypic categories of (non-)carriers: persistent, intermittent and non-colonised individuals [24]. Several humans will be nasal culture positive in over 90% of all culture attempts, others may be positive in only 10–80% of cultures whereas the remaining individuals will be repetitively culture negative. The persistent carriers comprise in the order of 30% of individuals. Interestingly, the intermittent carriers are characterised by a more extensive genotypic diversity among colonising strains, indicating that these individuals occasionally pick up a strain, carry it for a relatively short time and then either clear the strain or become re-colonised again [25, 26]. In this group of carriers the number of viable cells that can be collected from the nose is usually less (up to 1,000-fold) than the number collected from persistent carriers [24]. Several host factors define the differential host susceptibilities and simple ones are gender and age [8]. It has been found that males are more often persistent carriers and it has also been shown that with increasing age the fraction of persistent carriers declines. These are unexplained differences. Another unexplained but very intriguing difference is the influence of fasting glucose level. There is a linear association between the molarity of blood glucose and *S. aureus* nasal carriage: the higher the glucose level, the more likely an individual is to be a persistent carrier. Last but not least: there is a genetic basis for the predisposition to become a carrier. Certain HLA variants have since long been associated with *S. aureus* carriage [27–32], but modern genome-wide testing for genetic variation has recently facilitated more in-depth studies. Single nucleotide polymorphisms in several immune genes, the serine protease inhibitor and the glucocorticoid receptor gene have been shown to be associated with carriage in a statistically significant fashion [33–36].

In short, there is an enormous diversity in both the host and the bacterial factors that are important in establishing colonisation. These factors interact in a complex manner and it is this interaction that ultimately defines whether or not a host is susceptible to (long-term) colonisation. A number of essential colonisation factors have been identified through experimental approaches. Unfortunately, most of these experiments have been performed in animal models. Definite proof of the relevance of certain factors requires a human model of colonisation. Results to date from experiments in a human volunteer nasal colonisation model will be succinctly described below.

4 Artificial Colonisation Studies

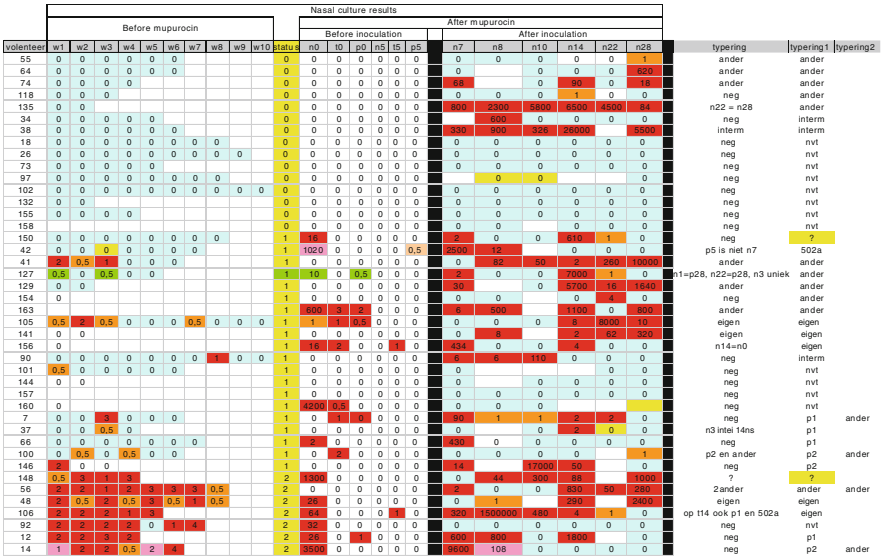
4.1 Setting Up a Nasal Colonisation Study

Artificial human colonisation with an opportunistic pathogen such as *S. aureus* needs careful consideration and preparation [37]. Individuals with certain risk factors have to be excluded from such experiments. These include, among others, diabetes, recurrent skin infections and other skin-related problems, pregnancy, frequent contact with susceptible patients (either work related or in the personal arena) and allergy to certain antibiotic or chlorhexidine [38, 39]. In addition, the inoculum strain(s) should be devoid of known virulence genes and resistance traits (SCC*mec*, superantigens and toxins such as Panton–Valentine leukocidin and others). An "intended release" genetically modified organisms (GMO) protocol with appropriate permissions is required for experiments involving the inoculation of genetic mutants of *S. aureus* [40]. Once ethical and GMO-related hurdles have been taken, colonisation studies are relatively simple to develop. Our studies include at least 16 people when comparing two parameters to provide data with sufficient statistical power. Only routine laboratory equipment found in a good clinical diagnostic microbiology facility is needed.

Our studies are usually staged: we start with assessing the nasal *S. aureus* carriage status of our volunteers. This means that participants are followed for 5–10 weeks and that at least five nasal cultures are taken. This identifies carriers, intermittent carriers and non-carriers [24, 26]. Once the cohort has been established and characterised, the elimination treatment can commence. This consists of nasal mupirocin ointment for 5 days and a 1 week supply of chlorhexidine soap and shampoo. After 6 weeks the colonisation state is re-assessed and individuals without carriage in the rectum, throat and nose can be included in the inoculation experiment.

Volunteers are then nasally challenged with 5×10^9 cells per strain to be used. The maximum number of strains combined to date is 5 which results in an inoculum of 2.5×10^{10}. To date, none of our volunteers has experienced any serious adverse events as a result of the inoculation. Participants have, however, remarked on a "fungus-like" smell of the bacterial inoculum. Volunteers are followed up closely, with an infectious disease specialist on call for the duration of the experiment. Nasal cultures are performed every day during the first week after inoculation and once weekly thereafter. The experiment continues for 3 months after which those individuals who are still colonised by a strain from the mixture are offered a new decolonisation course. The colonisation study protocol is shown in Fig. 1.

The microbiology aspects of the experiments are also quite simple. Samples are taken and diluted in physiological salt, part of which is plated on blood agar. The rest of the material is immersed in an enrichment broth. If the agar cultures show growth within 4 days, putative *S. aureus* colonies (all different colony morphologies included to a maximum number of 20) are selected and re-cultured. These are speciated by standard lab technology and up to 16 strains are frozen at –80°C for use at a later stage. These follow-up studies may involve overall genotyping, assessment of gene absence or more detailed microbiological analyses. When there is growth in



	Nasal culture results																												
	Before mupirocin											After mupirocin																	
												Before inoculation						After inoculation											
volenteer	w1	w2	w3	w4	w5	w6	w7	w8	w9	w10	status	n0	t0	p0	n5	t5	p5	n7	n8	n10	n14	n22	n28	typering	typering1	typering2
55	0	0	0	0	0	0					0	0	0	0	0	0	0	0	0	0	0	0	1	ander	ander	
64	0	0	0	0	0	0					0	0	0	0	0	0	0	0		0	0	0	620	ander	ander	
74	0	0	0	0							0	0	0	0	0	0	0	68		0	90	0	16	ander	ander	
118	0	0	0								0	0	0	0	0	0	0	0	0	0	1	0	0	neg	ander	
135	0	0									0	0	0	0	0	0	0	800	2300	5800	6500	4500	84	n22 = n28	ander	
34	0	0	0	0	0						0	0	0	0	0	0	0		600	0	0	0	0	neg	interm	
38	0	0	0	0	0	0					0	0	0	0	0	0	0	330	900	326	26000		5500	interm	interm	
18	0	0	0	0	0	0	0	0			0	0	0	0	0	0	0	0	0	0	0	0	0	neg	nvt	
26	0	0	0	0	0	0	0	0	0		0	0	0	0	0	0	0	0	0	0	0	0	0	neg	nvt	
73	0	0	0	0	0	0					0	0	0	0	0	0	0	0	0	0	0	0	0	neg	nvt	
97	0	0	0	0	0	0	0	0			0	0	0	0	0	0	0		0	0			0	neg	nvt	
102	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	neg	nvt	
132	0	0									0	0	0	0	0	0	0	0	0	0	0	0	0	neg	nvt	
155	0	0	0	0							0	0	0	0	0	0	0	0	0	0	0	0	0	neg	nvt	
158											0	0	0	0	0	0	0	0	0		0	0	0	neg	nvt	
150	0	0	0	0	0	0	0	0			1	16	0	0	0	0	0	2	0	0	610	1	0	neg	?	
42	0	0	0	0	0	0	0				1	1020	0	0	0	0	0,5	2500	12		0	0	0	p5 is niet n7	502a	
41	2	0,5	1	0	0	0					1	0	0	0	0	0	0	0	82	50	2	260	10000	ander	ander	
127	0,5	0	0,5	0	0						1	10	0	0,5	0	0	0	2	0	0	7000	1	0	n1=p28, n22=p28, n3 uniek	ander	
129	0	0									1	0	0	0	0	0	0	30		0	5700	16	1640	ander	ander	
154	0										1	0	0	0	0	0	0	0	0		0	4	0	neg	ander	
163											1	600	3	2	0	0	0	6	500		1100	0	800	ander	ander	
105	0,5	2	0,5	0	0	0	0,5	0	0	0	1	1	1	0,5	0	0	0	0	0	0	6	8000	10	eigen	eigen	
141	0	0									1	0	0	0	0	0	0	0	1		2	62	320	eigen	eigen	
156	0										1	16	2	0	0	1	0	434	0	0	4	0	0	n14=n0	eigen	
90	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	0	6	6	110	0	0	0	neg	interm	
101	0,5	0	0	0	0	0					1	0	0	0	0	0	0	0				0	0	neg	nvt	
144	0	0									1	0	0	0	0	0	0	0		0	0	0	0	neg	nvt	
157											1	0	0	0	0	0	0	0	0	0	0	0	0	neg	nvt	
160	0										1	4200	0,5	0	0	0	0	0	0	0				neg	nvt	
7	0	0	3	0	0	0					1	0	1	0	0	0	0	92	1	1	2	2	0	neg	p1	ander
37	0	0	0,5	0							1	0	0	0	0	0	0	0		0	2	0	0	n3 intel 14ns	p1	
66	0	0	0	0	0	0	0				1	2	0	0	0	0	0	430	0	0	0	0	0	neg	p1	
100	0	0,5	0	0,5	0	0					1	0	2	0	0	0	0	0	0	0	0		1	p2 en ander	p2	ander
146	2	0	0								1	0	0	0	0	0	0	14		17000	50		0	neg	p2	
148	0,5	3	1	3							2	1300	0	0	0	0	0	0	44	300	88		1000	?	?	
56	2	2	1	2	3	3	3	0,5			2	0	0	0	0	0	0	2	0	0	830	50	280	2 ander	ander	ander
48	2	0,5	2	0,5	3	0,5	1	0,5			2	26	0	0	0	0	0	0	1		290		2400	eigen	eigen	
106	2	2	2	1	3						2	64	0	0	0	1	0	320	1500000	480	4	1	0	op t14 ook p1 en 502a	eigen	
92	2	2	2	2	0	1	4				2	32	0	0	0	0	0	0	0	0	0	0	0	neg	nvt	
12	2	2	3	2							2	26	0	1	0	0	0	600	800	0	1800		0	neg	p1	
14	1	2	2	0,5	2	4					2	3500	0	0	0	0	0	9600	108	0	0	0	0	neg	p2	ander

Fig. 1 Table showing timing of nasal cultures for assessing the carriage rates among volunteers participating in artificial nasal colonisation studies with *S. aureus.* Studies can be divided into a number of distinct episodes: the before mupirocin/chlorhexidine and after mupirocin/chlorhexidine period and the before and after inoculation episodes. During the first episode the nasal carriage status of the volunteers is determined during a 10-week period. On the basis of the quantitative culture results the volunteers are then stratified into non-carriers, intermittent carriers and the persistent carriers. Before inoculation with *Staphylococci*, the volunteers are treated with mupirocin nasal ointment and chlorhexidine soap and shampoo. The success of this treatment is verified on the basis of nasal, throat and perineal *S. aureus*-specific cultures. Volunteers who are free of *S. aureus* can then proceed to the inoculation phase. Bacteria are instilled in the nose on day one and colonisation is followed up for 28 days. Finally, strains are typed to define colonising isolates

the enrichment broth, medium is plated on blood agar again and cultured bacteria are again speciated following which a single colony is stored. A pre-inoculation and end of study serum sample is also collected, primarily to assess infection parameters, though ultimately these samples will also be used for *S. aureus* antibody serological studies (see below).

4.2 Examples of Previous Colonisation Studies

Three artificial colonisation studies have been published from our laboratory thus far [37, 40, 41]. The first concerned a simple study involving 16 volunteers. Eight persistent and eight non-carriers were included and inoculated with a mixture of four strains. In carriers, their own strain was included in the inoculation mixture. Results clearly showed a human factor in nasal carriage: both the persistent and the non-carriers returned to their pre-inoculation carriage status. Interestingly, carriers tended to re-select their own strain, suggesting the existence of a very intricate

relatedness between host and guest. This was further corroborated by another study which also included intermittent carriers [41]. This study, involving the largest number of volunteer participants to date, showed that intermittent carriers behave in the same way as non-carriers: they eliminate the inoculum in a matter of days and, surprisingly, their anti-staphylococcal antibody profiles are similar to those of non-carriers. So both in humoral responses and nasal elimination kinetics the persistent carriers are a distinct group. This indicates that the paradigm needs changing: there are only two categories of nasal *S. aureus* carriers: those who do and those who do not persistently carry the organism. It is important to note that this implies that the majority of people are non-carriers and that these individuals have "solved" the problem of preventing auto-infection by ensuring that the nose is an inefficient reservoir for *S. aureus* bacteria. The third published inoculation study investigated the elimination kinetics of wild-type *S. aureus* and an isogenic *ClfB* mutant of the same strain [40]. It was shown that the ClfB mutant was eliminated at a statistically significant higher rate than the wild type. This indicates that ClfB, an adhesin belonging to the family of MSCRAMMs and capable of binding both fibrinogen and cytokeratin-10 [42–44], is an essential component in the array of molecules putatively involved in nasal colonisation.

4.3 Ongoing Experiments and Ideas for Future Colonisation Studies

We have preliminary evidence that *S. aureus* strains that harbour prophages that carry immune evasion clusters (IECs) [45, 46] do worse in colonising adult human volunteers. Counterintuitively, the strains that contain the evasion clusters are eliminated from the nasal cavity at a higher rate. Whether this is due to, for instance, elevated antibody levels against several of the IEC-coded proteins is the subject of current investigations. Our preliminary data seem to suggest that this is not the case and that there is no important role of humoral immunity in this selective phenomenon. It might thus be that the IECs play their most important role in immune-naïve individuals, i.e. children during their first encounters with colonising or even infecting *S. aureus* bacteria.

Multiple additional colonisation studies can be envisaged: each and every (potential) MSCRAMM can be assessed to investigate their importance in colonisation of humans (e.g. [47]). Also, it has been suggested that many strains are restricted to certain mammalian hosts (pigs, cow, horses, sheep, goats and many more) [48]. Inoculation studies could be used to corroborate these suggestions, primarily based on bacterial genotyping studies rather than real in vivo assessments. It is not known up to what extent *S. aureus* colonisation is inhibited by the presence of other bacterial species. The species that are most frequently mentioned in this respect are coagulase-negative staphylococci and Coryneform bacteria [49]. Artificial colonisation could be used to investigate this potentially therapeutic bacterial interference phenomenon [7, 50]. Finally, if voluntary participation is feasible, inoculation studies could be used to define the potential selective advantage of multi-resistant

bacteria including methicillin-resistant *S. aureus* (MRSA) or the effect of toxins on colonisation. Finally, inoculation with random mutant libraries could identify many novel adherence factors in a single large-scale in vivo expression technology (IVET)-like experiment [51].

In conclusion, over the years we have developed an important and useful model for the detailed study of the interaction between humans and *S. aureus* bacteria. In vivo selection of the fittest colonisers is feasible and this may help reveal why some *S. aureus* lineages are more successful (or epidemic) than others.

5 Example Of A Cohort Study

5.1 Relevance of Cohort Studies

Cohorting can be used to generate often unexpected clinically relevant data from complex environments and situations. Embedding a microbiological assessment of bacterial colonisation within a cohort study that focuses on other diseases is extremely productive [52, 53]. In Rotterdam in The Netherlands, a cohort of neonates was initiated in 2004 and primarily focused on determinants of non-infectious diseases. Within this so-called Generation R cohort a variety of features have been monitored [54]. These include host genetics, gestational age, immune parameters, gender and birth weight. In addition, day-care attendance, presence of siblings, parental smoking, socio-economic status and gender have been recorded. This has generated extensive databases enabling extrapolations and correlations between a variety of factors and microbiological culture data.

5.2 Microbiology in Generation R

The bacterial carriage study was embedded in the prospective Generation R cohort study. Overall, 10,000 pregnant women and their children were enrolled. Within the so-called Focus cohort, 1,232 women were eligible to participate. Complete follow-up in the Focus cohort of 1,079 infants was available. Visits to the study centre were scheduled at 1.5, 6, 14 and 24 months of age (with a visit rate of 82%). Questionnaires were done at age 2, 6, 12, 24 months and 2 and 5 years. The main goal of the microbiological research within Generation R was to study bacteriological and immunological determinants of nasopharyngeal bacterial carriage in young children and to assess their association with respiratory tract infections. For this reason, serial nasal and nasopharyngeal swabs were taken over extended periods of time and specifically cultivated for *S. aureus, Moraxella catarrhalis, Streptococcus pneumoniae* and *Haemophilus influenzae*. This is enabling the mapping of microbial colonisation dynamics, bacterial interference, the role of human genetic variability (SNP measurements), the definition of Ig and TCR repertoires and the definition of the intricate relations between colonisation, host immune response and respiratory tract infections [55].

5.3 Preliminary Results

Serial follow-up of young children with nasopharyngeal culture reveals that different bacterial opportunists show completely different longitudinal colonisation kinetics. For both *M. catarrhalis* and *H. influenzae* there is a continuous rise in prevalence from birth onwards, with peak incidences at the age of approximately 30% at the age of 2 years. At 6 weeks of age the colonisation rate peaks at 10%. For these two species increase in age is accompanied by an increasing colonisation rate until saturation levels are reached. For *Pneumococcus* and *S. aureus* the situation is a little more dynamic. The colonisation rates for the *Pneumococcus* at 1.5, 6, 14 and 24 months are 8, 31, 44 and 37%, respectively. The *S. aureus* rates are 52, 20, 15 and 35% [53]. This inverse relationship between the two species has also previously been demonstrated in large-scale cross-sectional population studies [50, 56]. Clearly, the colonisation of the nose and the nasopharynges by different bacterial species is a dynamic and evolving process. The situation is likely to even more complex: many additional bacterial species inhabit the same niche and further investigations should also include, for example, anaerobic bacterial species.

It is interesting to note that persistent carriage does not seem to exist in young children. Although the colonisation prevalence is quite high, serial cultures revealed that few children were persistently culture positive. In addition, genotyping revealed that even children who cultured positive on more than one occasion were colonised by genotypically distinct bacterial strains. This suggests that intermittent rather than persistent carriage is predominant among young children [53]. It might be that these children are "sensing" the microbiological diversity of staphylococci until they find their match and enter into a long-term relationship with a strain. Conversely, the initial encounters with *S. aureus* may also result in a more permanent non-carriage state. Whether this is imposed by humoral immunity, cellular immunity or another driving factor is currently unknown. Environmental, demographic or social determinants of staphylococcal carriage were not identified. Only male gender was associated with carriage. Features such as gestational age, birth weight, breast feeding habits, mothers educational level, (prenatal) smoking habits, the presence of siblings or day-care attendance were of limited importance. The studies in the paediatric cohort clearly reveal that staphylococcal carriage in children is significantly different to adults. Long-term persistence seems to occur in later life and the incidence of carriage shows a highly significant decrease in the first years of life. Whether this is immune regulated or due to other, physiological phenomena remains to be investigated.

Cohort studies are often designed to identify markers of disease. Within the Generation R cohort we specifically addressed the aetiology of atopic dermatitis (AD). We defined correlations between nasal *S. aureus* carriage and the development of AD in later stages of life [52]. When culture results for nasal swabs taken at 1.5, 6 and 14 months of age were correlated with AD determinants, significant associations were documented. For instance, a first positive *S. aureus* culture at the age of 6 months was associated with AD in both the first and the second year of life.

There was also an association with parentally reported AD severity. In addition, frequent colonisation resulted in a more than fourfold increased risk of moderate to severe AD in the second year of life. Although this is still mechanistically unexplained, it is clear that nasal colonisation with *S. aureus* predisposes to AD. We are currently investigating the correlation between nasal carriage and other relevant childhood diseases (including wheezing and asthma) and novel associations have already been identified. Future studies into these correlations should include detailed immunological and physiological assessments. Elucidating the mechanisms of such correlations may lead to the design of novel prophylactic measures for atopic diseases in general [57].

6 Humoral Immunity and *S. aureus* Carriage and Infection

6.1 Technical Aspects of Multiplex Anti-staphylococcal Antibody Measurements

Antibody detection can be done by a variety of long-established methods including a variety of immunodiffusion tests, membrane-based Western blotting assays and enzyme linked immunosorbent assays (ELISA) [58]. Disadvantage of the commonly used ELISA tests is that relatively large amounts of serum (or other test liquids) are required and that the opportunities for multiplex testing are limited. Innovative bead-based immunofluorescence tests, the so-called Luminex technology, overcome this limitation allowing the simultaneous measurement of a large number of antibodies or other factors [59, 60]. We have developed a 40-plex bead-based FACS test for a variety of staphylococcal antigens [61–63]. This can be used to study the immunogenicity of staphylococcal antigens during colonisation and infection and also to study the longitudinal maturation of the anti-staphylococcal antibody response in the early stages of life.

6.2 Application of the Luminex Technology and Microbiological Implications

The first tests involving the staphylococcal Luminex test concerned the identification of anti-staphylococcal antibody profiles in healthy adults who were established persistent or non-carriers of *S. aureus* [62]. This showed a number of biologically interesting features. First and foremost, antibody levels in persistent carriers were, on average, higher than in non-carriers and the profiles were quite unique to an individual. The profiles are unique to an individual and remain consistent over a period of at least 6 months. This suggests that the continuous challenging of the immune system by the presence of bacterial cells stimulates antibody production. Second, we showed that the overall antibody profiles, when comparing the two categories of carriers, differed significantly between the two groups. Despite the fact that the panel of staphylococcal antigens was limited to 20 in these initial studies we found

several antibody levels that differed in a statistically significant manner between the two groups. Antibody levels against toxic shock syndrome toxin (TSST), clumping factor A, clumping factor B and enterotoxin A were elevated among persistent carriers. We were also able to show that at least for the anti-TSST antibodies this elevation led to a more pronounced in vitro inhibition of toxin activity. In other words, the differences in antibody levels were also reflected in differences in antibody neutralising activity. It remains a challenge to define whether or not these quantitative and qualitative differences can be associated with the differences in infectious risk and outcome of infections that have been observed clinically between carriers and non-carriers [21, 64].

A second study involved the investigation of intermittent *S. aureus* carriers. The Luminex assay revealed that the antibody profiles of intermittent and non-carriers were essentially identical [41]. No significant differences could be established which led to the conclusion that intermittent carriers are contaminated non-carriers rather than "accidentally negative" persistent carriers. This fits well with the outcome of the artificial inoculation study and was also in agreement with previous studies in which infection risk in intermittent carriers was shown to be the same as that in non-carriers and not elevated like in persistent carriers.

We also used the Luminex system to investigate differences in antibody profiles between various groups of individuals with distinct staphylococcal infections [61]. These experiments focused on assessment of toxin antibody responses in patients with various diseases. For many of the toxins, clearly elevated levels of antibodies were documented in patients compared to healthy controls. This clearly suggests in vivo expression of these toxins during infection. Adults displayed higher anti-toxin antibody levels than children. If certain toxin genes were absent in the infectious strain, the antibody response was lower than that induced by gene-positive strains. Bacteriaemia was associated with a higher prevalence of enterotoxin A among the infectious isolates, whereas bone and joint infections were caused by strains enriched in Luk-PV. This led to the conclusion that during infection a variety of toxins is actively expressed and recognised by the immune system. In addition, certain toxin genes seem to be important aetiological factors during several types of infections.

Finally, defining the humoral immune kinetics at the early stages of life is important [63]. This could provide clues to the identity of major immunogens and it could also show whether (maternal) antibodies provide protection against colonisation or infection. In the Generation R cohort we studied serial serum samples from over 50 children. This showed that, again, the antibody profiles were extensively different between individuals. IgA and IgM levels clearly increased over the first 2 years of life, whereas maternal antibodies steeply decreased in the first half year of life. The maternal antibody levels did not provide any protection against colonisation of the child. Interestingly, the differences in antibody profiles documented for colonised vs. non-colonised children differed, but to a different extent than was defined for (non-)carrying adults. In children, the chemotaxis inhibiting protein of *S. aureus* (CHIPS), Efb and IsdA IgG levels were elevated among the carriers. Again, this shows that these antigens are expressed in vivo and stimulate

the humoral immune response. Their role in colonisation should still be defined further and further research is clearly warranted.

7 Conclusion

The interaction between *S. aureus* and its human host is complex. Both bacterial and human factors are important in establishing a quite individualised interaction, which may amount to an exclusive interaction between a host and a specific bacterial isolate. Although this interaction may be disrupted occasionally, leading to minor (skin lesions, boils, etc.) or more severe (bacteriaemia, sepsis, pneumonia, etc.) infections, in general the interaction is quite silent. *S. aureus* and the human host generally co-exist well, although it is notable that over 50% of individuals remain healthy without being colonised by *S. aureus* strains. More detailed, molecular insight into the factors associated with human non-colonisation may help identify novel measures that may prevent or cure staphylococcal infections. Our integrated model, comprising artificial inoculation studies, access to clinical materials (serum, DNA, nasal secretes and *S. aureus* strains), availability of human and bacterial transcriptomics facilities and the Luminex antibody-profiling system, provides a valuable set of tools for the future studies.

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A Practical Approach to Eosinophilia in a Child Arriving or Returning From the Tropics

Penelope Bryant and Nigel Curtis

1 Introduction

Eosinophilia is usually defined as an eosinophil count in the peripheral blood of greater than 0.35×10^9/L. The list of causes of eosinophilia is long and includes some that are rare in children (Table 1) [1–3]. Infection with helminthic parasites is the commonest cause of eosinophilia in children who are either immigrants from a developing country or returned travellers from tropical locations. However, determining the parasite causing eosinophilia can often be difficult for a number of reasons as elaborated below.

2 Why it is Often Difficult to Determine the Parasite Causing Eosinophilia

2.1 Patients with Helminthic Infection may be Asymptomatic

In retrospective studies of patients with helminthic infection, large proportions are asymptomatic [4–6]. When present, the commonest symptoms with helminthic infection are cough, skin manifestations, and gastrointestinal symptoms [6, 7]. These symptoms can be useful to guide diagnosis, although they frequently relate to the stage of helminthic disease, rather than being specific for a particular parasite. For example, fever and cough (Loeffler's syndrome) can be the presenting feature of larval migration through the lungs of several different parasites including nematodes, *Ascaris*, *Strongyloides*, and hookworm. In the absence of symptoms,

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Table 1 Causes of eosinophilia

Infectious	Allergic/skin	Drugs
Helminths	Asthma	Beta-lactam antibiotics
Less common:	Rhinitis	Sulfa-containing antibiotics
D. fragilis	Eczema/atopic dermatitis	Tetracycline
Isospora belli	Chronic urticaria	NSAIDs
Toxocariasis	Bullous pemphigoid	Salicylic acid
Toxoplasmosis	Toxic erythema of newborn	Carbamazepine
Leprosy	Eosinophilic cellulitis	Colchicine
Chronic tuberculosis		Nitrofurantoin
Coccidiomycosis		Dapsone
Myiasis		Minocycline
Scarlet fever		
HIV		
Hematological	**Rheumatological**	**Miscellaneous**
Lymphoma	Churg–Strauss syndrome	Allergic bronchopulmonary aspergillosis
Myeloid leukemia	Polyarteritis nodosa	Sarcoidosis
Eosinophilic leukemia	Wegener's granulomatosis	Addison's disease
Myelodysplastic syndrome	Rheumatoid arthritis	Ulcerative colitis
Hypereosinophilic syndrome	Other connective tissue diseases	Heavy metal poisoning
		Idiopathic eosinophilic pneumonia
		Irradiation

investigations should focus on the commonest infections and frequently an empiric approach to treatment is appropriate.

2.2 *Patients with Eosinophilia may have a Helminthic Infection that is too Early to Diagnose*

Helminthic infections have an early pre-patent phase when, despite the presence of the parasite in the host, they are almost impossible to detect. During this phase, the adult parasite may produce symptoms and eosinophilia, but definitive parasitological investigations are negative, as the progeny are not yet present in the stool, and the host has yet to mount a serological response. The duration of the pre-patent period is different for different parasites (Fig. 1).

2.3 *It is Difficult to Distinguish Between Different Helminthic Infections by Either Degree or Persistence of Eosinophilia*

Neither the degree nor the persistence of eosinophilia reliably differentiates between different helminths. The degree of eosinophilia is related more to migration and distribution of the parasite and the consequent host immune response than the particular parasite causing infection. Eosinophilia is highest when parasites come into

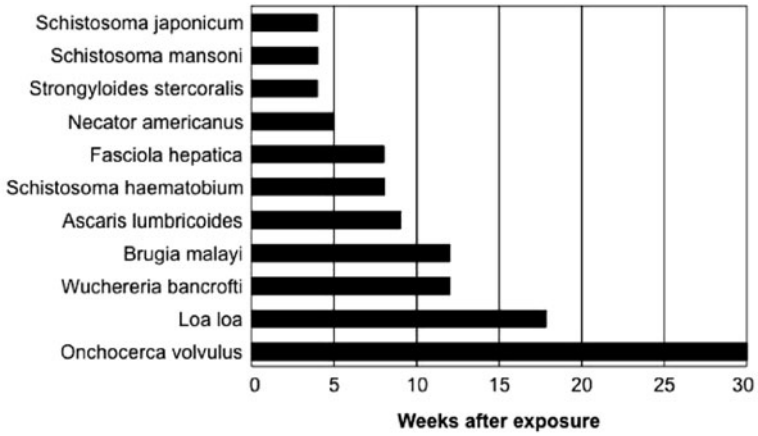

Fig. 1 Minimum duration of pre-patent period for different parasites (courtesy of Theodore E. Nash, MD)

contact with host immune cells in tissues, particularly during migration (for example, ascariasis, gnathostomiasis, and trichinosis). When the parasite is not invading tissue, the eosinophilic response is less pronounced (for example, intraintestinal tapeworms and encysted cysticercosis). Previous exposure of the host immune system to helminths also affects the degree of eosinophilia. In a returned traveller in whom infection probably reflects a first exposure, the eosinophilia may be very high. This contrasts with immigrants who have had chronic exposure to helminth infection, in whom the eosinophil count may be only slightly raised or even normal [8]. In one study of loiasis, temporary residents with the infection had a mean eosinophil count of 3.03×10^9/L, while for endemic residents it was only 0.37×10^9/L [9]. Volunteer studies of repeated exposure to parasitic infections show the highest eosinophil counts and most severe clinical symptoms following initial exposure, with decreasing counts and symptom severity following each subsequent exposure [10].

There are few helminthic infections in which there is extreme hypereosinophilia, although it has occasionally been reported, for example, in paragonimiasis and gnathostomiasis [11, 12]. In contrast, moderately high eosinophilia has been identified in patients with several parasitic diseases (Table 2) [2]. The persistence of eosinophilia is related more to chronicity of infection than infection with particular types of parasite, with a number of different parasites identified as causing persistent eosinophilia (Table 2) [2].

2.4 Whether a Child is an Immigrant or Returned Traveller does not help to Distinguish Between Different Helminthic Infections

For the same reasons as outlined above, although immigrants from endemic parts of the world are more likely to have persistent eosinophilia than returned travellers, this is not helpful in distinguishing between different parasites. Likewise, although the

Table 2 Helminthic infections associated with marked or prolonged eosinophilia

Parasite	Marked (>3×10^9/L)	Prolonged (several years)
Angiostrongylus	Yes	No
Ascaris	Yes – early migration	No
Clonorchis	Yes – early infection	Yes
Cysticercosis	No	Yes
Echinococcus	No	Yes – intermittent with cyst leakage
Fasciola	Yes – early infection	Yes
Gnathostoma	Yes	Yes – parasite migration
Hookworm	Yes – early migration	Yes – common cause
Loiasis	Yes – transocular migration	Yes
Onchocerca	Yes – dermatitis and ocular	Yes – dermatitis and ocular
Opisthorcis	No	Yes
Paragonimus	Yes – early infection	Yes
Schistosoma	Yes – early infection if not immune	Yes
Strongyloides	Yes	Yes – perpetuating auto-infection
Trichinella	Yes – muscle encystment	No
Visceral larva migrans	Yes	Yes

eosinophilic response due to acute infection is usually more pronounced in returned travellers compared to immigrants, this does not help to discriminate between parasites.

2.5 Serum IgE Level does not help to Distinguish Between Different Helminthic Infections or Exclude a Non-Parasitic Cause for Eosinophilia

Additional investigations such as serum IgE may also be abnormal in parasitic infection. In one study, serum IgE was raised in helminthic infections compared to most other causes of eosinophilia, but did not allow identification of the specific parasite or the exclusion of other diagnoses [3]. Serum IgE tends to be raised in the same situations as the eosinophil count, for example, during the lung migration phase of filaria.

2.6 Patients with Helminthic Infection may not have Eosinophilia

As the degree of eosinophilia is related to parasite location and host response, it is not uncommon for there to be helminthic infection in the absence of eosinophilia [4–6]. This occurs particularly in immigrants or expatriates who have had chronic exposure to any parasite (as discussed above) and with intraintestinal helminths such

as *Ascaris*, hookworm, and *Strongyloides*. In a study screening for helminthic infection in expatriates returning from the tropics, eosinophilia was present in only 27% of patients who were diagnosed with a parasite that usually causes eosinophilia [13]. The eosinophil count added nothing to stool culture and serology for diagnosis of *Strongyloides*, *Schistosoma* spp., and filaria.

2.7 The Degree of Eosinophilia does not Necessarily Relate to the Burden of Infection

Likewise, patients with chronic infection and a high burden of intestinal worms may have a relatively low eosinophilia, whereas returned travellers with a recent relatively mild exposure may exhibit a vigorous immune response and high eosinophil count.

2.8 Negative Investigations for Parasites do not Exclude Helminthic Infection

In addition to patients presenting in the pre-patent period, methods for diagnosing helminthic infections are relatively insensitive [14]. Detecting ova, cysts, or adult parasites in stool specimens is dependent on the experience of the examining scientist and the laboratory as a whole, whether or not the stool has been concentrated in addition to a direct smear and whether culture techniques are also used. Requests for ova, cyst, and parasite examination may be dealt with differently by the laboratory (e.g., the use of concentration techniques) depending on whether or not the words 'returned traveller' or 'immigrant' appear on the request form. It is particularly difficult to diagnose strongyloidiasis by microscopy of stool specimens as ova are almost never seen and larval production is usually low and intermittent. Agar culture techniques are more sensitive for the diagnosis of strongyloidiasis and hookworm infection than direct smear or concentration methods, but are not routine in many laboratories [15, 16].

The most sensitive investigation for diagnosing *Strongyloides* infection is serology: 85% compared to about 30% for stool microscopy. However, the sensitivity of serology for diagnosing this and other parasitic infections depends on a number of factors including whether it is too early in the disease for the host to have mounted a detectable immune response. Sensitivity may differ depending on where the parasite is located, for example, in hydatid disease: presence in the lung (90% of patients) or liver (60%) [17]; the number of parasites, for example, in neurocysticercosis: single (28%) or multiple (94%) cysts [18]; and for some parasites whether the infection is in a returned traveller (73%) or in an immigrant (98%) [5]. Serology is not always specific as a result of cross-reactivity between different helminths, for example, loiasis, strongyloidiasis, and filariasis.

2.9 Eosinophilia may Worsen Despite Appropriate Treatment

The lack of a decrease in eosinophilia or even an increase in eosinophil count with anti-helminthic treatment does not exclude helminthic infection as the cause. Products released from dying parasites can cause eosinophilia to worsen (in, for example, schistosomiasis and filariasis) and eosinophilia may persist for many months after effective treatment [19].

2.10 Patients from a Tropical Country may have an Alternative Explanation Other than Helminthic Infection for Their Eosinophilia

Although helminthic infection is the most likely cause of eosinophilia in a patient who has returned from a tropical country, this does not preclude an alternate diagnosis [6].

In general, protozoan parasitic infections do not cause eosinophilia but *Isospora* species and *Dientamoeba fragilis* are occasional exceptions. As these are both often acquired in the same regions of the world as helminthic infections, even if they are identified in the stool of a patient, it is important to exclude helminthic co-infection as a cause of eosinophilia.

Other infections associated with eosinophilia include viral (HIV), bacterial (leprosy), mycobacterial (chronic tuberculosis), and fungal (coccidiomycosis) infections.

In addition, travellers may take prophylactic or treatment drugs that cause eosinophilia including antimalarials such as quinine and antibiotics such as beta-lactams and sulfa-containing drugs.

Non-infectious causes of eosinophilia include allergic, immunological, rheumatological, and malignant diseases in addition to various other drugs and idiopathic hypereosinophilic syndromes (Table 1). Some non-infectious causes may resemble helminthic infection clinically, for example, ulcerative colitis.

Non-infectious causes of eosinophilia should be considered when the epidemiology or clinical picture is not consistent with helminthic infection, there is hypereosinophilia or eosinophilia that persists beyond 6 months after anti-helminthic treatment.

3 An Approach to Diagnosing and Treating Parasitic Infections in Children with Eosinophilia Returning From Tropical Countries

Despite all of these caveats, it is possible to formulate an approach to diagnosing parasitic infections in children with eosinophilia returning from tropical countries. First, it is important to take a precise history with targeted questions

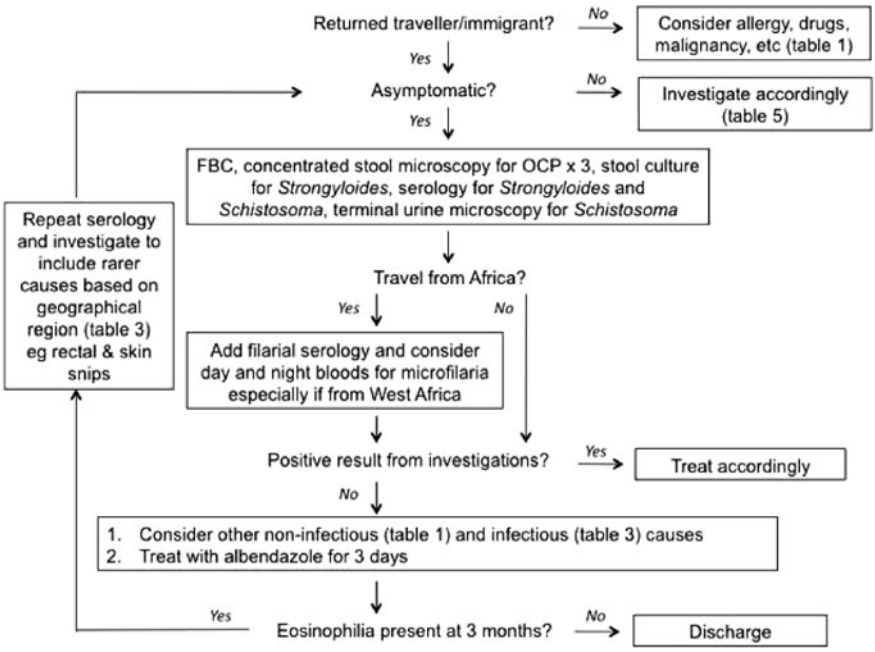

Fig. 2 Pragmatic approach to eosinophilia in children (adapted from UK guidelines [7]). FBC, full blood count; OCP, ova, cysts, and parasites

based on knowledge of epidemiology, life cycle, and clinical features associated with specific helminths. Second, investigations should be targeted if there are specific clinical features, but if asymptomatic should include broad first-line investigations and more specific second-line investigations if the former is not diagnostic (Fig. 2). Third, treatment should be given even if a definitive diagnosis is not made.

3.1 History

Targeted questions that will narrow down the possible diagnoses for helminthic infection are (i) which country the child has returned from, as some parasites are distributed only in specific geographical areas, or are more prevalent in a specific country (Table 3) [2, 7, 20]; (ii) how recently they travelled, which relates to the pre-patent period for different parasites [7]; (iii) what activities they did and what food they ate, which represent risk factors (Table 4) [21]; and (iv) what, if any, clinical symptoms they have had (Table 5) [2, 7]. Clinical syndromes with similar features may be common to several helminths. As already described, Loeffler's syndrome with fever, urticaria, cough, and wheeze is common to the larval migration stages of several parasites including the nematodes *Ascaris*, *Strongyloides*, and

Table 3 Helminthic infections by geographical region of travel or origin

Geographical region	Syndrome/common name (Helminth) (**bold denotes common**)
Worldwide	**Hookworm (*Ancylostoma/Necator*), roundworm (*Ascaris*), strongyloidiasis (*Strongyloides*),** hydatid (*Echinococcus*), pinworm (*Enterobius*), tapeworm (*Taenia*), whipworm (*Trichuris*), fascioliasis (*Fasciola*), trichinosis (*Trichinella*), visceral larva migrans (*Toxocara*), and cutaneous larva migrans (*Ancylostoma*)
Central and West Africa	**Bilharzia/schistosomiasis (*Schistosoma*), loiasis/eye worm (*L. loa*), river blindness/onchocerciasis (*Onchocerca*),** paragonimiasis (*Paragonimus*), lymphatic filariasis, and tropical pulmonary eosinophilia (*Wuchereria*)
North, South, and East Africa	**Bilharzia/schistosomiasis (*Schistosoma*),** lymphatic filariasis, tropical pulmonary eosinophilia (*Wuchereria*), and beef tapeworm (*Taenia saginata*)
Southeast Asia	Lymphatic filariasis (*Brugia*), paragonimiasis (*Paragonimus*), eosinophilic meningitis (*Angiostrongylus*), Katayama fever (*Schistosoma*), gnathostomiasis (*Gnathostoma*), clonorchiasis (*Clonorchis*), pork tapeworm (*Taenia solium*), fish tapeworm (*Anisakis/Pseudoterranova*), and opisthorciasis (*Opisthorcis*)
Middle East, Central Asia	Bilharzia/schistosomiasis (*Schistosoma*), river blindness/onchocerciasis (*Onchocerca*), hydatid (*Echinococcus*), and fascioliasis (*Fasciola*)
South America	Bilharzia/schistosomiasis (*Schistosoma*), fish tapeworm (*Anisakis/Pseudoterranova*), pork tapeworm (*Taenia solium*), paragonimiasis (*Paragonimus*), and gnathostomiasis (*Gnathostoma*)
Central America	River blindness/onchocerciasis (*Onchocerca*), paragonimiasis (*Paragonimus*), and gnathostomiasis (*Gnathostoma*)
Caribbean	Bilharzia/schistosomiasis (*Schistosoma*), eosinophilic meningitis (*Angiostrongylus*), and gnathostomiasis (*Gnathostoma*)
Oceania	Eosinophilic meningitis (*Angiostrongylus*) and lymphatic filariasis (*Brugia*)

hookworm. Likewise, different filarial parasites including *Wuchereria bancrofti* and *Brugia malayi* can cause the syndrome of dry cough, breathlessness, and wheeze that is tropical pulmonary eosinophilia.

3.2 Investigations

Investigation of eosinophilia in the returned traveller or immigrant who is symptomatic is guided by the clinical features (Table 5 and Fig. 2). In asymptomatic individuals, investigations should initially include samples for stool examination (including microscopy of a concentrated specimen) and serology for *Strongyloides stercoralis* and *Schistosoma* spp., regardless of geographical region, because these organisms are so widespread. Recently published UK guidelines [7] suggest that if the region of origin is Africa, and in particular West Africa where *Loa loa*, *W. bancrofti*, onchocerciasis, and lymphatic filariasis are more common, that

Table 4 Risk factors for helminthic infections associated with various activities

Activity	Helminthic infection
Food ingestion	
Raw or undercooked fish	Fish tapeworm, *Clonorchis*, *Opisthorcus*
Fish/frogs/snakes	*Gnathostoma*
Pork/beef	Pork/beef tapeworm
Unwashed salads	Hydatid
Watercress	*Fasciola*
Shellfish/crustaceans	*Paragonimus*, *Angiostrongylus*
Wild boar/bear/pork/walrus/horse	*Trichinella*
Insect bites	
Mosquito	Lymphatic filaria
Black fly (by rivers)	*Onchocerca*
Chrysops fly	*L. loa*
Swimming	
Fresh water	*Schistosoma*, swimmer's itch
Salt water	Swimmer's itch
Walking barefoot	Hookworm, *Strongyloides*, cutaneous larva migrans
Exposure to sheep	Hydatid

Table 5 Symptoms associated with helminthic infections (different symptoms may present at different stages of infection)

Symptoms	Helminthic infection
Fever	*Ascaris*, hookworm, *Strongyloides*, *Fasciola*, *Schistosoma*, lymphatic filaria, liver fluke
Gastrointestinal	*Ascaris*, hookworm, *Strongyloides*, *Trichuris*, *Trichinella*, *Schistosoma mansoni* and *S. japonicum*, *Paragonimus*, *Fasciola*, liver fluke
Dermatological	*Ascaris*, hookworm, *Strongyloides*, *Schistosoma*, *Paragonimus*, liver fluke, *Trichinella*, *Onchocerca*, lymphatic filaria, *L. loa*, *Gnathostoma*
Pulmonary	*Ascaris*, hookworm, *Strongyloides*, *Schistosoma*, *Paragonimus*, lymphatic filaria, hydatid
Hepatic	Hydatid, *Fasciola*, liver fluke, *Schistosoma*
Ophthalmological	*L. loa*, *Onchocerca*, Hydatid, *Trichinella*
CNS	Neurocysticercosis, *Angiostrongylus*, *Gnathostoma*, *Schistosoma*, hydatid
Genitourinary	*Schistosoma haematobium*
Anemia	Hookworm, *Trichuris*

filarial serology and day and night bloods for examination for microfilaria should be included. If results are negative, other rarer helminthic causes specific to the region of origin should be considered, as well as non-infectious causes. If eosinophilia persists beyond 3 months despite treatment, the UK guidelines recommend repeating serology and considering other causes.

3.3 Treatment

Specific treatment is available for most infectious causes of eosinophilia. Empiric treatment should be considered for persistent eosinophilia when investigations are negative for the reasons discussed above. Empiric treatment usually entails a short course of albendazole or mebendazole, which treats the commonest cause of asymptomatic eosinophilia in a returned traveller – strongyloidiasis – while also treating hookworm, *Ascaris*, and some other parasitic infections.

However, a cost-effectiveness analysis in recent immigrants to the USA comparing watchful waiting, investigating and treating, and presumptive treatment without investigation showed that the latter was the most cost-effective strategy, both in terms of lives and money saved [22]. While this analysis did not separate adults from children, it suggests that undertaking multiple investigations before treatment is likely not to be the best strategy in children with eosinophilia who have recently arrived from a tropical region. A more pragmatic approach has been adopted by the UK guidelines on the management of eosinophilia in immigrants or travellers returning from the tropics, as shown in an adapted form in Fig. 2 [7].

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Note: The letters 'f' and 't' following the locators refer to figures and tables respectively.

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