Gadi Borkow *Editor*

Use of Biocidal Surfaces for Reduction of Healthcare Acquired Infections

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Chapter 1
Preface

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Healthcare-acquired infections (HAI) have become a very significant medical concern both in developed and in developing countries, especially as microorganisms have developed high resistance to the existent antibiotics arsenal. While no exact numbers exist, it is assessed that millions of people worldwide acquire a HAI each year. These infections contribute significantly to morbidity, mortality and hospitalization costs. For example, in the United States alone, it was estimated that ~2 million HAI occur each year by all types of microorganisms, causing or contributing to ~100,000 deaths and adding ~$10 billion in additional healthcare expenses annually [1–3].

In order to reduce HAI rates, the medical community has developed aggressive measures – such as use of disposable equipment, healthcare staff education for improved hygiene, increased number of nurses and infection control personnel, isolation of infected patients, better ventilation management, use of high-efficiency particulate air filters, improved disinfection regimens, and use of aggressive antibiotic control programs. Indeed, all these measures have resulted in significantly lower HAI rates; however, even in hospitals where these infection control measures are rigorously implemented, the HAI rates are still unacceptably high, and it is clear that the current modalities to eliminate HAIs are not sufficient. The risk of an individual to acquire an infection while in the hospital is still intolerable and additional ways to fight HAI need to be developed (Fig. 1.1).

There is increasing evidence that potentially overlooked and neglected sources of nosocomial pathogens that significantly contribute to HAI are contaminated non-intrusive soft and hard surfaces located in the clinical surroundings, and that

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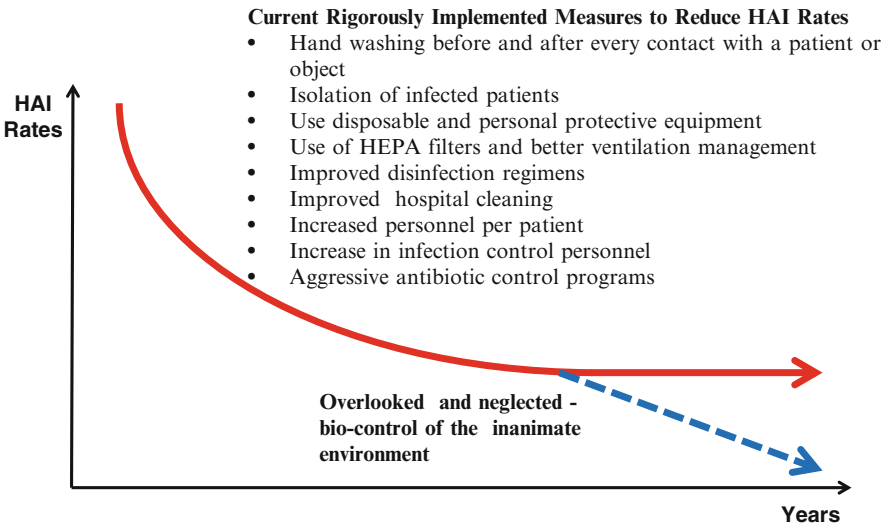

Fig. 1.1 HAI are not eliminated by the current implemented measures

there is a clear correlation between the environmental bioburden present in a clinical setting and the risk of patient of acquiring an infection [4–14]. Thus using self-disinfecting surfaces can be a very important adjunct in the fight against HAI [15, 16].

Copper is an essential trace element needed for the normal function of all aerobic life forms. Its ability to cycle between two oxidation states, Cu^{1+} and Cu^{2+}, is key to a wide array of metalloenzymes that catalyze electron transfer reactions. Conversely, copper can be highly toxic due in part to its ability to generate reactive oxygen species. Thus microorganisms have developed a complex series of mechanisms to regulate copper intracellular accumulation and distribution [17, 18]. However, above a certain threshold of exposure to copper, which varies between microorganisms, the microorganisms are killed, sometimes within minutes (e.g. [19–21]), via different multisite parallel mechanisms [22].

The ancient Greeks in the time of Hippocrates (400 BC) were the first to discover the sanitizing power of copper. They prescribed copper for pulmonary diseases and for purifying drinking water. Since then copper has been used as a biocide for treating sores and skin diseases and for purifying water by many civilizations, such as the Celts, Phoenicians, Egyptians, Hindus, and Aztecs [23]. By the eighteenth century copper had come into wide clinical use in the Western world for the treatment of mental disorders and afflictions of the lungs. Furthermore, in the eighteenth century it was discovered that no fungi grew on seed grains soaked in copper sulphate. Beginning in the early 1950s [e.g. [24–26]], the biocidal properties of copper and copper compounds were demonstrated in controlled laboratory studies. Notably, copper surfaces or copper compounds have been shown to be efficacious against hard-to-kill spores [27–33].

Today copper biocides have become indispensable and many thousands of tons are used annually all over the world for (i) prevention of roof moss formation [34];

(ii) wood preservation [35]; (iii) control of green slime in farm ponds, rice fields, irrigation and drainage canals, rivers, lakes and swimming pools [36]; (iv) prevention of downy mildew on grapes [37]; and (v) antifouling paints [38–40].

Non-soluble copper compounds, such as degradable phosphate glass fibres impregnated with CuO [41, 42], glass coated with thin films of CuO [43], or metallic and copper alloys [20, 32, 44–50] also exert potent biocidal properties, including against hard-to-kill spores [27–33]. Importantly, in March 2008 the U.S. Environmental Protection Agency (EPA) has approved the registration of copper alloys as materials with antimicrobial properties, thus allowing the Copper Development Association (CDA) to make public health claims [51]. More recently, Cupron Inc. received similar approvals by the EPA to make public health claims with its copper oxide infused countertops. These public health claims acknowledge that alloys containing above 60 % copper and surfaces impregnated with 16 % copper oxide particles are capable of killing more than 99.9 % of harmful, potentially deadly bacteria, such as Methicillin-resistant *S. aureus* (MRSA) within 2 h, and continue to kill more than 99 % of bacteria even after repeated contamination. Copper is the only metal that has received this type of EPA registrations.

This book discusses the role of the environment as a potential source for outbreaks of HAI and focuses on the utility of solid copper surfaces and copper oxide impregnated materials in reducing bioburden and fighting HAI. It also reviews other biocidal surface alternatives and the economics of using biocidal surfaces in a hospital environment. Finally, it discusses the pros and cons of existent disinfection modalities other than biocidal surfaces.

More specifically, in Chap. 2 of this book, Axel Kramer and Ojan Assadian, discuss the ability of pathogenic bacteria, fungi and viruses to persist and survive for long-term periods on inanimate surfaces. They discuss the factors influencing the survival of these pathogens in the environment and the mechanisms by which pathogens are transmitted from these inanimate surfaces to susceptible patients.

In Chap. 3, Jon Otter, Saber Yezli and Gary L. French provide proof that surface contamination by nosocomial pathogens shed by patients contributes to nosocomial cross-transmission and HAI. They review evidence that improved environmental hygiene can help bring HAI rates down and consider various options to address contaminated surfaces in healthcare facilities.

In Chap. 4, Michael G. Schmidt, Andrea L. Banks, and Cassandra D. Salgado, further discuss the effect of environmental contamination and HAI and review the studies showing the use of biocidal metallic copper surfaces resulting in dramatic reduction of bioburden and importantly of HAI rates.

In Chap. 5, I discuss how regular hospital linens, uniforms and other hospital textiles are a neglected source of nosocomial pathogens and how self-disinfecting biocidal textiles can significantly contribute to the reduction of HAI. Specifically I review the studies showing that incorporation of copper oxide in hospital textiles can reduce bioburden and HAI rates. I also briefly review the novel successful endowment of biocidal properties to non-porous solid surfaces by impregnating them with copper oxide particles.

In Chap. 6, Christophe Espírito Santo, Nadezhda German, Jutta Elguindi, Gregor Grass, and Christopher Rensing, discuss why on the one hand copper is an essential element to microorganisms and how copper homeostasis is achieved, and on the other hand how copper exerts its potent biocidal properties, with special focus on the molecular mechanisms underlying bactericidal properties of solid copper surfaces.

In Chap. 7, Jon Otter reviews potential and existent biocidal surface alternatives to copper. He discusses what should be the ideal biocidal surface candidate and discusses the pros and cons of each of the existent candidate alternatives, the optimal deployment modes, the surfaces that should be made self-disinfecting surfaces, and how do we test and compare efficacy of antimicrobial surfaces.

In Chap. 8, Panos A. Efstathiou evaluates the impact of using biocidal surfaces in a hospital environment, specifically discussing the use of metallic copper surfaces in the intensive care units, reaching the conclusion that the use of biocidal surfaces has significant positive economic advantages.

Finally, in Chap. 9, George Byrns reviews the pros and cons of using chemical fumigation and germicidal UVC irradiation in healthcare and other related settings. He raises the concern that while both fumigation and UV irradiation are capable of killing microorganisms, it is uncertain whether the benefits in terms of overall hospital patient infection rates outweigh the risks and costs associated with these methods, further strengthening the importance of using biocidal self-disinfecting surfaces to combat environmental contamination.

I hope this book will give significant support to the notion that the inclusion in clinical settings of self-disinfecting biocidal hard and soft surfaces can significantly help in the fight against healthcare-acquired infections. I also hope you will find this book informative, comprehensive and interesting.

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Chapter 2
Survival of Microorganisms on Inanimate Surfaces

Axel Kramer and Ojan Assadian

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Abstract In healthcare settings microbial contaminated surfaces play an important role in indirect transmission of infection. Especially surfaces close to the patients' environment may be touched at high frequencies, allowing transmission from animated sources to others via contaminated inanimate surfaces.

Therefore, the knowledge on the survival of bacteria, fungi, viruses and protozoa on surfaces, and hence, in a broader sense, in the human environment, is important for implementing tactics for prevention of Healthcare-acquired Infections (HAI).

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This chapter will elaborate the role of surfaces in the transmission of pathogens. Particular emphasis is laid on the current knowledge of the survival time and conditions favouring survival of the pathogens. Finally, mechanisms of transmission from inanimate surfaces to patients are highlighted.

Within the multi-barrier strategy of the prevention of HAI, environmental disinfection policies should be based on risk assessments for surfaces with different risks for cross contamination such as high- and low-touched surfaces with appropriate standards for adequate disinfection measures under consideration of the persistence and infectious dose of the pathogens. As a result, surface disinfection is indicated in the following situations:

- Frequently touched surfaces adjacent to patients
- Surfaces with assumed or visible contamination
- Terminal disinfection in rooms or areas where infected or colonized patients with easily transferable nosocomial pathogens are cared for, and
- in outbreak situations.

Furthermore, the knowledge of the persistence of pathogens will also support ensuring the biosafety in microbiological and biomedical laboratories, food-handling settings, and for hygienic behaviour in the everyday life to prevent transmission of infectious diseases.

Keywords Persistence • Bacteria • Fungi • Viruses • Protozoa transmission mechanisms • Surface disinfection

List of Abbreviations

HAI	Healthcare-acquired infections
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSSA	Methicillin-sensible *Staphylococcus aureus*
RH	Relative humidity
SARS	Severe acute respiratory syndrome
VRE	Vancomycin-resistant enterococci

2.1 Introduction

Microorganisms may be transmitted from animated sources to inanimate environmental sources, which may become secondary reservoirs if they meet the needs of transmitted pathogens to survive and to multiply. In healthcare settings, however, contaminated surfaces, which may not always be optimal for microbial survival and multiplication, still may play a role in the chain of infection, since surfaces close to the patients' environment may be touched at high frequencies, allowing transmission from animated sources to others via contaminated inanimate surfaces.

Because of this, the knowledge on the survival of bacteria, fungi, viruses and protozoa on surfaces, and hence, in a broader sense, in the human environment, is important for planning and implementing tactics for prevention of Healthcare-acquired Infections (HAI). Furthermore, such knowledge will also assist ensuring the biosafety in microbiological and biomedical laboratories, food-handling settings, and for hygienic behaviour in the everyday life to prevent transmission of infectious diseases.

One example of microorganisms with relatively short ability of persisting in the environment is the severe acute respiratory syndrome (SARS) coronavirus (CoV), which became pandemic within months in China in 2002. This virus retains infectivity on different substrates up to 9 days, as compared to the influenza virus, which demonstrates a relatively long persistence in the environment up to 4 weeks [112]. Both viruses are airborne transmitted infectious agents, however, they may also be transmitted via hand-surface contacts, supporting the relevance of hand hygiene and personal protection against infection.

Because of a number of microorganisms' ability to persist and survive for long-term periods on surfaces, particularly in healthcare settings, the usage of antimicrobially impregnated surfaces is increasingly discussed [82]. However, because of the required long contact times of microorganisms on antimicrobial surfaces [64, 65, 25, 45], such technologies may be useful for surfaces with low frequency of hand contacts.

2.2 The Role of Surfaces in the Transmission of Pathogenic Microorganisms Causing Healthcare-Acquired Infections (HAI)

In healthcare settings, bacteria, bacterial spores, viruses and yeasts are mainly transmitted from infected and/or colonized patients, but also from staff, and in some situations from visitors to the inanimate hospital environment, particularly to areas adjacent to patients and frequently touched surfaces by hands ("high-touch surfaces"). Potential pathogenic microbial flora of the respiratory tract and of the vestibulum nasi, such as methicillin- sensible (MSSA) or resistant *Staphylococcus aureus* (MRSA), is correlated with a higher risk of contamination of surrounding surfaces through direct or indirect contact with hands [81]. Intestinal infections caused i.e. by *Clostridium difficile* and Norovirus, or enteral colonization with nosocomial pathogens such as vancomycin-resistant enterococci (VRE) may also be associated with a risk of widespread environmental contamination [30]. Compared with the large number of published literature on environmental contamination with MRSA, VRE, and *C. difficile*, there are relatively few published studies on environmental contamination by Gram-negative bacteria [64, 65]. Aside of a possible publication bias in the past, one reason for this is the different ability of Gram-positive and Gram-negative bacteria to survive in the inanimate environment.

The level of microbial bio-burden on surface in healthcare settings is low compared to the numbers on patients' skin or in faeces. However, even at low particle numbers

Table 2.1 Infectious doses for selected pathogens

Infectious dose	Organisms	Reference
(1)-10–100 viable particles	Norovirus, Rotavirus, EHEC, ETEC, *C. difficile*, Enterococci incl. VRE	Ward et al. [122], Paton and Paton [88], Pang et al. [85], Lawley et al. [68], Porter et al. [92], Yezli and Otter [130], Robine et al. [97]
≥1 viable particle in water	Oocysts of cryptosporidia	Chappell et al. [17]
$>10^5$ viable particles	*Salmonella enteritidis*	Craven et al. [24]

there is a risk of transmission (Table 2.1). In immuno-compromised patients, the required numbers of microorganisms for causing infectious diseases is even lower, increasing the risk of HAI in these populations. Inanimate surfaces have been described as source for HAI-outbreaks. Hayden et al. [49] demonstrated that touching the environment contaminated with relatively low pathogen concentrations in a room occupied by a patient colonized with VRE is associated with approximately the same risk of VRE acquisition on hands as touching an affected patient directly. Evidence of the importance of environmental transmission is further provided by studies showing an increased risk of infection in patients admitted to the same rooms previously occupied by other infected/colonised cases. This has been shown for *C. difficile* [101], VRE and MRSA ([54, 55], and also own observations). Environmental Norovirus contamination has been repeatedly found to be correlated with continuing outbreaks [128], although the significance of this pathway has not been fully elucidated.

The importance of surface contamination is also shown by reduction in the rate of HAI when effective measures of environmental disinfection are implemented [50, 10, 26]. A recent observational study showed a significant reduction in *C. difficile* infection rates following the introduction of sporicidal wipes in an environmental cleaning regimen in an acute London trust [16]. However, not all studies have shown a direct link between surface disinfection and reduction in infection rates, probably because of the complex interactions and transmission routes in the clinical practice.

Yet, in summary it is undisputed that contaminated surfaces may contribute to the transmission of pathogens and may thus pose a critical element in the chain of transmission of microorganisms [41].

2.3 Persistence of Microorganisms on Inanimate Surfaces

The risk for transmission of HAI depends of the persistence of nosocomial pathogens on surfaces. The longer a microorganism may persist on a surface, the longer the contaminated surface may be a source of transmission and thus endanger a susceptible patient or healthcare worker of becoming the target of infection. In order to estimate the risk of cross contamination, Kramer et al. [64, 65] have published a systematic review on persistence of pathogens on surfaces.

The following findings are based on this review; however, knowledge on persistence of microorganisms on inanimate surfaces is now expanded by additional findings published after 2005/2006.

2.3.1 Persistence of Bacteria

In most reports, persistence was studied on dry surfaces using artificial contamination of a standardized type of surface in a laboratory. Bacteria were prepared in broth, water or saline.

Most Gram-positive bacteria, such as *Enterococcus* spp. including VRE, *S. aureus* including MRSA, or *Streptococcus pyogenes* survive for months on dry surfaces (Table 2.2). In general, there is no observable difference in survival between multi-resistant and susceptible strains of *S. aureus* and *Enterococcus* spp. [78]. Only in one study [118] a difference of survival time between antibiotic resistant and susceptible bacteria was suggested, yet, the susceptible strains demonstrated only a non-significant shorter survival time on surfaces. The factors why the same bacteria may persist more or less on a surface (i.e. from hours to days as detailed in Table 2.2) will be discussed later in Sect. 2.3.5.

Many Gram-negative species, such as *Acinetobacter* spp., *Escherichia coli*, *Klebsiella* spp., *Pseudomonas aeruginosa*, *Serratia marcescens*, or *Shigella* spp. can survive on inanimate surfaces even for months (Table 2.2). These species are found among the most frequent isolates from patients with HAI [64, 65]. However, a few others Gram-negative bacteria, such as *Bordetella pertussis*, *Haemophilus influenzae*, *Proteus vulgaris*, or *Vibrio cholera* persist only for days (Table 2.2).

Mycobacteria, including *Mycobacterium tuberculosis,* and spore-forming bacteria, such as *C. difficile,* can survive for many months on surfaces (Table 2.2).

Because paper still is omnipresent in healthcare settings worldwide today, Hübner et al. [56] have analysed the persistence of various Gram-positive and Gram-negative bacteria including *E. coli*, *S. aureus*, *P. aeruginosa*, and *Enterococcus hirae* on office paper after contamination with standardised inocula of bacterial suspensions in the range of 2.8×10^7 cfu/mL. Opposite to *E. coli*, all other organisms were more stabile at room conditions and were reduced on paper only by 3 $\log_{10}$ after 7 days, whereas *E. coli* was reduced by 5 $\log_{10}$ within 24 h. Furthermore, the transmissibility of bacteria from hands to paper and back could be demonstrated for all bacteria strains. Similar investigations showed that paper money notes could harbour and transmit pathogens [62, 111, 115].

2.3.2 Persistence of Viruses

In order to estimate the persistence of viruses on inanimate surfaces, usually cell culture media are prepared [64, 65]. Most viruses from the respiratory tract such as Corona-, Coxsackie-, or Influenza virus, SARS, or rhinovirus can persist on surfaces

Table 2.2 Published data on survival of nosocomial and community acquired pathogens on various inanimate surfaces

Organism	Range of survival (environment)	Reference
Acinetobacter spp.	3 days to 1 year (in-vitro) 36 days within biofilm vs. 15 days for non-biofilm-forming strains	Wagenvoort and Joosten [117], Espinal et al. [36][a]
Bordetella pertussis	3 to >10 days; in pernasal swabs: >4 days	Hunter [57], Walther and Ewald [121][a]
Campylobacter jejuni	>6 days, in water >60 days	González and Hänninen [44][a]
Clostridium difficile spores	5 months	Weber et al. [123][a]
C. difficile, vegetative form	15 min (dry surface) 6 h (moist surface)	
Chlamydia pneumoniae	≤96 h	Fukumoto et al. [40], Haider et al. [51], Matsuo et al. [70][a]
C. trachomatis	<1 week	
Chlamydia psittaci	15 days to months (environment)	Wendel [125][a]
Corynebacterium diphtheriae	7 days to 6 months	Walther and Ewald [121][a]
Corynebacterium pseudotuberculosis	1–8 days, up to several weeks (environment)	Yeruham et al. [129][a], Dorella et al. [31]
Enterococcus spp. including VRE	5 days up to 30 months	Robine et al. [97], Wagenvoort et al. [116][a]
Escherichia coli	1.5 h to 16 months	Guan and Holley [46], Erickson et al. [35], Chauret [19][a], Duffitt et al. [33]
E. coli O157:H7	27 days on spinach leaves, 179 days in soil, 98 days in water	
Haemophilus influenzae	12 days	[a]
Helicobacter pylori	≤90 min; in water: 2–30 days	West et al. [124], Percival and Thomas [89][a]
Klebsiella spp.	2 h to >30 months, ≤144 h in detergent solution	Beadle and Verran [6][a]
Listeria spp.	1 day–months, 141 days in water	Budzińska et al. [13][a]
Mycobacterium bovis	>2 months	[a]
Mycobacterium tuberculosis	1 day up to 4 months	Walther and Ewald [121][a]
Neisseria gonorrhoeae	1–3 days	[a]
Neisseria meningitidis	72 h	Tzeng et al. [110][a]
Parachlamydia acanthamoebae	<4 weeks, in presence of blood <7 weeks	Fukumoto et al. [40][a]
Proteus vulgaris	1–2 days	[a]
Pseudomonas aeruginosa	6 h up to 16 months; on dry floor: 5 weeks; in aerosol: few hours	Clifton et al. [21][a]
Salmonella typhi	6 h up to 4 weeks	[a]
Salmonella typhimurium	10 days up to 4.2 years	[a]

(continued)

Table 2.2 (continued)

Organism	Range of survival (environment)	Reference
Salmonella spp.	1 day	[a]
non typhoid *Salmonella* spp.	336 days	Morita et al. [76][a]
Salmonella enteritidis (broiler farms)	1 year	Davies and Wray [27][a]
Salmonella enteritica sv. Tennessie	30 days (dried in desiccated milk powder)	Aviles et al. [1][a]
Serratia marcescens	3 days up to 2 months; on dry floor: 5 weeks	[a]
Shigella spp.	2 days up to 5 months 3–11 days in water	Ghosh and Sehgal [42][a]
Staphylococcus aureus including MRSA and MSSA	7 days up to 1 year (in-vitro) 9–12 days (plastic surfaces) 72 h (stainless steel) 6 h (copper) ≤28 days (dry mops) ≤14 days (in water)	Oie and Kamiya [81], Wagenvoort and Penders [118], Huang et al. [54, 55], Noyce et al. [80], Tolba et al. [108], Petti et al. [90][a]
Streptococcus pneumoniae	1 day up to 30 month	Walsh and Camilli [120][a]
Streptococcus pyogenes	3 days up to 6.5 months	Wagenvoort et al. [119][a]
Vibrio cholerae	1–7 days	[a]
Yersinia enterocolitica	Up to 64 weeks (in water)	Guan and Holley [46][a]
Yersinia pestis	Up to 5 days	Rose et al. [98][a]

[a]Additional references in Kramer et al. [64, 65]

only for a few days [18]. Herpes viruses such as Cytomegalie virus or Herpes simplex virus type 1 and 2 have been shown to persist from only a few hours up to 7 days.

Viruses from the gastrointestinal tract, such as Astrovirus, Hepatitis A virus, Polio- and Rotavirus persist significantly longer for approximately 2 months. Blood-borne viruses, such as Hepatitis B virus or Human Immunodeficiency virus can persist for more than 1 week (Table 2.3).

2.3.3 Persistence of Fungi

Candida albicans, the most important nosocomial yeast, can survive up to 4 months on surfaces. Persistence of other yeasts was described to be similar (*Torulopsis glabrata:* 5 months) or shorter (*Candida parapsilosis:* 14 days) (Table 2.4). The survival of fungi in the environment, however, is strongly influenced by physical factors in nature, such as temperature and relative humidity (see Sect. 2.3.5).

Table 2.3 Survival of clinically relevant viruses on dry inanimate surfaces

Organisms	Range of survival (environment)	Reference
Adenovirus	<6 h up to 3 months (type dependent), ≤301 days (in water)	Hara et al. [48], Rigotto et al. [95][a]
Astrovirus	7–90 days	[a]
Avian metapneumonovirus	~48 h up to 6 days	Tiwari et al. [107][a]
SARS Coronavirus	<5 min up to 24 h (on paper) 5–28 days (at room temp.) 28 days (at 4 °C)	Lai et al. [66], Rabenau et al. [93], Guionie et al. [47]
Coxsackievirus	7–10 days, up to >2 weeks	Wong et al. [127][a]
Cytomegalovirus	1–8 h	Faix [37], Stowell et al. [102][a]
Echovirus	Up to 7 days	[a]
Hepatitis A virus	2 h up to 60 days	[a]
Hepatitis B virus	≥1 week	[a]
Human immunodeficiency virus	Up to 7 days, 7 days (in peritoneal dialysis effluent), 48 h (on peritoneal dialysis exchange and tubing), 4–8 weeks (on glass cover slides)	Van Bueren et al. [113], Farzadegan et al. [38][a]
Herpes simplex virus, Type 1 & 2	<2 h up to 8 weeks	Larson and Bryson [67], Bardell [2], Rabenau et al. [93][a]
Influenza virus	1–28 days (strain dependent) 1–3 days (on banknotes), up to 8 days (admixed in mucous)	Edward and Derrick [34], Walther and Ewald [121], Tiwari et al. [107][a], Thomas et al. [106]
Marburg virus (strain Popp)	4–5 days	Belanov et al. [7][a]
Para-influenza virus	10 h	Brady et al. [11][a]
Norovirus, Feline calici virus (FCV), Murine norovirus (MNV)	8 h up to 7 days, MNV > 40 days (in diapers and gauze)	Cannon et al. [14], Lee et al. [69][a]
Papillomavirus 16	≤7 days	Hsueh [53][a]
Papovavirus	8 days	[a]
Parvovirus	>1 year	[a]
Poliovirus type 1	4 h to <8 days	[a]
Poliovirus type 2	1 day up to 8 weeks	[a]
Pseudorabies virus	≥7 days, <1 h (in aerosol infectivity decreases by 50 % per hour)	Schoenbaum et al. [100]
Respiratory syncytial virus	up to 6 h	[a]
Rhinovirus	2 h up to 7 days	[a]
Rotavirus	30 min, 6–60 days	Keswick et al. [61][a]
Vacciniavirus	3 weeks up to >20 weeks	[a]

[a]Additional references in Kramer et al. [64, 65]

Table 2.4 Survival of clinically relevant fungi on dry inanimate surfaces

Organisms	Range of survival (environment)	Reference
Aspergillus spp.	>30 days	Neely and Orloff [79][a]
Candida albicans	1 up to 120 days, 24 weeks (in soil-water mixture)	Neely and Orloff [79], Théraud et al. [105][a]
Candida parapsilosis	>30 days	Neely and Orloff [79][a]
Candida krusei	11 days	
Cryptococcus spp.	24 weeks (in soil-water mixture)	Théraud et al. [105][a]
Fusarium spp.	>30 days	Neely and Orloff [79][a]
Mucor spp.	>30 days	
Paecilomyces spp.	11 days	
Torulopsis glabrata	102–150 days	Kane et al. [59]

[a]Additional references in Kramer et al. [64, 65]

Moulds are ubiquitous in nature, thermo-tolerant, and can survive in house dust for long time. Indoor airborne mould measurements underline the survival for several months [4, 5].

2.3.4 Persistence of Other Pathogenic Microorganisms

Cryptosporidium spp. can induce water-born infection. Their oocysts can survive for months in surface water [96, 20, 75, 15], and up to 120 days in soil [60].

Acanthamoeba are one of the most common protozoa in soil, and frequently found in fresh water and other environmental habitats. An important habitat and vector for infection are hydrogel contact lenses, resulting in contact lens associated keratitis caused by acanthamoeba and fusarium [87], particularly since the contact lenses' moist condition supports survival protozoa.

2.3.5 Factors Influencing the Survival of Microorganisms in the Environment

2.3.5.1 Relative Humidity (RH)

Generally, viruses with lipid envelops, such as most respiratory viruses including Influenza virus, Para-Influenza virus, Corona virus, Respiratory syncytial virus, Herpes simplex virus, Measles virus, Rubella virus, and Varicella zoster virus will tend to survive longer at lower relative humidity (20–30 % RH) [103]. However,

Cytomegalie virus makes an exception, as it was more likely isolated from moist surfaces [102].

Conversely to enveloped viruses, non-lipid enveloped viruses such as Adenovirus, Enterovirus, and Rhinoviruses tend to survive longer at higher relative humidity (70–90 % RH) [103]. For Rotavirus and Poliovirus conflicting results were reported [64, 65].

S. aureus can persist longer at low humidity [74]. However, for *Enterococcus faecalis* the survival kinetic is decreased at 25 % RH compared to 0 % RH [97].

The survival of aerosolized Gram-negative bacteria including *Pseudomonas* spp., *Enterobacter* spp. and *Klebsiella* spp. improved at higher relative humidity and low temperature [103]. Studies on airborne Gram-negative bacteria such as *S. marcescens*, *E. coli*, *Salmonella pullorum*, *Salmonella derby*, and *Proteus vulgaris* showed decreased survival at intermediate (approx. 50–70 % RH) to high (approx. 70–90 % RH) relative humidity. For some airborne Gram-positive bacteria, such as *Staphylococcus epidermidis*, *Streptococcus haemolyticus*, *Bacillus subtilis*, and *Streptococcus pneumoniae*, their survival rate also decreased at intermediate relative humidity ranging at 50–70 % RH [103]. Gram-positive cocci were most prevalent in indoor air, followed by Gram-positive rods (e.g. *Bacillus* spp. and *Actinomycetes* spp.), Gram-negative rods and Gram-negative cocci [103]. The reason for this bacterial behaviour is the design of bacterial cell wall, which allows Gram-positive organisms to tolerate dry conditions better than Gram-negative organisms. Because of a lipid double-layer structure with a thin peptidoglycan (Murein) layer consisting of alternating residues of β-(1,4) N-acetylglucosamine and N-acetylmuramic acid, the later are not so well protected against physical stress and need higher RH in order to survive.

2.3.5.2 Temperature

The viral genome (viral DNA or RNA) is sensitive to the surrounding temperature. Indeed, temperature is an important factor influencing the survival of a number of viruses. Higher temperatures impact viral proteins and enzymes, as well as the viral genome. In general, DNA viruses are more stable than RNA viruses; yet, high temperature also will affect DNA integrity.

For most viruses, such as Astrovirus, Adenovirus, Poliovirus, Herpes simplex virus, and Hepatitis A virus, low temperature is associated with a longer persistence [64, 65]. Constant temperatures >24 °C appear universally to decrease airborne bacterial survival [103].

2.3.5.3 Biofilm

Biofilm is the predominate form of life for microorganisms in a nutrient-sufficient ecosystem. Adhesion triggers the expression of a sigma factor that depresses a large number of genes so that bacteria within the biofilm are at least 500 times more

tolerable against antimicrobial agents [23] as well as against physical cold plasma [71, 72]. The reason for the unspecific increased tolerance is the production of extracellular substances like polysaccharides, proteins and DNA after attachment to surfaces. A precondition for biofilm formation is the presence of certain amounts of humidity. The biofilm matrix restrains water and nutrients and protects the microorganisms against environmental influences [28, 39]. Because of that, once formed biofilms are an important factor of persistence of microorganisms on surfaces in nature as well as in industrial or medical areas [22, 29, 12]. The persistence on inanimate surfaces is prolonged and depends of the environmental conditions, especially the humidity. Also on hospital surfaces biofilms were demonstrated on a number of objects and surfaces, such as sterile supply buckets, opaque plastic doors, venetian blind cords, and sink rubbers, and it was possible to cultivate viable bacteria. Currently, there is not enough research to elucidate whether presence or absence of biofilm affect the risk of transmission or possibility for cross-transmission. However, multi-drug resistant bacteria may not only be protected within biofilms, which may be the mechanism why they persist within the hospital environment [114], but may also exchange virulence factors among their own species or to other species present in biofilms as well [29, 43, 109].

2.3.5.4 Other Factors

A number of other factors may influence the survival of microorganisms on surfaces. Clearly, the material character of a surface itself may play in important role. However, inconsistent results are reported for the influence of type of materials on microbial survival. Some authors described that the type of material did not affect the persistence of Echovirus, Adenovirus, Para-Influenza virus, Rotavirus, Respiratory syncytial virus, Poliovirus, or Norovirus. Other investigators found that persistence was favoured on non-porous surfaces for Influenza virus on formica and gloves for Respiratory syncytial virus, and on hand pieces of telephones for Feline calicivirus [64, 65]. Other factors for a longer persistence of viruses include the presence of faecal suspension and a higher bio-inoculum [66, 64, 65]. Interestingly and by nature, Urease activity enhances the survival of *Haemophilus influenzae* at a reduced pH [77].

2.3.6 Limitations on the Knowledge of Microbial Survival on Inanimate Surfaces

Laboratory studies to determine the survival and persistence do not reflect the clinical situation, in which surfaces can be simultaneously contaminated with various nosocomial pathogens, different types of bodily and other fluids, secretions,

and antimicrobial residues, i.e. from the last surfaces disinfection. However, little dispute exists that beside the hands of healthcare workers surfaces in the close vicinity of patients may play a key role for the transfer of nosocomial pathogens.

2.4 Mechanisms of Transmission from Inanimate Surfaces to Susceptible Patients and Consequences Thereof

The main route of transmission of HAI is via transiently contaminated hands of healthcare workers, but contaminated surfaces may serve as important vectors for cross transmission after hand contact as well (Fig. 2.1).

A single hand contact with a contaminated surface results in a variable degree of pathogen transfer. Transmission from surfaces to hands was most successful with *E. coli*, *Salmonella* spp., *S. aureus* (all 100 %), *C. albicans* (90 %), Rhinovirus (61 %), Hepatitis A virus (22–33 %), and Rotavirus (16 %) [64, 65]. Other transfer rates were calculated for Echovirus, Poliovirus, and Rotavirus with 50 % transmissibility, and for *Salmonella enteritidis*, *Shigella* spp., and *E. coli* O157:H7 with 33 % [104]. Contaminated hands can transfer viruses to 5 more surfaces or 14 other subjects. Contaminated hands can also be the source of re-contamination of the surface, as demonstrated with Hepatitis A virus [64, 65].

Because of this, it is critical to note that healthcare workers' compliance with hand hygiene varies between 13 % and 94 % with a median of less than 50 % [91]. Moreover hand hygiene is performed less frequently after contact with the environment than with the patient [94]. Both facts underline the necessity to perform additional surface decontamination procedures to interrupt the transmission of nosocomial pathogens. Due to the overwhelming evidence of low compliance of hand disinfection, the risk from contaminated surfaces cannot be overlooked and must not be down played by hospital administrations.

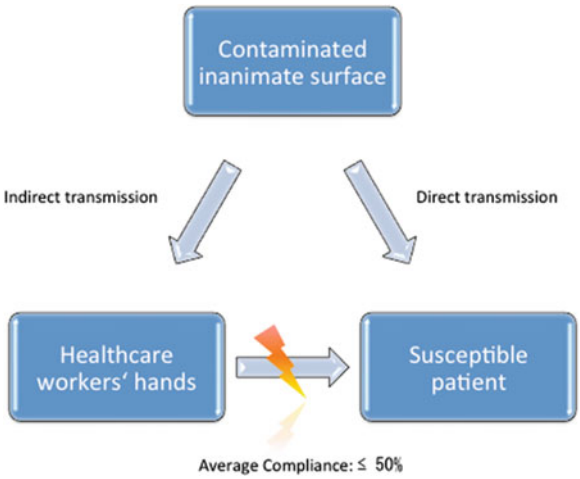

Fig. 2.1 Transmission routes for nosocomial pathogens

During outbreaks, the role of the patients' environment is particularly evident, as suggested by observed evidence for *Acinetobacter baumannii*, *C. difficile*, MRSA, *P. aeruginosa*, VRE, Adenovirus, SARS virus, Rotavirus, and Norovirus [64, 65, 54, 55, 99, 9, 123, 83, 58]. The role of contaminated surfaces is also underlined by the observation that after environmental disinfection, significant decrease of transmissions and HAI have been shown, i.e. for *C. difficile* [73, 126], for VRE [50], for MRSA [32], for multidrug-resistant *A. baumanii* [84], for *S. marcescens* [3], and for other multidrug-resistant Gram-negative rods [86].

If performed correctly, also the burden of microbial airborne transmission can be significantly decreased by surface disinfection. This again may have an impact on healthcare organisations, resulting in i.e. higher clean room class of drug manufacturing areas [8] by elimination of critical bacterial and fungal contamination [63]. As consequence for the successful interruption of cross contamination and infections a multi-barrier approach is required with the key points of hand hygiene and surface disinfection, appropriate used of antisepsis, barrier nursing, and safe reprocessing of contaminated medical devices. Within such multi-barrier strategy, environmental disinfection policies should be based on risk assessments for surfaces with different risks for cross contamination such as high- and low-touched surfaces with appropriate standards for adequate disinfection measures. Generally, surface disinfection is indicated in the following situations:

- Frequently touched surfaces adjacent to patients
- Surfaces with assumed or visible contamination
- Terminal disinfection in rooms or areas where infected or colonized patients with easily transferable nosocomial pathogens are cared for, and
- in outbreak situations.

The purpose of preventive or targeted disinfection on inanimate surfaces is the killing or irreversible inactivation of pathogens to an extent which prevents subsequent infection transmission [41]. In order to ensure the success of environmental disinfection, education, training [52], and targeted microbiological control are important measures and have been shown to improve both, cleaning performance and infection prevention [50]. Increasingly, novel technologies are introduced, which may be used additionally to cleaning. Such technologies may include antimicrobial surfaces on basis of different antimicrobial compounds and are provided for hospital door handles, alarm knobs, curtains, and other objects with high frequencies of hand contact. However, such technologies must be used appropriately and as an adjunct measure to meaningful cleaning and disinfection processes.

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Chapter 3
The Role of Contaminated Surfaces in the Transmission of Nosocomial Pathogens

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Abstract Studies in the 1970s and 1980s suggested that environmental surface contamination had a negligible role in the endemic transmission of healthcare-associated infections. However, recent studies demonstrate that several major nosocomial pathogens are shed by patients and contaminate hospital surfaces at concentrations sufficient for transmission, survive for extended periods, persist despite attempts to disinfect or remove them and can be transferred to the hands of healthcare workers. Evidence is accumulating that contaminated surfaces make an important contribution to the epidemic and endemic transmission of *C. difficile*, vancomycin-resistant enterococci, methicillin-resistant *Staphylococcus aureus*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and norovirus and that improved environmental decontamination contributes to the control of outbreaks. Efforts to improve environmental hygiene should include enhancing the efficacy of cleaning and disinfection and reducing the shedding of pathogens. Further high quality studies are needed to clarify the role of surfaces in nosocomial transmission and determine the effectiveness of different interventions in reducing associated infection rates.

Keywords Environmental contamination • Cleaning • Disinfection • MRSA • VRE • *Acinetobacter baumannii* • *Pseudomonas aeruginosa* • *Clostridium difficile* • Norovirus

List of Abbreviations

aHP	Aerosolized hydrogen peroxide
CDC	Centers for Disease Control and Prevention
CDI	*Clostridium difficile* infection
CFU	Colony forming units
CPE	Carbapenemase-producing Enterobacteriaceae
ESBL	Extended spectrum beta-lactamase
GNRs	Gram-negative rods
HAI	Healthcare-associated infections
HCPs	Healthcare personnel
HPV	Hydrogen peroxide vapor
ICU	Intensive care units
QAC	Quaternary ammonium compounds

MRSA	Methicillin-resistant *Staphylococcus aureus*
MDR-GNRs	Multidrug-resistant Gram-negative rods
MDROs	Multidrug-resistant organisms
NTD	"No-touch" automated room disinfection
RLU	Relative light unit
VRE	Vancomycin-resistant enterococci

3.1 Introduction

Contamination of hospital equipment, medicines and water supplies with hospital pathogens is a well-recognized cause of common-source outbreaks of infection [3, 4]. There is extensive guidance on prevention and control of such contamination available from manufacturers, Specialist Societies and Health Departments and often a legal requirement to comply with associated health and safety regulations. In contrast, the degree to which ongoing contamination of the surface environment contributes to the development of healthcare-associated infections (HAI) is unclear and approaches to control uncertain.

Hospital patients shed pathogens into their surrounding environments but there is debate over the importance of the resulting surface contamination as a source for subsequent transmission. Since the 1950s, hospital design and hygienic practices have been largely directed at controlling nosocomial pathogens contaminating air, hands, equipment and surfaces [5]. However, several studies in the 1970s and early 1980s suggested that the hospital environment contributed negligibly to endemic transmission [6, 7]. Routine surveillance cultures of the hospital environment were regarded as unjustified and the significance of environmental cultures made during outbreaks was questioned [8, 9]. Consequently, the frequency of routine environmental sampling reduced from three quarters of US hospitals in 1975 [8] to virtually none today. Indeed, in recent USA Centers for Disease Control and Prevention (CDC) guidelines, environmental sampling is currently recommended only during outbreaks [10]. Recently, however, there has been a reassessment of the role of contaminated surfaces in the transmission of nosocomial pathogens [4, 11]. The epidemiological finding that admission to a room previously occupied by certain environmentally-associated pathogens such as *Clostridium difficile*, methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE) and *Acinetobacter baumannii* increases the risk of acquisition for incoming patients is perhaps the most compelling evidence that contaminated surfaces contribute to transmission [1, 2]. Furthermore, intervention studies demonstrate that improvements to terminal (discharge) disinfection mitigate – to a lesser or greater degree – the increased risk from the prior occupant cements the epidemiological association [12, 13].

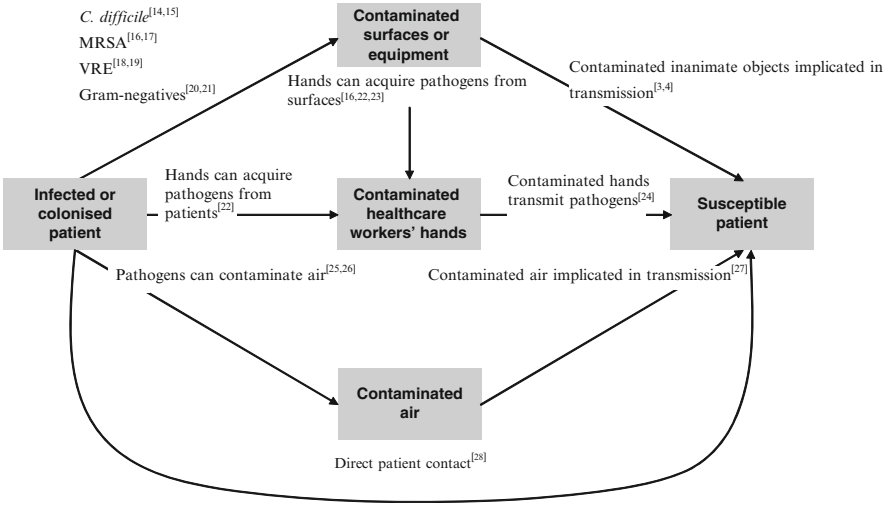

Fig. 3.1 Generic transmission routes

Pathogen transfer from an affected patient to a susceptible host occurs most commonly via the hands of healthcare personnel (HCPs) but contaminated objects, surfaces and air can be either directly or indirectly involved in the transmission pathway (Fig. 3.1). Here we review evidence that nosocomial pathogens are shed by patients and can contaminate hospital surfaces at concentrations sufficient for transmission, can survive for extended periods, can persist despite attempts to disinfect or remove them and can be transferred to the hands of HCPs. We also review evidence that improved environmental hygiene can help to bring outbreaks under control and reduce endemic nosocomial transmission, and consider the various options to address contaminated surfaces in healthcare facilities.

3.2 Pathogens Are Shed into the Hospital Environment

Several important pathogens including *C. difficile*, MRSA, VRE, *A. baumannii, P. aeruginosa* and norovirus are shed by patients and contaminate surfaces in hospitals, which may serve as a source for transmission. Fungi, in particular *Aspergillus* spp., can also contaminate the hospital environment and cause HAI; however, fungi are a special group with unusual features that have been well-reviewed elsewhere and will not be considered here [27].

Bacteria, spores and viruses are shed from infected and/or colonized patients (and sometimes staff) into the hospital environment. Wide variation in the reported frequency of environmental contamination can be explained by several factors, including the culturability of the organism, the degree of shedding by the patient, the sampling methodology, the ease of contamination (or difficulty of cleaning) of

the particular environment, and whether there is an ongoing outbreak at the time of sampling. Methodological differences in sample collection and culture make comparisons between studies difficult and in some cases the true level of environmental contamination may be underestimated.

Patients are the prime source of contamination, so surfaces in the vicinity of patients that are touched frequently by healthcare workers and patients, termed "high-touch surfaces", have a higher frequency of contamination than other sites [16, 22, 29, 30]. For example, a recent study defined high-touch surfaces as the bed rails, the bed surface and the supply cart, based on their observed frequency of contact [29]. Developing an understanding of which sites are more likely to be contaminated with pathogens can guide infection control practice and direct new innovations.

Areas around patients are frequently contaminated with MRSA, VRE and *C. difficile* [17, 31, 32]. The frequency of MRSA and VRE contamination correlates with the number of culture positive body sites [16, 18, 33]. Infected patients shed more pathogens than those who are only colonized, and diarrhea results in widespread contamination [16, 34, 35].

Contamination of rooms of unaffected patients has been reported for *C. difficile*, MRSA and VRE. *C. difficile* was identified on 16–17 % of samples from the rooms of patients without known *C. difficile* infection (CDI) [14, 36], MRSA was cultured from 43 % of beds used by patients not known to be MRSA positive [17] and VRE was cultured from 13 % of surfaces in the rooms of patients with unknown VRE status [37]. Contamination of rooms of unaffected patients is most likely to be due to continued viability of organisms shed by previous occupants [12, 17, 38, 39] but may also result from importation by HCPs or visitors, or shedding from asymptomatic carriers [40, 41].

Relatively few prospective studies have evaluated surfaces contamination with Gram-negative or norovirus. The frequency of contamination is approximately 5–10 % of surfaces for Gram-negative bacteria [20, 21, 42–44] and was found to be highly variable, although usually less than 20 % of surfaces for norovirus RNA [45, 46].

Highly variable levels of contamination have been reported during outbreaks. Frequent environmental contamination has been identified and implicated as a contributory factor during continuing outbreaks of *C. difficile*, MRSA, VRE, *A. baumannii* and norovirus [35, 47–50].

Contamination of air has been reported but the interchange between contaminated air and surfaces is not well defined [25, 26, 51–53].

3.3 The Concentration of Contamination Is Sufficient for Transmission

In general, colonized or infected patients have a higher concentration of contamination than their surrounding surfaces [18, 30, 54, 55]. The concentration of VRE on patients' skin is approximately 10^3 colony forming units (CFU) per 50 cm^2 [56]

whereas the concentration of *C. difficile*, VRE and MRSA in stool ranges from 10^3 to 10^9 CFU per gram [34, 57, 58]. The concentration of norovirus in stool can be $>10^{12}$ particles per gram [59] and patients can vomit $>10^7$ norovirus particles assuming a vomit volume of 20–30 ml and the fact that 10^6 particles/ml need to be present for detection by electron microscopy [60]. In contrast, the concentration of nosocomial pathogens on surfaces is generally in the range of <1 to 100 CFU/cm^2 [61, 62] and is often detected only by broth enrichment [16, 17]. Reports of higher concentrations of surface contamination do occur and include total aerobic counts of 10^4 CFU per cm^2 on some intensive care units (ICU) surfaces [63], >200 CFU/cm^2 both before and after cleaning [64] and >15 to >100 MRSA colonies from 23 % of sites positive by direct plating in the rooms of MRSA-positive patients with diarrhea [34].

The presence of a pathogen on a surface does not necessarily represent a transmission risk [10]. However, the infectious dose for most environmentally-associated nosocomial pathogens appears to be low. For example, less than 15 *S. aureus* cells were sufficient to cause infection in experimental lesions [65], <1 CFU/cm^2 was sufficient to cause *C. difficile* disease in mice [66] and a single norovirus particle is thought to have the capacity to cause infection [67]. Importantly, despite the comparatively low concentration of contamination on surfaces compared with patients, touching a contaminated surface carries approximately the same risk for the acquisition of MRSA, VRE and *C. difficile* on hands as touching an affected patient [22, 30, 54, 55]. Therefore, the presence of a pathogen on a surface in any concentration may be a risk for transmission, and this is reflected in proposed guidelines for microbiological hygiene standards [68].

3.4 Nosocomial Pathogens Can Survive on Surfaces for Long Periods

Studies investigating the survival of nosocomial pathogens on surfaces have recently been reviewed by Kramer et al. (Table 3.1 and Chap. 2) [73]. Under certain conditions, *C. difficile* spores, VRE, MRSA and *Acinetobacter* spp. can survive for 4–5 months or more on dry surfaces and norovirus can survive for a week or more.

Table 3.1 Survival of hospital pathogens on dry hospital surfaces

Organism	Survival time
Clostridium difficile (spores)	>5 months
Acinetobacter spp.	3 days to 11 months [69]
Enterococcus spp. including VRE	5 days to >46 months [70]
Pseudomonas aeruginosa	6 h to 16 months
Klebsiella spp.	2 h to > 30 months
Staphylococcus aureus, including MRSA	7 days to > 12 months [71]
Norovirus (and feline calicivirus)	8 h to > 2 weeks [72]

Adapted from Kramer et al. [73].

Large variations in survival times in different reports is partly due to species and strain variation but also to differences in experimental conditions, including inoculum size, humidity, the suspending medium and the surface material [73].

3.5 Limitations of Cleaning and Disinfection

Cleaning is the removal of soil and contaminants from surfaces whereas disinfection relates to the inactivation of pathogens by using a disinfectant [8]. Microorganisms vary in their resistance to disinfectants, so agents must be chosen carefully for their effectiveness, particularly for *C. difficile* spores and norovirus [74]. Furthermore, the hospital environment is complex and often difficult to clean and the use of a cleaning agent that is not effective against the target organism can spread pathogens to other surfaces [74–76].

Liquid disinfectants may damage equipment, especially electronics, and chlorine-containing materials may corrode metals [77]. Disinfectants can potentially harm users and the discharge of waste biocides into the environment may encourage the development of both biocide and antibiotic resistance and have other more general environmentally damaging effects [77]. For these reasons some authorities have questioned the use of routine disinfectant decontamination of the hospital environment and favor instead the use of only detergents [77]. There has been a tendency for disinfectants to be used in the USA and detergents in Europe [77, 78]. Recently, UK and European workers have moved more towards the use of disinfectants to control MRSA and *C. difficile*, but the debate continues while awaiting more evidence for the effective use of particular agents.

Cleaning and disinfection does not always eliminate pathogens from surfaces. This is illustrated by a study from St. Louis, USA, showing that one or more site remained contaminated with either MRSA of *A. baumannii* in 26.6 % of more than 300 rooms sampled following four consecutive rounds of bleach disinfection [79]. In other studies, *C. difficile* was cultured from 44 % of 54 surfaces after bleach disinfection in 9 rooms [32] and from 16 % of 243 cultures after bleach disinfection implemented during an outbreak [50]. VRE was cultured from 71 % of 102 samples after bleach disinfection in 17 rooms [32] and it took an average of 2.8 bleach treatments to eradicate VRE in another study [80]. MRSA was cultured from 66 % of 124 surfaces in MRSA patient rooms after cleaning with a detergent sanitizer [17], from 16 % of 65 sites following bleach and steam cleaning during an outbreak on a surgical ward [49] and was found at a concentration of 0.7 CFU per plate following phenolic disinfection during an outbreak on a burns unit [62]. Norovirus RNA was identified on 31 % of 239 surfaces after bleach disinfection, and 16 % of surfaces remained contaminated after double bleach disinfection [45]. Contamination has been identified on apparently clean surfaces during outbreaks due to *Acinetobacter* spp [81]. and viruses [47]. The frequent finding of contamination in empty rooms and rooms occupied by patients unaffected by pathogens suggests residual contamination from previous occupants [39, 82].

However, the thoroughness of cleaning and disinfection was not evaluated in these studies, meaning that it is difficult to determine whether it is the products, the procedures or a combination of the two that is responsible for the failure to eliminate pathogens from surfaces. Nonetheless, the procedure rather than the product is implicated by the fact that many of these studies were performed using agents that are effective *in vitro* against the microorganisms cultured from surfaces after the process [83].

The physiological state of bacteria cultured from dry hospital surfaces has not been studied in detail. A recent study from Australia 'destructively sampled' several hospital surfaces (i.e. cut the materials out of the hospital environment and took them to the lab for analysis) after cleaning and disinfection using bleach and identified biofilms on 5/6 surfaces [84]. Furthermore, MRSA was identified in the biofilm on three of the surfaces. The presence of biofilms may partly explain why vegetative bacteria can survive on dry hospital surfaces for so long, why they are so difficult to remove or inactivate using disinfectants (bacteria in biofilms can be 1,000× more difficult to kill than corresponding planktonic bacteria) and why it is often difficult to recover environmental pathogens by surface sampling [85].

3.6 Nosocomial Pathogens Can Be Transferred from Contaminated Surfaces to the Hands of Healthcare Workers

In vitro studies present a picture of rapid dynamic transfer from surfaces to hands and vice versa (Table 3.2). For example, DNA markers dried onto toys were transferred readily to the hands of researchers and subsequently onto clean toys, and the markers spread rapidly when introduced into a child care center [87, 90]. Similarly, experimentally contaminated fingers serially contaminated multiple surfaces with norovirus [75]. Similar findings have been reported using surfaces experimentally contaminated with bacteria and bacteriophage [86, 88]. Importantly, experimentally contaminated fingers have been shown to transfer more than 30 % of inoculated bacteria and bacteriophage to the mouths of volunteers, with clear implications for the fecal-oral transmission of nosocomial pathogens [86].

Several studies have shown that various bacterial pathogens can be acquired on the hands of HCP through contact with environmental surfaces in the absence of direct patient contact (Table 3.2) [16, 22, 23, 54, 55, 89]. Patients and contaminated surfaces can transfer VRE, MRSA and *C. difficile* to HCP hands at similar frequencies [22, 30, 54, 55]. However, in a recent study, compliance with hand hygiene was 80 % of 142 opportunities after patient contact compared with only 50 % of 196 opportunities after contact with a patient's environment ($p = 0.01$, Fisher's exact test) meaning that contamination acquired from a patient's environment is less likely to be dealt with by hand hygiene [91].

Table 3.2 Transfer of pathogens and surrogate markers from surfaces to hands

Reference	Setting, location	Organism	Method	n	Contaminated (%)	Comment
Rusin et al. [86]	Laboratory, USA	Bacteria and phage	Fomites experimentally contaminated with a mixture of bacteria and phage and touched by volunteers	10–20	–	Transfer efficiency higher for non-porous fomites (28–66 %); Gram-positive bacteria had the highest transfer efficiency (41 %)
Jiang et al. [87]	Child care center, USA	Virus surrogate	DNA was dried onto toys, which were passed to researchers to hold	5	5 (100)	Subsequent DNA transfer to clean toys occurred on 3/5 occasions
Rheinbaben et al. [88]	Laboratory, Germany	Phage	Volunteers contacted an experimentally contaminated door handle	14	14 (100)	30–66 % of the inoculated virus was recovered from the hands of volunteers
Boyce et al. [16]	Ward side rooms, USA	MRSA	Hands cultured after routine patient care without direct patient contact	12	5 (42)	All 12 healthcare workers wore gloves
Ray et al. [89]	Wards side rooms, USA	VRE	Hands cultured after 5 s contact with the bed rail and bedside table in VRE patients' rooms	13	6 (46)	5/6 hand cultures were indistinguishable from environmental cultures by pulsed-field gel electrophoresis
Barker et al. [75]	Laboratory, UK	Norovirus	Clean fingertips touched contaminated surfaces and then other objects	30	12 (40)	4/10 door handles, 5/10 telephones and 3/10 taps became contaminated
Bhalla et al. [23]	8 wards, USA	Pathogens	Hands cultured after 5 s contact with the bed rail and bedside table	64	34 (53)	Positive hand cultures obtained from 24 % of 25 rooms that had been cleaned after patient discharge
Hayden et al. [22]	Intensive care unit, USA	VRE	Hands cultures from 44 healthcare workers who had negative hand cultures at study entry and touched only environmental surfaces during routine patient care in the rooms of patients with VRE	44	23 (52)	Each contact with patient or environmental surface represented a 10 % risk of picking up VRE
Stiefel et al. [55]	Hospital-wide, USA	MRSA	Gloved hand print cultures obtained from healthcare personnel following contact with patient sites or environmental surfaces in the rooms of patients with MRSA	40	18 (45)	Risk of contamination of gloved hands was not significantly different following contact with environmental surfaces (45 %) than with the patient (40 %)
Guerrero et al. [54]	Hospital-wide, USA	*C. difficile*	Gloved hand print cultures obtained from healthcare personnel following contact with patient sites or environmental surfaces in the rooms of patients with *C. difficile*	30	15 (50)	Risk of contamination of gloved hands was not significantly different following contact with environmental surfaces (50 %) than with the patient (50 %)

3.7 Evidence That Surface Contamination Contributes to Nosocomial Cross-Transmission

If environmental surfaces are involved in transmission, inadequate disinfection after discharge of an infected or colonized patient will increase the risk of acquisition of the same pathogen in the subsequent room occupant. This risk of increased transmission to subsequent occupants has been shown in several studies for a range of organisms, including *C. difficile*, MRSA, VRE and some multidrug-resistant Gram-negative rods (MDR-GNRs), including *A. baumannii* (Table 3.3, Fig. 3.2) [39, 93–95].

The fact that conventional terminal cleaning and disinfection does not reliably eliminate pathogens supports the findings of these 'prior room occupancy' studies. Inadequate terminal disinfection may also result in a room becoming contaminated with more than one strain of a particular pathogen due to a "build up" over time. For example, MRSA with an average of 2.3 antibiograms were found in each patient room in one study where there was sub-optimal terminal cleaning [17]. Similarly, in other studies approximately 30 % of MRSA environmental types were not closely related to the MRSA type affecting the patient in the room [31, 34]. Also, pathogens can be identified in empty rooms [39, 96] and can be transferred to the hands of healthcare personnel from surfaces in empty rooms [23].

These 'prior room occupancy' studies allow the assessment of the risks of environmental contamination independent of common confounding variables of hospital infection, such as patient age, co-morbidities and length of stay. In addition, since in these studies the source patients were already discharged, patient acquisition directly from surfaces or via hand transfer from healthcare personnel is most likely to have come from contaminated surfaces.

A further strand of evidence suggesting that the contaminated surface environment contributes to the transmission of nosocomial pathogens is the impact of improved cleaning and disinfection on overall infection rates [4]. Specifically, the findings of the prior room occupancy studies are supported by evidence that improved terminal cleaning and disinfection can reduce the risk of infection for the next occupant [13, 96]. Datta et al. performed a retrospective cohort intervention study on 10 ICUs at a US hospital to evaluate the impact of improved cleaning and disinfection [13]. The intervention consisted of targeted feedback using a black-light marker, the introduction of a "bucket method" for wetting cleaning cloths, and increased education of housekeeping staff. Patient acquisition was compared during 20-month baseline and intervention periods separated by 16 months. The acquisition of both MRSA and VRE fell significantly during the intervention periods, by 50 % and 27 %, respectively. The risk associated with the prior room occupant was successfully reduced for MRSA but not for VRE.

Passaretti et al. performed a prospective 30-month cohort intervention study on six high-risk units in a US hospital to evaluate the impact of introducing hydrogen peroxide vapor (HPV) for the terminal disinfection of select patient rooms [12]. HPV was introduced to disinfect the rooms of patients known to be infected or colonized with multidrug-resistant organisms (MDROs) on three units following

Table 3.3 The impact of the prior room occupant's colonisation or infection status on the acquisition of pathogens by subsequent occupants of the same room

Reference	Setting[a]/Study design	Findings	Variables	Acquired	Did not acquire	Percentage difference	Adjusted ratio (95 % confidence interval)[b]
Martinez et al. [92]	ICU, USA/9 month retrospective case-control/2003	Placement within a room from which VRE had been cultured was associated with VRE acquisition in the subsequent room occupant	Admitted to a room from which VRE had been cultured	13 % of 30	2 % of 60	87.5 %	OR: 81.7 (2.2–3092)
Drees et al. [39]	ICU, USA/14 month prospective cohort	Positive room cultures or previous VRE-positive room occupants were associated with VRE acquisition	Positive culture prior to admission or acquisition	8.0 % of 50	4.8 % of 588	40.5 %	HR: 4.3 (1.5–12.5)
			VRE prior room occupant	38.0 % of 50	20.2 % of 588	46.7 %	HR: 3.8 (2.0–7.3)
			VRE prior room occupant in the previous 2 weeks	60.0 % of 50	41.8 % of 588	30.3 %	HR: 2.7 (1.4–5.3)
Nseir et al. [93]	ICU, France/12 month prospective cohort	Admission to a room previously occupied by an *A. baumannii*- or *P. aeruginosa*-positive was associated with acquisition of these pathogens	*A. baumannii* prior room occupant	28.1 % of 57	7.9 % of 454	71.8 %	OR: 4.2 (2.0–8.8)
			P. aeruginosa prior room occupant	25.6 % of 82	14.9 % of 429	41.7 %	OR: 2.3 (1.2–4.3)
				% acquired, prior room occupant			
				Positive	Negative		
Huang et al. [94]	ICU, USA/20 month retrospective cohort	Admission to a room previously occupied by an MRSA- or VRE-positive was associated with acquisition of these pathogens	VRE prior room occupant	4.5 % of 1,291	2.8 % of 9,058	37.1 %	OR: 1.4 (1.0–1.9)
			MRSA prior room occupant	3.9 % of 1,454	2.9 % of 8,697	28.8 %	OR: 1.4 (1.1–1.8)
Shaughnessy et al. [95]	ICU, USA/18 month retrospective cohort	Admission to a room previously occupied by a *C. difficile* patient was associated with *C. difficile* acquisition	*C. difficile* prior room occupant	11.0 % of 91	4.6 % of 1,679	58.3 %	HR: 2.3 (1.2–4.5)

[a] *ICU* Intensive care unit
[b] *OR* Odds ratio, *HR* Hazard ratio

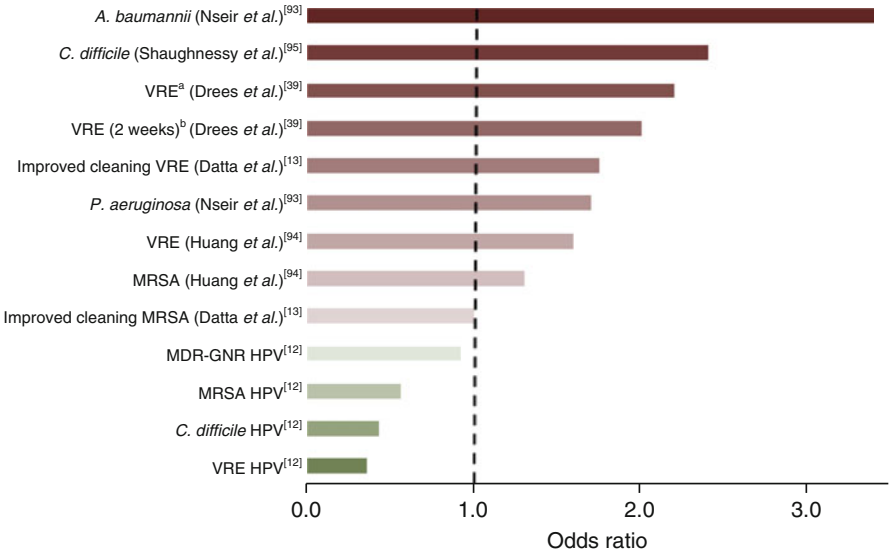

Fig. 3.2 Chart showing the increased risk associated with the prior room occupant
The figures of difference in risk are unadjusted based on raw data. Several of the studies included adjusted measures of risk but these were not included due to differences in study design
(**a**) The immediate prior room occupant was known to be infected or colonized with VRE
(**b**) Any patient infected or colonized with VRE in the 2 weeks prior to admission

a 12-month pre-intervention phase. Patients admitted to rooms decontaminated using HPV were significantly less likely to acquire any MDRO (64 % reduction) than patients admitted to rooms disinfected using standard methods. There was a significant reduction in the risk of acquiring VRE from the prior room occupant (80 % reduction), and non-significant reductions in the risk of acquiring MRSA, *C. difficile* and MDR-GNRs. HPV decontamination significantly reduced the proportion of rooms environmentally contaminated with MDROs. In particular, rooms contaminated with multiple MDROs, MDROs cultured from a room that differed from the room occupant's known MDRO, and MDROs cultured from empty rooms were less frequent on HPV units during the intervention phase. These environmental findings are consistent with improved terminal disinfection by HPV.

The next sections review evidence that contaminated surfaces are important in the transmission of *C. difficile*, VRE, MRSA, norovirus and certain Gram-negative rods (Table 3.4).

3.7.1 Clostridium Difficile

Outbreaks of *C. difficile* were first linked to contaminated surfaces in the 1980s [50]. Samore et al. [109]. conducted a detailed 6-month prospective study of all *C. difficile* cases in a US hospital. The frequency of positive hand cultures and

Table 3.4 Intervention studies investigating the role of contaminated surfaces in the endemic transmission of nosocomial pathogens

Reference	Setting, location	Organism	Study design	Key findings
Mayfield et al.[97]	Three units, USA	*C. difficile*	18-month before-after study of a switch from QAC to bleach disinfection	Significant reduction in CDI incidence on the highest risk unit from 8.6 to 3.3 cases per 1,000 patient-days
Wilcox et al.[15]	Two units, UK	*C. difficile*	2-year ward cross-over study of a switch from detergent to bleach disinfection	Significant reduction in CDI incidence on one of the units (from 8.9 to 5.3 cases per 100 admissions), but not on the other
McMullen et al.[98]	Medical and surgical intensive care units (MICU, SICU), USA	*C. difficile*	2-month before-after evaluation of bleach disinfection of CDI rooms on SICU and 4-month evaluation of bleach disinfection of all rooms on MICU in a hyper-endemic setting	Significant reduction in CDI incidence on both units (10.4 to 3.9 cases per 1,000 patient days on SICU; 16.6 to 3.7 cases per 1,000 patient days on MICU)
Valiquette et al.[99]	Hospital-wide, Canada	*C. difficile*	5-month evaluation of enhanced infection control and disinfection, including a switch to bleach, and a subsequent switch to 'accelerated' hydrogen peroxide	Neither environment intervention made a significant impact on the incidence of CDI; a reduction in the use of high-risk antibiotics significantly reduced the incidence of CDI
Boyce et al.[61]	Hospital-wide, USA	*C. difficile*	20-month before-after study on the use of HPV disinfection for terminal disinfection of CDI rooms	Significant reduction in CDI incidence on five high incidence units (from 2.3 to 1.3 cases per 1,000 patient-days). Lesser reduction in CDI incidence hospital wide
Hacek et al.[100]	Three hospitals, USA	*C. difficile*	3-year before-after study on switching from QAC to bleach for terminal disinfection of CDI rooms	Significant reduction in the incidence of CDI (from 0.85 to 0.45 per 1,000 patient days)
Orenstein et al.[101]	Two medical units, USA	*C. difficile*	2-year before-after study on switching to bleach wipes for daily and terminal disinfection of all rooms	Significant reduction in the incidence of CDI (from 24.2 to 3.6 per 1,000 patient days)
Manian et al.[102]	Hospital-wide, USA	*C. difficile*	3-year before-after study on enhanced terminal disinfection of CDI rooms using HPV and bleach	Significant reduction in the incidence of CDI (from 0.88 to 0.55 cases per 1,000 patient days)
Hayden et al.[82]	ICU, USA	VRE	9-month before-after study on educational improvement of cleaning and hand hygiene	The frequency of environmental contamination and patient acquisition of VRE were reduced from 33 to 17 acquisitions per 1,000 patient-days during the improved cleaning phase
Datta et al.[13]	ICU, USA	VRE / MRSA	3-year before-after study of an intervention (fluorescent markers, "bucket method" and education) to enhance daily and terminal cleaning	Significant reduction of MRSA (3.0 - 1.5% of admissions) and VRE (3.0 - 2.2% of admissions) acquisitions; intervention significantly reduced the increased risk from the prior occupant for MRSA but not VRE
Perugini et al.[103]	Hospital-wide, Brazil	VRE	4-year before-after study of an educational and observational intervention for cleaners	Significant reduction in VRE infection (from 7.7 to 1.9 per 1,000 patient days) and environmental contamination
Grabsch et al.[104]	Hospital-wide, Australia	VRE	18-month before-after study of a multimodal intervention (switch to bleach, improved monitoring of cleaners, modification of VRE contact isolation, periodic 'super-clean-disinfection' of high-risk wards)	Significant reduction of VRE colonization (from 10.7 to 8.0% of patients) and VRE environmental contamination
Passaretti et al.[12]	ICU, USA	VRE / all MDROs	30-month cohort study on the impact of HPV decontamination	Patient admitted to rooms disinfected using HPV significantly less likely to acquire an MDRO (15.7 to 6.2 per 1,000 patient days) and VRE (11.6 to 2.4 per 1,000 patient days)
Mahamat et al.[105]	Hospital-wide, UK	MRSA	8-year interrupted time series analysis of multiple infection control interventions	Introduction of bleach disinfection, environmental sampling, alcohol gels and admission screening all reduced the prevalence of MRSA
Dancer et al.[106]	Two wards, UK	MRSA	12-month cross over-study on the impact of one extra cleaner	Enhanced cleaning was associated with significant reductions surface contamination, hygiene fails and MRSA acquisition
Wilson et al.[107]	ICU, UK	MRSA	12-month randomized cross-over study on the impact of additional twice daily cleaning of hand contact surfaces	Significant reduction in the detection of MRSA on surfaces and hands, but no significant change in MRSA acquisition was detected
Dharan et al.[108]	5 medical wards, Switzerland	-	4-month controlled study where 3-wards received an intervention (including an active oxygen based compound) and 2 wards continued current practice	Intervention associated with reduced contamination but not reduced nosocomial infection or MRSA infection / colonization

Rows marked in grey indicate studies that did not find a significant reduction in transmission associated with the environmental intervention

clinical cultures that matched the pulsotype of the index case among contacts (either roommates, neighbors or subsequent room occupants of index cases) correlated with the intensity of environmental contamination, suggesting that the transmission risk was related to the intensity of contamination.

Several studies have investigated the impact of switching to bleach for disinfection on the incidence of CDI [15, 97, 98, 100, 101]. For example, Mayfield et al. showed that switching from quaternary ammonium compounds (QAC) to bleach disinfection reduced the incidence of CDI for high risk bone marrow transplant patients [97]. However, no significant reduction in infection rates occurred for lower risk patient groups and environmental contamination was not quantified. Wilcox et al. conducted a cross-over study in elderly care wards to compare the impact of detergent cleaning versus bleach disinfection, and demonstrated a significant reduction in infection rates on one ward [15]. No significant reduction was demonstrated on the other ward and the frequency of environmental contamination was not reduced in either study arm, suggesting that other factors were involved. Boyce et al. found that the use of HPV to decontaminate rooms following the discharge of patients with *C. difficile* reduced the incidence of CDI on five high-incidence wards [61]. The hospital-wide incidence of CDI was also reduced, but this was only statistically significant when the analysis was limited to the months when the epidemic NAP1 *C. difficile* strain was known to be present. Meanwhile, a before-and-after study by Manian et al. found that improved terminal disinfection using a combination of multiple rounds of bleach disinfection and HPV significantly reduced hospital-wide incidence of *C. difficile* [102].

Evidence from an *in vitro* model provides proof of concept that *C. difficile* spores can be transmitted via environmental surfaces [66]. Mice exposed to an experimentally contaminated enclosure became colonized in a dose-dependent manner and oxidizing agents including a chlorine-containing liquid disinfectant and HPV reduced effectively the level of contamination and blocked transmission. A recent study found that prior room occupancy by patients with CDI increased the risk of *C. difficile* acquisition, providing evidence that the *in vitro* concept translates into the clinical setting [95].

3.7.2 Vancomycin-Resistant Enterococci (VRE)

Evidence from several studies suggests that the acquisition of VRE is associated with environmental contamination. Huang et al. found that admission to a room previously occupied by a patient with VRE significantly increased the risk of acquiring VRE [94]. In this study, other routes of transmission (which may involve contaminated surfaces indirectly) accounted for the majority of nosocomial transmission. Drees et al. found that VRE acquisition was associated with a positive room culture prior to admittance to the room, a prior room occupant positive for VRE or any VRE-positive room occupants within the 2 weeks prior to admission [39]. A study from Boston, USA, showed that enhanced cleaning reduced overall transmission of VRE on an ICU, but did not mitigate the increased risk from the

prior room occupant [13]. In contrast, a recent cohort study of HPV decontamination on six ICUs found that patients admitted to rooms decontaminated by HPV were less likely to acquire VRE than patients admitted to rooms cleaned by using standard methods when the prior room occupant was positive for VRE (incidence rate ratio, 0.22) [110]. This contrast perhaps illustrates the difficulty in eliminating VRE from surfaces using conventional methods [80].

In addition to patient-level analysis, several studies have shown that improved environmental hygiene can reduce the general incidence of VRE [13, 103, 104]. For example, Hayden et al. investigated the impact of environmental and hand hygiene improvements on VRE infections in an ICU [82]. An educational improvement program for environmental cleaning reduced the frequency of contamination in the rooms of patients with and without VRE and the incidence of VRE acquisition fell. The reduction in contamination was sustained through a "washout" period where no further intervention occurred and through a subsequent hand hygiene educational improvement program. A recent 4-year before-after study from Brazil showed that an educational and observational intervention for cleaners resulted in impressive reductions in both VRE infection and environmental contamination [103].

3.7.3 MRSA

Dancer recently reviewed evidence that environmental contamination makes an important contribution to the transmission of MRSA [111]. That review summarized evidence that staphylococci are carried by people and shed into the environment, can survive for extended periods on surfaces and can spread between people and the environment, and that improved hygiene reduces staphylococcal infection rates. More recently, Dancer et al. conducted a ward cross-over study to investigate the impact of an extra cleaner focusing on hand touch sites [106]. The enhanced cleaning was associated with a significant reduction in the total aerobic counts on surfaces and the number of failures to reach a hygienic standard of >2.5 CFU/cm^2 and with a significant reduction in MRSA acquisitions by patients. However, there was no significant reduction in surface contamination with methicillin-susceptible *S. aureus* and admission screening was not universally applied, so the true MRSA acquisition rate was uncertain. Nonetheless, the study provides further evidence to support the view that reducing surface contamination reduces MRSA nosocomial transmission.

3.7.4 Gram-Negative Rods (GNRs)

3.7.4.1 Non-fermenting Gram-negative bacteria (*Acinetobacter* and *Pseudomonas*)

A recent prospective cohort study showed that prior room occupancy with a patient colonized or infected with *A. baumannii* or *P. aeruginosa* was a significant

risk factor for the acquisition of these pathogens. This was the first evidence from an endemic setting that contaminated surfaces contribute to the transmission of GNRs [93].

Numerous outbreaks of *A. baumannii* have been associated with contaminated inanimate fomites, which resolve once the common source was identified and removed, replaced or adequately disinfected [4]. Several outbreaks, where environmental surfaces were contaminated but a common source was not identified, offer limited evidence that surface contamination also plays a role in continued transmission [48, 112, 113]. For example, during an outbreak of *A. baumannii* in the UK affecting 19 patients on a neurosurgical unit, 53 % of 51 surfaces in the unit were contaminated with the outbreak strain and monthly screens showed that the frequency of contamination correlated with the number of affected patients on the unit [48]. Crucially, failure to maintain low levels of contamination resulted in increases in patient colonization, suggesting that the contamination was contributing to the outbreak. However, it was not possible to prove causality because neither molecular epidemiological analysis nor hand cultures were performed.

Further evidence for the role of contaminated surfaces in such transmission comes from an investigation of a multi-institutional outbreak of *A. baumannii* among war-wounded US soldiers [114]. In this study, outbreak strains of *A. baumannii* were cultured from 21 % of 175 surfaces in seven field hospitals but a very low frequency of contamination was identified in soil samples and on healthy soldiers' skin.

3.7.4.2 Enterobacteriaceae

Two recent studies have shown that admission to a room previously occupied by extended spectrum beta-lactamase (ESBL) producing Enterobacteriaceae did not increase the risk of acquisition for incoming patients, suggesting that the environment may have a less important role in the transmission of Enterobacteriaciae than for non-fermenting GNRs (Fig. 3.3) [93, 115]. This is supported by laboratory findings that Enterobacteriaceae tend to survive less well on dry surfaces than non-fermenters, Gram-positive bacteria and bacterial endospores [73, 116]. However, several recent studies have identified environmental contamination with ESBL and carbapenemase-producing Enterobacteriaceae (CPE) on hospital surfaces [42, 43]. Resistant Enterobacteriaceae are shed into the hospital environment and do have the capacity to survive on dry surfaces, so surfaces may be involved in their transmission [1, 117]. The major concern with CPE relates to *K. pneumoniae* [117, 118], which seems to be more closely associated with environmental contamination than other Enterobacteriaceae; [42, 43] thus, environmental contamination may be more important with CRE than ESBL carriers.

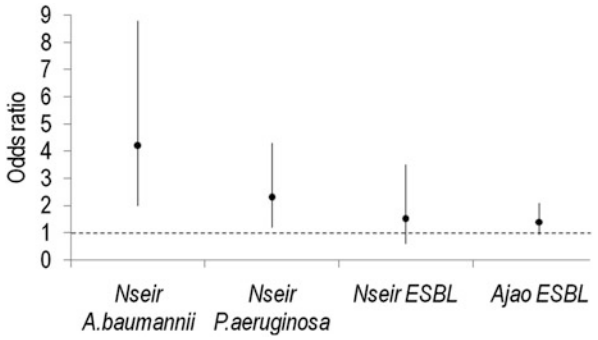

Fig. 3.3 Comparing the role of the prior room occupant for non-fermenting Gram-negative bacteria and Enterobacteriaceae
Odds ratio (*circles*) with 95 % confidence intervals (*lines*) comparing the rate of acquisition for patients admitted to rooms where the prior room occupant had the pathogen of interest vs. patients admitted to rooms were the prior room occupant did not have the pathogen of interest; from studies by Nseir et al. [93] and Ajao et al. [115]

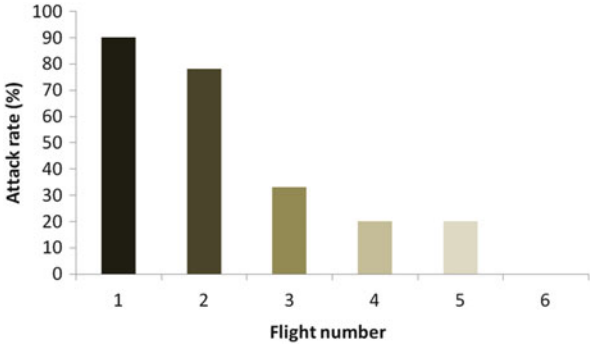

Fig. 3.4 Norovirus attack rate among staff on subsequent flights following an episode of vomiting in the economy cabin

3.7.5 Norovirus

Compelling evidence for the role of surface contamination in the transmission of norovirus comes from outbreaks affecting epidemiologically distinct cohorts of passengers on boat and plane trips [119–122]. For example, an outbreak affected 74 % of guests on three consecutive houseboat trips [120]. An environmental investigation identified norovirus on 71 % of surfaces in bathrooms, kitchens and door handles and fomite contamination appeared to contribute to continuation of the outbreak over the three trips. Similarly, there was a sequentially decreasing attack rate of norovirus among distinct cohorts of cabin crew following an episode of vomiting on an airplane (Fig. 3.4) [119].

Several investigations have identified surface contamination with norovirus in the absence of other potential reservoirs during continuing outbreaks [45, 47].

During one outbreak of norovirus in a long-term care facility, five of ten environmental samples collected after phenolic disinfection were positive, suggesting widespread persistent contamination [47]. Positive sites included an elevator call button used only by staff. The outbreak resolved following a second more thorough facility-wide disinfection, suggesting that environmental contamination contributed to transmission. Various community outbreaks with norovirus have been linked to shared computer keyboards [123], specific episodes of vomiting [124, 125] (for example, a kitchen assistant vomited into a sink that was used to prepare vegetables) [124], contamination of carpets following a hospital outbreak [126] and persistent widespread contamination in a UK hotel [127]. However, a key limitation of these studies is that the role of symptomatic or asymptomatic staff carriage in transmission often was not investigated.

Mathematical models that include the role of contaminated surfaces are rare, but one study evaluated the likely economic impact of various control strategies for norovirus including improved disinfection [128]. The model found that increased disinfection alone or in combination with increased hand hygiene and using protective apparel were the most effective strategies for the control and containment of norovirus outbreaks. However, prospective studies are required to quantify the role of surface contamination in the spread of norovirus.

3.7.6 Revaluating "Negative" Studies

It is important to note that some studies report that environmental intervention is ineffective in controlling various pathogens, indicated by the grey rows on Table 3.4. A critical re-evaluation of these studies suggests reasons why they did not identify a significant reduction in transmission:

- Wilcox et al. [15]. There was virtually no impact on the frequency of *C. difficile* environmental contamination on the wards when they switched from using a QAC to bleach. Thus, it is not surprising that a significant reduction in CDI was not consistently demonstrated in this cross-over study.
- Valiquette et al. [99]. A bundle of interventions were implemented over a period of a few months, some of which were environmental. The bundle of interventions was only given a few months to be effective.
- Dharan et al. [108]. The intervention was focused mainly on improving the cleaning and disinfection of floors, which are not the high-touch, high-risk sites that are likely to contribute most to transmission.
- Wilson et al. [107]. The cross-over study was performed in a declining prevalence of MRSA in the UK, so could have been underpowered to detect a clinical impact.

3.8 Environmental Cleaning, Disinfection and Infection Control

3.8.1 Improving the Efficacy of Cleaning and Disinfection

Cleaning and disinfection rarely eliminates pathogens and the baseline cleaning rate for high-risk objects in a large study of 36 acute US hospitals was <50 %, as determined by removal of a fluorescent marker [129]. Several studies have demonstrated that focused efforts can improve the efficacy of cleaning. For example, Eckstein et al. found that a research team was able to eliminate persistent VRE and *C. difficile* from surfaces while a housekeeping team did not [32]. Furthermore, a number of studies have shown that systematic education and monitoring of the cleaning and disinfection process can reduce contamination of surfaces and transmission (Table 3.4) [13, 82, 103, 106].

There are several different options to monitor the effectiveness of cleaning, each with advantages and disadvantages. These include visual monitoring, microbiological sampling, fluorescent markers and ATP bioluminescence assays (Table 3.5) [130, 131].

Visual assessment of hospital cleaning is performed by measuring the apparent cleanliness of a room against a checklist [132, 133]. Visual inspection is important since a room needs to be visually clean to be acceptable to the current and subsequent occupant. However, visual assessment of hygiene does not correlate with microbial contamination, and can thus be a misleading measure of cleanliness [64, 134, 135].

Microbiological surface cultures can be qualitative (pathogen presence or absence) or quantitative (aerobic colony counts). Several different sampling methods are available; usually swabs (with or without enrichment) or contact plates. Quality standards for both aerobic colony counts (<2.5 CFU/cm^2) and specific indicator organisms (<1 CFU/cm^2) have been proposed [133, 136]. Standards exist for the quality of air in operating theatres [137] but cost and practicality mean that routine microbiological sampling outside of operating theatres is rarely performed.

Table 3.5 Comparing the options for assessing the efficacy of conventional cleaning and disinfection

	Visual	Micro	ATP	Fluorescent
Ease of use	High	Low-moderate	High	High
Quantitative	No	Yes/no	Yes	No
Correlation with microbial contamination	Poor	Accurate	Indirect	Indirect
Identifies pathogens	No	Yes/no	No	No
Risk of "gaming" by staff	Low	Low	Low	Moderate
Identifies 'dirty' surfaces[a]	Yes	No	Yes	No
Published evidence of attributable clinical impact	No	Yes [106]	No	No

[a]non-microbial soiling

ATP bioluminescence assays are performed by swabbing surfaces and using a hand-held sensor to give a real-time quantitative measurement of ATP from the surface. Several "quality standards" have been set as relative light unit (RLU) thresholds, ranging from 100 to 500 [131, 133]. There is no direct correlation between RLU and microbial contamination, but "hygiene fails" determined by aerobic colony count and ATP do correlate [131, 133].

Fluorescent material in the form of gel, powder or lotion can be applied to a surface and its removal assessed by a 'black light' illumination. The percentage of spots removed is used to evaluate cleaning performance [129, 131] and can be improved by educational interventions [129, 138]. The removal of marked spots has been shown to correlate with microbial contamination in some studies [131, 138]. However, altering the location of fluorescent dye spots reduced the proportion of objects that were cleaned from 90 % to approximately 60 %, indicating that staff may "get wise" to the location of the markers and preferentially target them [139]. Furthermore, the sustainability of cleaning improvement by using fluorescent markers, and probably other assessment methods too, is questionable. One study showed that cleaning performance measured by the removal of a fluorescent marker increased from a baseline of 52 to 80–85 % through training and monthly feedback; [140] however, compliance soon returned towards baseline (57–66 %) when the monthly feedback ceased.

3.8.2 Evaluating and Implementing New Technology

Technological developments to assist with cleaning and disinfection include the introduction of microfiber cleaning materials, which may be more effective than standard cloths for removing pathogens from surfaces [141]. Designers and manufacturers of hospital equipment can help by producing hospitals which are easier to clean [142]. For example, the 'Design Bugs Out' initiative in the UK aims to design hospital furniture and equipment that are easier and quicker to clean (http://www.designcouncil.org.uk/designbugsout).

New liquid disinfectants boast improved efficacy and practicability, reducing the risk for human error during formulation [77, 101, 143–145]. The emergence of wipes impregnated with bleach [101] or hydrogen peroxide [144] are promising developments, which seem to be better tolerated by cleaners and effective for surface disinfection. Emerging new agents include reformulated hydrogen peroxide solutions (sometimes called "activated" or "improved" hydrogen peroxide) [143, 146] and electrolyzed water [145].

The manual application of liquid detergents and disinfectants is limited by reliance on the operator to ensure appropriate selection, formulation, distribution and contact time of the agent. These problems can be reduced by the use of "no-touch" automated room disinfection (NTD) systems [83]. A number of NTD systems have emerged, which remove or reduce reliance on the operator to ensure distribution, contact time and process repeatability. These aim to improve the

level of room disinfection and thus reduce the increased risk of environmental contamination by the prior room occupant [12]. Because areas or rooms must be vacated for all NTD systems, they are best suited for terminal disinfection following the transfer or discharge of patients infected or colonized with pathogens. Available NTD systems include HPV systems [12, 61], aerosolized hydrogen peroxide (aHP) [147, 148], and UVC [149, 150] and pulsed-xenon (PX-UV) [151, 152] ultraviolet radiation. These systems have important differences in their active agent, delivery mechanism, efficacy, process time and ease of use [83]. Typically, there is a trade-off between time and effectiveness, with the hydrogen peroxide-based systems being more efficacious but the UV systems faster and easier to use [83]. The choice of NTD system should be influenced by the intended application, the evidence base for effectiveness, practicalities of implementation and cost constraints (See also Chap. 9).

3.8.3 Reducing and Controlling the Extent of Environmental Contamination

In addition to improving the efficacy of cleaning and disinfection once contamination has occurred, steps can be taken to prevent, reduce or improve the containment of shed pathogens. Rapid identification and isolation of affected patients could reduce contamination of bays and open ward areas shared by unaffected patients [153]. Identification and isolation of asymptomatic shedders may also have a role. Asymptomatic carriers of *C. difficile* were a source of widespread contamination in one study [41] and asymptomatic fecal carriage of small round virus (probably norovirus) was common in another long-term care facility study [40]. Further work is required to determine the extent and length of time that patients continue to shed pathogens into the environment after the resolution of symptoms, particularly for *C. difficile* and norovirus.

While hospitals in the US generally have a high proportion of single rooms, hospitals in other countries typically have a much lower proportion of single rooms [154]. The lack of isolation facilities hampers effective isolation of patients known to be infected or colonized with pathogens. Where single rooms are not available, cohorting of patients affected with the same pathogen within a multi-occupancy area is often practiced [155, 156]. However, increasing the number of single rooms has been associated with reduced transmission [157]. Thus, hospitals and healthcare administrators should ensure the adequate provision of isolation facilities through building hospitals with a high proportion of single occupancy rooms or modifying existing facilities to increase the proportion of single occupancy rooms [154, 156–158].

'Source control' through daily bathing with chlorhexidine is another approach to reducing the shedding of pathogens, and this has been shown to reduce the transmission of certain pathogens [56, 159–162]. However, most studies of the

Table 3.6 An overview of candidates for antimicrobial surfaces

Candidate	Pros	Cons
Metal		
Copper	Rapidly microbicidal Reduces acquisition	? Sporicidal Acceptability/retrofitting
Silver	Rapidly microbicidal	? Sporicidal Tolerance development
Chemical		
Organosilane	Easy to apply	Limited microbicidal activity Durability
Light-activated	Broadly microbicidal	? Sporicidal
Topography		
"Liquid glass"	Reduces deposition Improves 'cleanability'	Not microbicidal
Sharklet pattern	Reduces deposition Reduced biofilms	Not microbicidal

effectiveness of this intervention have been performed in ICU settings, so studies are required outside of the ICU. 'Source control' could be used in conjunction with complementary strategies aimed at improving cleaning and disinfection to further reduce transmission. However, chlorhexidine is not effective against spores and reduced susceptibility may emerge [163] so novel 'source control' strategies and agents are needed for these pathogens.

3.8.4 Antimicrobial Surfaces

Improvements in hospital design and surface science can help to reduce the potential for contamination [164]. Copper, silver and other antimicrobial impregnated materials reduce bacterial survival *in vitro* and numerous antibacterial surface materials or treatments are now available (Table 3.6, See also Chaps. 4, 5 and 7) [63, 165–167]. Salgado et al. performed a multi-center evaluation of the clinical impact of introducing 6 copper alloy high-touch sites into the rooms of patients on three ICUs. Patients (n = 614 following exclusions) were randomized to intervention 'copper' rooms and control 'non-copper' rooms in three USA ICUs over an 11 month period. The only difference between the rooms was the presence of six items made of copper alloy. Patients admitted to 'copper rooms' were significantly less likely to acquire HAI or colonization with MRSA/VRE (see also Chap. 4). Subsequent correspondence has criticized the methods used to report clinical outcomes [168, 169], but notwithstanding the limitations, the study does suggest that the introduction of a small number of copper surfaces reduces transmission. The results of further studies are awaited.

Some data indicate textiles impregnated with biocides could help to reduce the degree of contamination that is shed by patients and this may help to reduce transmission, but more detailed studies are required [170, 171].

3.8.5 *Improving the Quality of the Evidence*

The quality of evidence supporting the role of surface contamination in the transmission has improved from outbreak reports to large, well-designed intervention studies (Table 3.4). Structured reporting of future outbreaks, for example using the ORION guidance [172], will help, but large, prospective controlled trials are needed to properly elucidate the role of surface and air contamination (and decontamination) in the transmission of nosocomial pathogens.

Although the role of contaminated air in transmission is uncertain, nosocomial pathogens can be detected in hospital air [26, 51, 53, 173]. The introduction of portable HEPA filtration using reduced the amount of MRSA present in the air [173]. However, further research is required to determine whether better containment of pathogens in air will reduce transmission.

Most of the evidence investigating the role of contaminated surfaces in transmission comes from acute care facilities. However, environmental contamination may play an important role in transmission in long-term care facilities and other non-acute healthcare facilities [41, 47]. There is considerable evidence that contaminated environmental surfaces are involved in the transmission of norovirus in the community [119, 124–127] and emerging evidence that contamination with pathogens such as community-associated MRSA may be involved in transmission in outpatient and community settings [174–177]. Environmental interventions that are effective for the prevention and control of the transmission of pathogens in acute healthcare facilities may not be effective in community and non-acute settings. Therefore, further research is required to investigate the role of surface contamination in the transmission of pathogens in non-acute and community settings.

3.9 Conclusion

The historical perspective that contaminated surfaces contribute negligibly to nosocomial transmission has been revaluated in light of new information. There is now compelling evidence that contaminated surfaces make an important contribution to the epidemic and endemic transmission of *C. difficile*, VRE, MRSA, *A. baumannii* and *P. aeruginosa* (Tables 3.3 and 3.4, Fig. 3.2), and to the epidemic transmission of norovirus. However, few studies have quantified the link between contaminated surfaces and the risk of transmission. This is in part due to the difficulties in conducting research in this area because of the multifaceted nature of nosocomial transmission (Fig. 3.1). In addition, the widespread view that contaminated surfaces are relatively unimportant in transmission has meant that fundholders and administrators have not commissioned research in this area until relatively recently. There is now sufficient evidence to support further studies in this area to identify the best methods of achieving and maintaining clean hospitals

and to evaluate the cost and effectiveness of such interventions on reducing the incidence of hospital associated infections. In particular there is a need to conduct large, high quality prospective controlled trials to identify interventions that significantly reduce surface contamination and transmission.

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Chapter 4
Role of the Microbial Burden in the Acquisition and Control of Healthcare Associated Infections: The Utility of Solid Copper Surfaces

Michael G. Schmidt, Andrea L. Banks, and Cassandra D. Salgado

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Abstract For more than a century, healthcare has been challenged to keep environmental surfaces clean to control microbes and improve patient outcomes. However despite an annual cost exceeding ten billion dollars cleaning with disinfection has done little to reduce the incidence of healthcare-associated infections (HAI). This chapter will review the scientific evidence delineating the role that the environment and healthcare workers play in the acquisition and movement of

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the microbes implicated in HAI and how through controlling the microbial burden of the built clinical environment it is possible to mitigate the rate of HAI acquisition. Specifically evidence demonstrating the effectiveness of solid copper surfaces for its ability to continuously limit the concentration of bacteria found on surfaces and objects within the built environment will be reviewed in concert with a discussion of how through the mitigation of the environmental burden copper surfaces are able to concomitantly reduce the incidence of HAI. Insights provided by this chapter are intended to facilitate an understanding and importance of the need to use a comprehensive or systems based approach to fight healthcare associated infections.

Keywords Hospital Associated Infections (HAI) • Antimicrobial Copper

List of Abbreviations

CA-ASB	Catheter associated bacteriuria
CAUTI	Catheter-associated urinary tract infections
CFU	Colony-forming units
CI	Confidence interval
CLABSI	Central line-associated bloodstream infections
CDI	*Clostridium difficile* infection
EPA	Environmental Protection Agency
HAI	Hospital associated infections
HCWs	healthcare workers
HPV	Hydrogen peroxide
HTOs	High touch objects
ICU	Intensive care unit
IDSA	Infectious Disease Society of America
IV	Intravenous
KPC	*Klebsiella pneumoniae* carbapenemase
MRSA	Methicillin resistant *Staphylococcus aureus*
MDR	Multi-drug resistant bacteria
PFGE	Pulsed field gel electrophoresis
PMF	Proton motive force
PPE	Personal protective equipment
OR	Odds ratio
SSI	Surgical site infections
UTI	Urinary tract infections
UV	Ultraviolet
VAP	Ventilator-associated pneumonia
VRE	Vancomycin resistant *enterococci*

4.1 Introduction

Hospital associated infections (HAI) continue to be a common and significant complication of hospitalization, leading to increased morbidity and mortality. It was estimated that in 2002, there were approximately 1.7 million healthcare-associated infections, which resulted in approximately 99,000 deaths [41]. A more recent meta-analysis of the costs and financial impact of HAI on the US healthcare system reported that the total annual costs for the five major infections (central line-associated bloodstream infections (CLABSI), ventilator-associated pneumonia (VAP), surgical site infections (SSI), *Clostridium difficile* infection (CDI), and catheter-associated urinary tract infections (CAUTI)) were $9.8 billion (95 % confidence interval (CI) $8.3 to $11.5 billion) [106]. There has been an unprecedented movement for healthcare facilities to improve patient safety and certainly prevention of HAI represents a major portion of that effort.

The process by which a patient acquires an infection while hospitalized is complex. This has been elegantly illustrated and described by Dr. Weinstein (Fig. 4.1), highlighting the role of the patient's endogenous flora, exposure to exogenous flora, as well as the influence of devices and pressure from antibiotic use [101]. Recent development and implementation of strategies to prevent HAI have included such efforts as antimicrobial stewardship, interrupting transmission of epidemiologically important organisms, and infection specific prevention bundles; however, there is renewed interest in defining the role of environmental contamination in transmission of nosocomial pathogens and development of HAI.

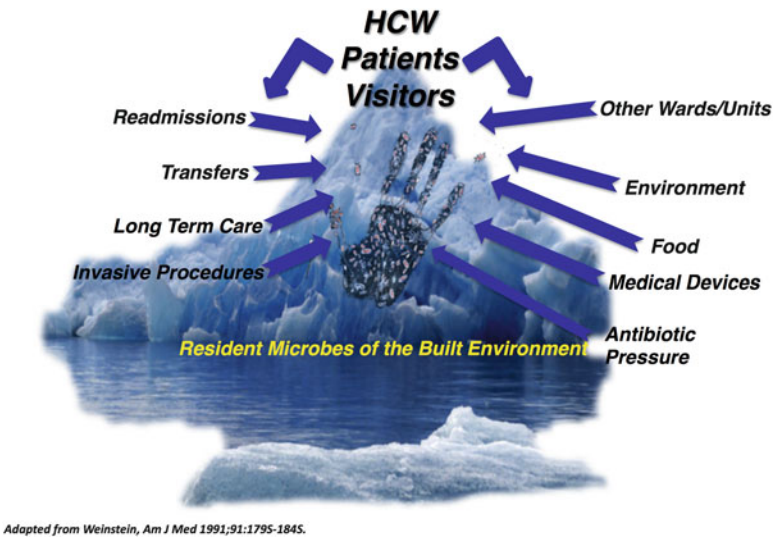


Fig. 4.1 Hazards in the hospital (Adapted from the figure by Weinstein [101]). The complexity and dynamic nature of the microbial pressure being introduced into the built clinical environment is dependent on stochastic nature inherent to healthcare

The purpose of this chapter is to review the role of the environment of care as it pertains to microbial contamination and risk of HAI to patients as well as describe the novel use and efficacy of antimicrobial copper surfaces in mitigating this risk. We will discuss problematic pathogens in healthcare, their ability to contaminate and persist in the environment, their ability to contaminate the healthcare provider, and ultimately their ability to directly or indirectly result in colonization and infection in the patient. We will briefly review the traditional measures utilized to reduce the microbial burden associated with the healthcare environment but focus our discussion on the use of continuously active antimicrobial solid copper for this purpose. Given that, we describe the proposed mechanism of action for copper's antimicrobial property, its activity against pathogens commonly found in healthcare, as well as the clinical efficacy of placing solid copper surfaces into the patient care environment.

4.2 Role of the Environment in Healthcare Infection

The majority of healthcare associated infections are thought to occur via transmission from the patient's own endogenous flora. However, there is increasing evidence that there exists significant transmission of microbes from healthcare personnel and the hospital environment to vulnerable patients. A study published in 1991 estimated that the causative source of an HAI in the Intensive Care Unit (ICU) was the patients' endogenous flora 40–60 % of the time and antibiotic driven changes in flora 20–25 % of the time. Cross-infection via the hands of personnel accounted for 20–40 % of cases and other sources, including contamination from the environment, accounted for the remaining 20 % [101]. It has been established that the inanimate hospital environment can become contaminated with nosocomial pathogens after exposure to colonized patients [36]. This environment includes surfaces within the hospital room (bedrails, bedside tables, etc.) and medical equipment. A review of the available literature in 2002 concluded that personal and environmental hygiene reduced the spread of infections [1]. More recent literature has provided additional evidence that contaminated hospital surfaces are a source of transmission of nosocomial pathogens [57]. Otter and colleagues delineated the continuous, omni-directional and complex nature of how microbes can easily move between infected or colonized patients, healthcare workers, and objects resident in the built environment (Chap. 3 and ref [57]) (Fig. 4.2).

4.2.1 Microbes in the Built Environment

Microbes have an innate ability to contaminate and potentially establish residence on any surface. Surfaces with frequent hand contact and in close proximity to the patient are often colonized with nosocomial pathogens, and most of these pathogens can

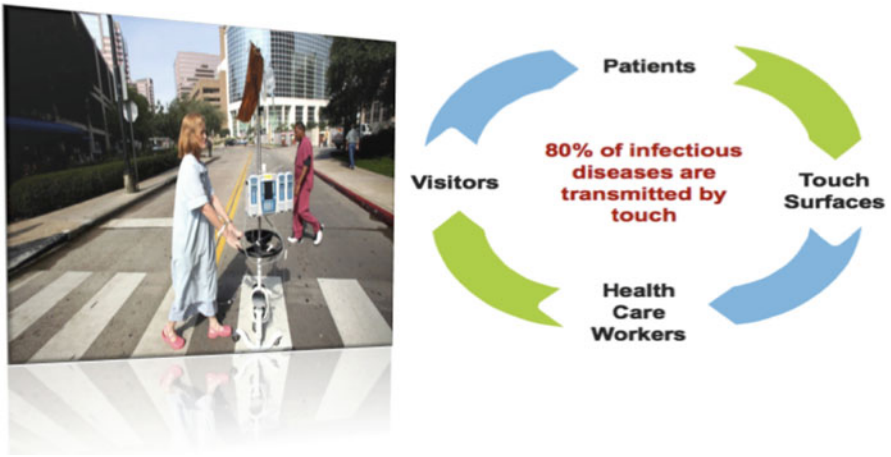


Fig. 4.2 Transmission dynamic of microbes resident in the clinical environment. the ubiquitous distribution of microbes, coupled with the stochastic nature of care, facilitates a continuous risk of the patient, healthcare worker or high touch object introducing, acquiring or spreading unwanted microorganisms

remain viable on these inanimate surfaces for weeks to months (Chap. 2 and ref [42]). Further, the distribution and dispersal of the microbes from healthcare workers, visitors and patients can contribute to the resident microbial flora of the built environment. Humans shed a minimum of ten million of their 100 million skin cells per day. Routine activities such as walking can result in the loss of approximately 10^4 skin particles per minute with a complete layer of skin cells being lost and replaced from healthy individuals on average approximately every 4 days [51]. The displaced skin cells are covered with the endogenous flora of the individual. Not all individuals shed skin equally. In one study of microbial dispersal by skin in a hospital ward, Noble defined a '*Staph aureus* disperser' as a patient who contributed greater than six *S. aureus* per cubic meter of air [52]. Given that the mean concentration of bacteria within the ward was the equivalent of 800 viable bacteria per cubic meter, the concentration of S. *aureus* observed was thought to represent 1 % of the total flora [52]. Over the years the number has been revised to suggest that an individual is a '*Staph aureus* disperser' when they are able to disseminate more than four viable particles per microbe per cubic meter of air [9]. Causality, or the linkage of an environmental isolate to that organism responsible for disease in individuals has been demonstrated as early as 1945 when deForest and Kerr reported cases of eczema which occurred amongst nurses that were caused by streptococci that were shed [26]. With the advent of molecular techniques, such as Pulsed Field Gel Electrophoresis (PFGE) and whole genome sequencing, the ability to demonstrate casualty has now become much more straightforward but is still nevertheless time intensive and cost prohibitive.

Once established within the built environment the microbe must then be able to resist the perturbations introduced as a consequence of cleaning and other infection control measures. To that end, some pathogens have become resistant to

disinfectants used on environmental surfaces, thus leading to their persistence and continued distribution within the built environment and presenting a continued risk of being transferred to a patient, healthcare worker or object within the built hospital environment [100].

4.2.2 *Transmission of Pathogens to Patients and Healthcare Workers*

Certain factors must be met for a microbe to transition from its role as an inhabitant of the surfaces associated with the built clinical environment to pathogen that can be transmitted to a patient or healthcare worker. First, the pathogen must be able to survive on the objects and surfaces within the environment for a sufficiently long period of time while retaining its ability to be virulent or its ability to colonize a susceptible host after its subsequent liberation from the surface and resulting transmission/establishment. Second, contamination of the environment by a particular pathogen must be sufficiently frequent to account for its loss from the object or surfaces as a consequence of routine cleaning, desiccation, or starvation. Third, the agent must be present at a concentration sufficient to establish itself upon encountering the new host or location. Certain nosocomial pathogens, such as Norovirus, have incredibly small infectious doses with a median dose of 18 viruses [86] while the environmental dose of the causative agent of the majority of CAUTI, *Escherichia coli*, is not as evident. The Infectious Disease Society of America (IDSA) has classified that in the absence of symptoms a concentration of $\geq 10^5$ colony forming units (CFU) per ml coupled where ≥ 1 bacterial species is present in the urine of a catheterized patient that the individual has an asymptomatic catheter associated bacteriuria (CA-ASB) [35]. A CAUTI is defined as the "presence of symptoms or signs compatible with urinary tract infections (UTI) with no other identified source of infection along with $\geq 10^3$ CFU/mL of ≥ 1 bacterial species" from a catheterized or previously catheterized ($\leq$48 h) urine sample [35]. However the guidelines are silent as to the origin and/or concentration of the microbe(s) required to establish the CA-ASB or CAUTI. The concept of infectious dose from the environment as it pertains to nosocomial infection has not been rigorously studied for the majority of the HAI. Further study is warranted.

Hospitals have put into place measures in an attempt to decrease the contamination or likelihood of colonization of healthcare workers with infectious pathogens. Focus has been placed on increased hand hygiene, contact precautions, and enhanced environmental cleaning. In 2006 Pittet and colleagues presented an evidence-based model arguing for improved hand hygiene practices during patient care as being the most important method for preventing HAI and spread of antimicrobial resistant pathogens [61]. In their model, five steps are required for the transmission of pathogens within the clinical care setting. Collectively, the model considers the microbes and their transmission from objects, healthcare workers, and patients to the next individual or object. The first step requires that the microbe be present or resident

on the patients'/healthcare workers' skin or immediate environment. The concentration of the nosocomial pathogen can vary from as few as 1 to over 10^6 CFU per cm^2. Subsequently, the microbe must be transferred to the healthcare worker. Simple acts such as lifting a patient, obtaining a blood pressure, pulse, or assessing a temperature can easily result in the transfer of between 100 and 1,000 CFU of a common Gram-negative pathogen *Klebsiella* spp. [19]. In fact these authors learned that 17 % of the staff of an intensive care unit were found to have *Klebsiella* contaminating their hands when screened and that the serotypes were related to those isolated from infected or colonized patients within the ICU on the same day [19]. Further advancing the importance of hand hygiene was a study that found healthcare workers were as likely to contaminate their hands or gloves from commonly-touched environmental surfaces as from direct contact with colonized patients [85].

The third aspect of the model is dependent upon the biology of the microbe. Some microbes can survive for longer periods of time on hands than others. Epidemic and non-epidemic strains of *E. coli* and *Klebsiella* spp. were found to have significantly different survival times [30] supporting the argument that bacterial properties other than the survival of a typed strain under defined conditions may contribute to the ability of a microbe to be easily transmitted and retained within healthcare setting. In other studies workers found that bacterial colonization of the hands of healthcare workers progressively increased with time [60, 62]. In these two studies they found that the concentration of commensal and pathogenic flora increased as a consequence of patient care. Additionally, the authors reported that the dynamics of hand contamination were independent of whether or not the healthcare worker was working while gloved or ungloved [60, 62].

Such an establishment of causality in the development of HAI, and an intrinsic ability to survive on the hands of the healthcare workers, provides strong support for a role for hand hygiene for limiting the incidence and controlling the spread of HAI. The fourth and fifth aspects of the model advanced by Pittet and colleagues addresses the issue of defective and/or absent hand cleansing and how it can lead to the cross transmission of the microbes [61]. Here they have raised the issue of the need to microbiologically validate proper hand cleansing in order to control the spread of microbes regardless of their source. In citing a study by Sala and colleagues, they describe how an outbreak of Norovirus was traced to an infected food handler within a hospital cafeteria. Here the implicated foodstuffs consumed during the outbreak were handmade by the infected worker [69]. Independently, it has been shown that Norovirus contaminated fingers can sequentially transfer this virus to up to seven surfaces [7]. Sequential transfer is not only confined to human to surface transfer. In the same study, the virus was found to move from contaminated cleaning cloths to clean hands and surfaces [7]. Recently, Snitkin and colleagues used whole genome sequencing to track an outbreak of Carbapenem-Resistant *Klebsiella pneumonia* that occurred at the U.S. National Institutes of Health Clinical Center where they learned that despite early implementation of infection control procedures, including aggressive hand hygiene controls, the microbe persisted in the environment [83]. Consequently, the built environment can serve as a reservoir from which clean hands can serve as a source of HAI.

4.2.3 Contamination of Medical Equipment

When a patient is known to be colonized or infected with a transmittable pathogen, dedicated equipment (i.e. stethoscopes) should be used when possible along with other personal protective equipment such as gowns, gloves and masks. Frequently touched hospital surfaces and medical equipment, such as doorknobs, bed rails, faucet handles, and intravenous (IV) poles, have been identified as reservoirs of pathogenic microbes [10, 56]. In addition to medical equipment and healthy or intact skin, there have been reports of the transfer of bacteria to the gloves and gowns of healthcare workers after patient contact [6, 18, 33, 49]. Specifically, Morgan and colleagues reported that the transfer of multi-drug resistant bacteria (MDR) to the gowns and gloves of healthcare workers occurred after routine contact, and that this was found to increase as environmental contamination increased [49]. The intent of the study was to evaluate the differential rate of contamination by a MDR variant of *Acinetobacter baumannii* compared with other MDR bacteria while attempting to understand the importance of environmental contamination in the transfer of MDR bacteria to personal protective equipment (PPE, (gowns and gloves)) of healthcare workers. Here the microbe most frequently recovered was the extremely recalcitrant multidrug resistant variant of *A. baumannii*. Most striking however, were the conclusions that resulted from the modeling of their data. Here a positive environmental culture was found to be the strongest risk factor associated with the contamination of the clothing of the healthcare worker by MDR bacteria (Odds ratio (OR) 4.2; 95 % CI 2.7–6.5) [49]. Other independent variables, such as presence in the patient's room for greater than 5 min (OR 2.0; $p = 0.014$), performing a physical examination (OR 1.7; $p = 0.019$) or contact with a ventilator (OR 1.8; $p = 0.014$) were similarly significant in raising the likelihood or risk of transfer of MDR bacteria but at rates lower than the rate observed for a positive environmental culture [49]. Intuition would suggest transfer was greater when interacting with a patient. However, the higher risk associated with a positive environmental culture serves to reinforce the importance that the microbial burden of the built clinical environment represents to the set of circumstances required for colonization and infection of patients while hospitalized.

4.2.4 Risk to Patient When Prior Room Occupant Colonized or Infected with Epidemiologically Important Organisms

Even with environmental cleaning, studies have suggested that certain organisms can be transmitted to the subsequent occupants in the setting of patient care. Specifically, methicillin resistant *Staphylococcus aureus* (MRSA), vancomycin resistant *enterococci* (VRE), *C. difficile,* and Gram negative pathogens have been implicated. In a study by Martinez, and others in 2003 an epidemiologic link was

made between contaminated surfaces by VRE and subsequent VRE infection [46]. In another study where the environments of patients colonized or infected with VRE were evaluated upwards of 37 % of the environmental samples collected were found to harbor VRE [31]. The samples included patient gowns, medical equipment used for care, as well as environmental surfaces [31]. Controlling the spread of VRE to subsequent room occupants is challenging in that this microbe can be resistant to the disinfectants used for routine and terminal cleaning; even the use of bleach-based products have been reported to fail in their ability to eradicate the microbe from surfaces [22, 24] (See also Chap. 9).

Independent of cleaning, the issue of transference of pathogens from the environment to subsequent occupants can be inferred from studies demonstrating the long-term survival of the microbes on surfaces within the built environment. MRSA and other nosocomial pathogens, including VRE and *C. difficile* can survive for months on dry surfaces (Chap. 2 and ref [42]). MRSA has been documented for its ability to survive within hospital dust for up to a year [89]. Further, frequently touched hospital surfaces, such as doorknobs, have been implicated as reservoirs from which pathogens can be routinely recovered and thus transferred [56]. MRSA, like VRE, is ubiquitous in the hospital environment, especially in the vicinity of patients known to be colonized or infected [22, 24]. The chief method of spread is poor compliance with infection control measures, such as hand hygiene, by healthcare workers. Several studies have described endemic and epidemic contamination of the environment with MRSA. A recent review by Dancer and colleagues found that the site contamination mean for common objects in the patient's room with MRSA was 37 %, with high percentages found for such surfaces as overbed tables (40 %), bed rails (27 %), and other furniture (27 %) [23].

The risk of acquiring MRSA or VRE by a patient being admitted into a room that was previously occupied by a patient known to harbor MRSA or VRE was described by Huang and colleagues [37]. The added risk of acquisition of MRSA to the 10,151 'eligible' patients examined by their study was found to increase by an adjusted odds ratio of 1.4 ($p = 0.04$). Specifically, amongst the patients whose prior room occupant was MRSA positive ($n = 1{,}454$), 3.9 % of this cohort acquired MRSA, while only 2.9 % of the patients who occupied a room previously housing a MRSA negative patient ($n = 8697$) acquired MRSA. A similar risk profile of acquisition of the drug resistant microbe was similarly observed with VRE. Here 4.5 % of patients who occupied a room that previously housed a VRE positive patient ($n = 1{,}291$) developed VRE while the infection rate in patients housed in rooms previously occupied by a VRE negative patient ($n = 9{,}058$) had an attack rate of 2.8 % (adjusted odds ratio of 1.4; $p = 0.02$). The authors concluded that acquisition from previous occupants accounted for 40 % increased odds of transmission of MRSA and VRE strongly suggesting a role for environmental contamination, despite room cleaning methods that exceeded the national standard [37]. A review of the topic of the risk of nosocomial pathogen acquisition from prior room occupants was recently published [58]. Here Otter and colleagues reviewed the increased risk associated with other MDR microbes. Again the trend was the same. Patients who occupied rooms where the former patient was infected or colonized

with *Pseudomonas aeruginosa* or *A. baumannii* [55], or *C. difficile* [81] resulted in a similar increase risk of acquiring the previous occupants pathogen.

A study in 2011 showed that a prior room occupant with a CDI was a significant risk factor for CDI acquisition by the subsequent occupant [81]. This spore-forming anaerobic bacterium can survive for many months on hospital surfaces and is recalcitrant to usual cleaning methods [36]. Studies have shown very high environmental surface contamination rates, particularly in areas within close proximity to the patient. In a trial conducted in France, approximately 25 % of healthcare workers who were caring for patients with a CDI were found to have *C. difficile* spores associated with their hands [43]. The authors concluded that contamination of the hands was positively associated with exposure to fecal soiling and lack of glove use.

Several Gram-negative nosocomial pathogens, such as *P. aeruginosa* and *A. baumannii*, increasingly associated with multi-drug resistance, have similarly been recovered from high touch surfaces such as beds, tables, and infusion pumps [5]. Outbreaks, thought to have occurred because of patient to patient spread of MDR Gram negatives, can be devastating to patients and hospitals, resulting in high numbers of cases and high morbidity and mortality. Responses have included robust and aggressive approaches towards infection control often including enhanced environmental cleaning and in extreme cases closure of the affected unit or substantial areas of the hospital [25, 27, 45]. Fortunately, the majority of the clinically relevant Gram-negative microbes associated with the built clinical environment are not viable after drying. Half-lives routinely encountered are 7 h or less [36].

An emerging nosocomial fungal pathogen, which has become a common cause of central line associated bacteremia in healthcare, is *Candida albicans*. There are fewer studies documenting the extent of environmental contamination with fungi; however, *C. albicans* has been shown to be able to survive anywhere from 3 days to up to 4 months on inanimate surfaces [42]. The majority of *Candida* infections are likely from endogenous sources. However, through molecular typing, evidence of transmission via environmental sources has been suggested; identical strain types were recovered from patients infected with *Candida* and from hospital surfaces from the rooms of the affected patients [88].

There are several classes of pathogenic viruses that can be found on hospital surfaces. Respiratory viruses such as influenza, coronavirus, and rhinovirus can persist on surfaces for a few days [42]. Viable influenza virus can be transferred from surface to skin, leading to the potential transfer to patients [36]. Gastrointestinal tract viruses, such as rotavirus and astrovirus, can persist for around 2 months [42]. Rotavirus is a well-known cause of gastrointestinal illness outbreaks, especially in day care centers where it is spread through contamination of toys [36]. Norovirus has been shown in several studies to be consistently transferred to frequently touched sites in a hospital, such as door handles and telephones [24]. Closure of units and deep environmental cleaning similar in scope, time and expense seen with MDR-Gram negative outbreaks are often needed to control Norovirus outbreaks in hospitals.

4.3 No-Touch Disinfection Technologies

In summary, since the seminal paper by Weinstein in 1991, substantial evidence implicating the environment as a continuous source of risk for the acquisition of HAI has accumulated to such an extent that there now exists significant interest in learning how to manage and provide best-practice applications for infection control for hospitals [8, 12, 13, 15]. Evident from the previous discussion, microbes have an intrinsic ability to survive and ultimately colonize common touch surfaces where acquisition and transport from surfaces to humans is common. Healthcare workers have the potential to transfer these microbiological contaminants not only from patient to patient but amongst themselves and back to surfaces, refreshing or adding to the complexity of the microbial reservoir involved in transmission. There have been many studies looking at the control of contamination of common hospital touch surfaces both from hand to surface contact and vice versa. Investigators have shown that the gloves of nurses frequently collected viable MRSA after touching inanimate objects near colonized patients [16]. In concert with aggressive hand hygiene campaigns recent hygiene guidelines specifically recommend that particular attention be paid to the disinfection of patient-care surfaces, especially surfaces designated "high touch objects" (HTOs) as a target of infection prevention and control [78]. The guidelines note that such objects could potentially contribute to secondary transmission by contaminating hands of healthcare workers (HCWs) or by contacting medical equipment that subsequently contacts patients [8, 29, 64, 67, 72, 73, 90]. Routine or daily cleaning coupled with cleaning immediately after patient discharge (terminal cleaning) of the surfaces and objects within the room with subsequent application of a hospital grade disinfectant has been an accepted method for controlling and limiting the spread of infectious agents [68]. A concentration of between 2.5 and 5 aerobic CFU per square centimeter has been proposed as the benchmark where bacterial levels below this value are considered to represent a minimum of risk while concentrations greater are suggestive of an increased risk of HAI acquisition [22, 44].

No touch solutions for the disinfection of at-risk environments within healthcare settings are quickly gaining acceptance as technologies that have been found to be an effective and comprehensive addition to systems-based solutions for infection control. The technologies have been studied in concert with aggressive hand hygiene campaigns, appropriate routine and terminal cleaning of patient care environments, and an active surveillance and isolation protocol for patients entering care who are already colonized with VRE, MRSA, *C. difficile* or other multi-drug resistant microbes such as *Klebsiella pneumoniae* carbapenemase (KPC). As a consequence of this, one is left to wonder whether or not the antimicrobial effectiveness is providing an additive effect or whether the antimicrobial effectiveness of these 'no-touch technologies' are acting synergistically.

As the name suggests, no-touch technologies do not come in direct contact with colonized, contaminated or soiled surfaces. Rather, they distribute their microbiocidal activity through the atmosphere by either delivering a lethal

concentration of electromagnetic energy in the ultraviolet spectrum or by the real-time distribution of reactive oxygen species, such as hydrogen peroxide, singlet oxygen, hydroxyl radical or oxyanions. In general both systems have been found to effectively reduce the concentration of microbes by at least 4 $\log_{10}$ [34]. Both systems have their limitations (See Chap. 9). Each requires skilled labor to place the equipment and commence the disinfection cycle in the location subjected to disinfection.

The disinfection reach of ultraviolet light is subject to the effects of shadowing and 'cornering'. This typically requires that the equipment be placed in the center of the room to insure uninform distribution of the lethal ultraviolet energy. Additionally, the room must be vacant and any associated ultraviolet energy need be prevented from leaking into areas occupied by people as the ultraviolet (UV) light energy can damage eyesight and result in skin burns. The energy can also shorten the life of equipment in the room as routine exposure to UV light can accelerate decay by increasing the brittleness of many of the plastics used in the fabrication of healthcare associated equipment.

The use of an automated UV-C light emitting system for the inactivation of VRE, *C. difficile* and species of *Acinetobacter* has been found to be effective in debulking the built environment of these pathogens. In one study, employing an automated emitter in two hospitals, the concentrations of bacteria were reduced for all 9 of the environmental sites tested and occurred regardless of whether the sampled location was in direct or indirect line of sight of the UV source [3]. Further, the extent of the reduction to the microbial burden was found to be significant for VRE and C. *difficile* but not *Acinetobacter* spp. [3]. However, the data were sufficiently compelling to lead the authors to conclude that the use of an automated UV-C no-touch disinfection device can lead to a decrease in the bioburden of important nosocomial pathogens in 'real-world' active clinical environments [3].

Another multi-hospital intervention used a pulsed xenon based UV delivery mechanism in concert with screening and hand hygiene education, together, the three were able to significantly reduce (56 %, $p = 0.001$) the incidence of hospital associated MRSA infections in the study population [82]. Given that this was a bundled intervention the contribution of the individual components of the bundle cannot be discerned. However, the data do reinforce the common belief that any effective infection control program requires a systematic approach in order to be effective.

As early as 1990 vapor phase hydrogen peroxide (HPV) has been advocated as an effective surface decontaminant and sterilant [40]. In the intervening years a number of devices have been developed to deploy this disinfectant/sterilant as a vapor into the built clinical environment. In one study conducted by Passaretti and others, an evaluation of the environmental and clinical impact of this no-touch technology was assessed [59]. In a 30 month prospective cohort intervention trial involving 6 high risk units from a 994 bed tertiary care hospital, they learned that patients admitted to rooms decontaminated using HPV were 64 % less likely ($p < 0.001$) to acquire any multidrug resistant microbe and 80 % less likely to acquire VRE ($p < 0.001$) after adjusting for other factors [59]. Again, the complexity inherent to the transmission and distribution of microbes within the built environment, coupled with the stochastic nature of care, well illustrates that the risk of acquiring *C. difficile*, MRSA, and

multidrug-resistant Gram-negative rods were reduced, but failed to reach significance. However, in spite of the failure to reach significance the effectiveness of this no-touch infection control solution was able to significantly alter the proportion of rooms environmentally contaminated with MDRs. Here the concentration of MDRs in the HPV treated units were significantly reduced (relative risk, 0.65, $p = 0.03$), but not on non-HPV treated units leading the authors to conclude that the use of HPV can reduce the risk of acquiring MDRs compared with standard cleaning protocols [59].

In spite of the success demonstrated here and in other studies [14, 21] vapor-phase disinfection of the built environment has limitations in that the ventilation to the room must be controlled/and or limited for the duration of the disinfection cycle. This time can vary depending upon the concentration of peroxide or disinfecting gas used. These two technologies, HPV and UV, have been found to be effective for the disinfection of inanimate objects and surfaces. However, neither technology is intended as a substitute for cleaning or for the removal of soil from the resident objects and surfaces within the built patient care environment (see also Chap. 9). An appropriately trained environmental service team must accomplish cleaning, with subsequent disinfection of the built environment.

4.4 Antimicrobial Copper: A Continuously Active No-Touch Disinfection Solution for Healthcare

Recently, we have begun to witness the incorporation of another 'no-touch' technology. However, unlike UV and vapor phase oxygen radicals (H_2O_2) that distribute their antimicrobial activity through the atmosphere, this technology requires the microbe come in contact or be in close proximity with the material in order to facilitate its antimicrobial activity. In contrast to UV and HVP, once placed, this no-touch system simply requires that the fugitive microbe come in contact with the surface in order to effect disinfection. Thus, the inactivation or killing of the microbe does not require any user intervention once deployed. One such example of this type of no-touch technology is solid antimicrobial copper. The resident microbial burden associated with the built environment is continuously reduced through the strategic placement of solid copper surfaces onto critical high touch surfaces within the patient care setting [75].

Copper has been used by humans for millennia, first as tools and then as a measure to fight the spread of infectious agents. Metallic copper intrinsically displays a strong antibacterial activity in aquatic systems [2, 38] as well as on dry surfaces [32, 54, 96, 102, 104]. In 2008 the United States Environmental Protection Agency (EPA) registered five families of copper-containing alloys as antimicrobial, establishing that products manufactured from one of these registered alloys can make public health claims wherein the label indication states that the alloys kill greater than 99.9 % of bacteria within 2 h of exposure [87]. It is anticipated that the solid antimicrobial copper surfaces will remain microbiocidal for the life of the product (>10 years). A variety of controlled studies have looked at the antimicrobial activity

Table 4.1 Microorganisms sensitive to the antimicrobial properties intrinsic to solid metallic copper

Microbe	Reference(s)	EPA registered
Acinetobacter baumanii	[47]	
Aspergillus flavus	[96]	
Aspergillus fumigatus	[96]	
Aspergillus spp.	[96]	
Campylobacter jejuni	[28]	
Candida albicans	[47, 96]	
Clostridium difficile	[97]	
Clostridium difficile spores	[97]	
Carbapenem-resistant *Enterobacteriaceae (CRE)*	[84]	
Enterobacter aerogenes	[87]	*
E. coli O157:H7	[87, 104]	*
Escherichia coli-NDM1	[93]	
Fusarium culmonium	[96]	
Fusarium oxysporium	[96]	
Fusarium solani	[10]	
Fusarium spp.	[96]	
Influenza A (including H1N1)	[53]	
Klebsiella pneumoniae	[47]	
Klebsiella pneumoniae-NDM-1	[93]	
Legionella pneumonphila	[65, 66]	
Listeria monocytogenes	[105]	
Methicillin resistant *Staphylococcus aureus* (*MRSA*)	[87]	*
Methylobacterium spp.	[76]	
Mycobacterium tuberculosis	[47]	
Norovirus	[94]	
Penicillium chrysogenum	[96]	
Penicillium spp.	[96]	
Pseudomonas aurginosa	[87, 96]	*
Rhinovirus	[11]	
Rotavirus	[11]	
Salmonella enterica	[28]	
Salmonella typhi	[79, 80]	
Spingomonoas spp.	[76]	
Staphylococcus auerus	[87]	*
Serratia marcescens	[11]	
Vancomycin resistant Enterococci (VRE)	[87]	*
Vibrio cholerae	[79, 80]	

*Designates EPA registered

of copper surfaces against specific human pathogens [54, 63, 92, 98, 102, 104, 105]. In fact solid copper surfaces have been found to be microbicidal to well over 30 bacteria, fungi and viruses. Of the microbes listed in Table 4.1, five were evaluated in the studies used to grant the public health registration by the United States EPA. The public health claims granted illustrate the robust nature of the antimicrobial activity. Alloys granted registration contain greater than 60 % metallic copper and

were found to continuously kill greater than 99.9 % of Gram-negative and Gram-positive bacteria within 2 h of exposure even after repeated contamination illustrating how solid copper surfaces will inhibit the buildup of microorganisms between routine cleaning and sanitizing steps.

The public health claims attributed to solid copper have been evaluated to limit the bacterial burden found on commonly touched surfaces and objects in active healthcare environments. In a recent hospital trial bacterial reductions up to one third were recorded using copper alloys in place of plastic or aluminum surfaces on light switches, door knobs and push plates [48]. Casey and others [20] observed a median microbial reduction of between 90 and 100 % ($\log_{10}$ 1.95–2.0) on copper surfaced push plates, faucet handles, and toilet seats while Schmidt and colleagues demonstrated significantly lower bacterial burdens on six HTOs, averaging an 83 % ($\log_{10}$ 1.93) reduction for all of the objects over the course of a 43 month multi-center trial [75].

Current cleaning methods can effectively remove pathogens from surfaces but studies have shown that more than half of the trial surfaces were not adequately terminally cleaned, and became re-contaminated within minutes [4, 17]. The rails of hospital beds, as a consequence of coincident interactions with patients, HCWs, and visitors are one of the most frequently touched items found in the built patient care environment. Schmidt and colleagues found when they quantitatively assessed the bacterial burden present on bed rails that, through the surfacing of the rail with metallic copper, the concentration of bacteria resident on this frequently touched surface was continuously at or below the threshold representing a risk of transfer regardless of whether or not the surface was measured before or after routine cleaning [77].

Further, the environmental monitoring of bed frames has consistently shown that the rails of hospital beds typically exceed a suggested threshold of risk more than any other object in the patient's room [4, 50, 75, 77, 103]. It was evident that bed rails covered with solid copper are able to augment cleaning and thereby continuously support the control of the concentration of associated aerobic bacteria. This observation was consistently maintained in spite of the kinetic nature of care present in the environment of the ICU. Lower risk concentrations, less than 2.5 CFU/cm^2, were associated with over 83 % of the sampled beds [77]. Further, MRSA and VRE were absent from all but 7 of the 3,938 copper objects sampled arguing that the risk mitigation provided by copper surfaces might be greater than the average concentrations reported suggest [77].

Weber and Rutala [99] in their commentary of the evaluation of no-touch copper conducted by Karpanen and colleagues argued that it was impractical or impossible to coat each of the environmental surfaces with copper [39]. However, the data provided by Schmidt and colleagues suggest that the strategic placement of solid copper surfaces in high touch areas is key, and offers a novel strategy to limit the bacterial burden on a continuous basis [75]. Copper-alloyed surfaces offer a continuous way to limit and/or control the environmental burden. Hospital and environmental services need not perform additional steps, follow complex treatment algorithms, obtain "buy-in" from other providers or require additional training or oversight. The other 'no touch' methods presently in wide scale use for room

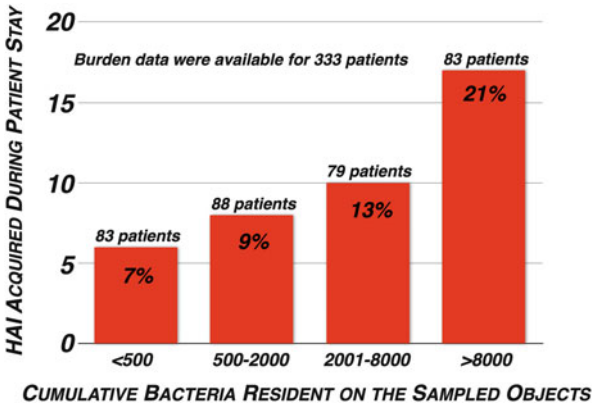


Fig. 4.3 Concentration of bacteria associated with high touch objects associated with the built clinical environment and HAI are linked. A significant association ($p = 0.038$) was observed between the microbial burden and the incidence of HAI acquired during patient stay. Briefly, the burden data from 333 patients were evaluated in the context of an acquisition of a HAI. It was found that 89 % of HAI occurred amongst patients cared for in rooms where the burden observed on six high touch objects exceeded a concentration of 500 CFU. *Percentage values* listed for the individual quartiles are reflective of the percentage quartile population acquiring an HAI (Adapted after the figure of Salgado and colleagues [70])

disinfection rely on discontinuous modalities of application in order to reduce the environmental bacterial burden [34]. Hydrogen peroxide vapor is introduced as a gas into a sealed room. Ultraviolet light achieves its effectiveness through the transient transmission of germicidal radiation within an unoccupied room. Consequently, like the EPA registered disinfectants regularly used to disinfect patient rooms subsequent to cleaning, both UV and HPV will likely suffer from the same limitations of the rapid restoration of the bacterial burden intrinsic to high touch objects.

In addressing the question of whether or not the strategic placement of copper might ameliorate the rate with which HAI are acquired, Salgado and colleagues [70] found from the conduct of a multi-center trial that the limited placement of copper as described by Schmidt and colleagues [75] resulted in a significant reduction to the HAI rate and/or MRSA or VRE colonization rate in medical intensive care rooms (ICU). The collective rate for HAI infection or MRSA/VRE colonization was found to be significantly lower by 42 % (7.1 %) in the copper arm of the study when compared against the (12.3 %) rate observed in the control rooms ($p = 0.02$). When the data were considered separately for HAI alone, the rate of infection was significantly reduced (58 %) from 8.1 to 3.4 % ($p = 0.013$).

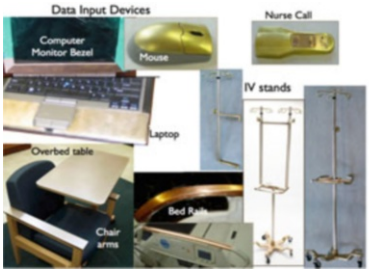
More importantly, these investigators were able to demonstrate that burden and infection were directly linked. In the analysis of the quartile distribution of HAIs stratified by microbial burden measured in the ICU rooms during the patient's stay they learned that there was a significant association between burden and HAI risk ($p = 0.038$), with 89 % of HAI occurring among patients cared for in a room with a burden of more than 500 CFU (Fig. 4.3) [70].

4.5 Postulated Mechanism of Action of Solid Metallic Cooper

The mechanism of action associated with the antimicrobial properties of solid copper surfaces is multifaceted (Chap. 6). Upon coming in contact with the metallic copper surfaces of objects, the electron potential of the microbe in concert with copper facilitates a cascade of irreversible events leading to the rapid death of the bacterium. Given the inherent ability of solid metallic copper and its alloys containing greater than 60 % copper for the conduction of electricity, the electrons resident in the membrane of the bacterium that are sufficiently close to the metallic surface coupled with the high flux required by living cells result in the rapid collapse of the proton motive force of the microbe. The subsequent dissipation of the proton motive force (PMF) has been observed through the use of dyes that measure the membrane potential. Warnes and others have reported on this observation on numerous occasions for both Gram positive and Gram negative bacteria [91–93, 95]. Subsequent to the collapse of the membrane potential a concomitant production of free radicals immediately develops within the cytoplasm of the bacterium. The free radicals facilitate the peroxidation of the membrane, bleaching of cellular proteins and the cleavage and subsequent complete destruction of the nucleic acids resident in the cytoplasm of the effected microbes. Additionally upon peroxidation of the membrane there is a loss of membrane integrity resulting in the subsequent leakage of the cytoplasm from the cell and diffusion mediated transport of copper ions into the cytoplasm. The copper ions then act in concert with the free radicals resulting in a Fenton reaction that leads to further irreparable damage to the cell [91]. The entire process occurs quickly resulting in the collapse of a population within minutes. Thus, the likelihood that the population will develop resistance to this multifaceted mechanism of death is unlikely. There have been reports in the literature of bacteria being isolated from copper coins but upon challenging the 'resistant' isolates they were found to be uniformly sensitive to metallic copper [71]. A likely explanation for their recovery from the surface is likely a consequence of a failure to sufficiently collapse the PMF of the entire community as either a function of proximity of the surviving microbes to the metallic surface or the absence of sufficient electron flux through the membrane to initiate the cascade required for death.

4.6 Use of Copper Surfaces in Healthcare

In the study conducted by Salgado and colleagues, six highly touched objects within the ICUs were selected based from a limited survey where the contact surfaces being the most highly contaminated were identified. The six items were then fabricated from a variety of antimicrobial copper alloys, where the criteria for alloy selection were reflective of the ability of the antimicrobial alloys to be readily



	Copper Alloy	Component part	Description	% Copper Content
IV stands	C710 and C706 C693 C87610 C706 C706	Pole(s) IV hanger loops Base Handle Brackets	Copper nickel Brass Silicon bronze Copper nickel Copper nickel	80% / 90% 75% 90% 90% 90%
Patient bed—Side rails	C110	Top of rails	Copper	99.99%
Over-bed table	C706 C110 C464	Table top Table bottom Release lever	Copper nickel Copper Naval brass	90% 99.9% 60%
Visitors' chair (arms)	C706		Copper nickel	90%
Nurse call button	C638 C260	Buttons Clamshell	Aluminum bronze Cartridge brass	80% 80%
Computer data input devices (keyboard laptop, mouse and touch-screen monitor)	C260 C524 C710	Computer mouse Monitor bezel Laptop palm rest	Cartridge brass Phosphor Bronze Copper nickel	70% 90% 80%

Fig. 4.4 EPA registered antimicrobial cooper alloys used in the fabrication or surfacing of high touch items. Items were fabricated from a variety of EPA registered antimicrobial copper alloys as listed. The criterion used to select an alloy was reflective of the ability of the antimicrobial alloy to be readily fabricated into that particular component and withstand the rigors of healthcare

fabricated into that particular component (Fig. 4.4). Properties of strength and durability were operationally defined such that the resulting component would be able to withstand the rigors placed on the finished goods within the built environment of an active clinical setting and for the ability of the materials to withstand standard hospital cleaners, including sodium hypochlorite. Additionally, the surface finish was to provide consistent wear and aesthetics over the lifespan of the product. All of the copper alloys used for component fabrication were made from solid alloys registered with the EPA [87]. Subsequent to the published report, manufacturers have introduced numerous products fabricated from EPA registered solid copper that meet or exceed the criteria used by the referenced authors [70, 74, 75].

From a design standpoint, it is important to note that these results were 'additive' to other infection-control implementations already in place. Single patient rooms, hand-washing sinks, hand sanitizing alcohol dispensers, contact precautions required of MRSA and VRE carriers/infected patient(s), and an active hand hygiene staff education program were already in place in the units of the hospitals studied. Should these conclusions expand to other areas of the hospital, then employing inherently antimicrobial surfaces could represent a significant enhancement to mitigating infectious bacteria within hospitals. For example, by instituting a 'best practices' approach that implemented cleaning and hand hygiene designs and protocols, the California's Healthcare-Associated Infection Prevention Initiative showed a reduction of HAI by 3.2 %. With many of these best practices already in place, the initial findings from the clinical trials are showing an additional double-digit reduction in infections.

Although the relative infection rate in the medical ICUs where the clinical effectiveness of antimicrobial copper surfaces were evaluated is generally higher than hospitals at large, patients in ICUs are typically not mobile, and their interaction with the built environment is very limited. Consequently, items where antimicrobial copper alloys might have been easily incorporated, e.g. grab bars, sinks, faucets, paper dispensers, shelves and towel racks were not present. The further evaluation of antimicrobial copper surfaces is warranted beyond the medical ICU to include, but not be limited to, the effect of inherently antimicrobial materials in

general wards where patients have greater interaction with other objects in the built environment. Similarly, investigations should also be conducted in emergency and recovery rooms, in hospital rehabilitation units, pediatric and neonatal units, dialysis centers, burn units, transplant units and cancer centers with immune-compromised patients. At issue is the central theme that antimicrobial copper surfaces continuously and passively limit the concentration of bacteria within the built environment. Salgado and colleagues were able to demonstrate that infections were correlated with burden. Thus, other healthcare environments that may arguably receive less day-to-day hygienic oversight than hospital patient rooms, such as visiting area, long-term care facilities, long-term rehab centers, outpatient clinics and elder care facilities should also be investigated as they too may directly benefit from the antimicrobial activity of copper.

4.7 Summary

The study of pathogen transmission in the hospital and the impact of colonization and infection with nosocomial organisms have established the epidemiologic importance of the environmental microbial burden associated with the built clinical environment. These studies have outlined the complexity of this concept and have led to robust recommendations for infection prevention that have undoubtedly prevented undo morbidity and mortality. However, with renewed interest and study the risk contribution provided by the built environment towards patient care warrants a better understanding of the dynamics of colonization and infection. Through our discussion here we hope that we have been able to identify potential avenues for improvement with adjunctive use of newer technologies. These include the use of UV light and HPV disinfection, and the potential value of the use of solid antimicrobial copper surfaces.

Through a multifaceted and continuously active mechanism of action, solid copper surfaces placed in key locations within the patient room can significantly reduce the overall microbial burden; have demonstrated their ability to continuously maintain this concentration at a level representing a minimal risk for HAI acquisition and most importantly, have translated meaningful benefit to patients by their association with significant reduction of HAI. Further study to identify the optimal amount of copper surfaces needed as well as the optimal placement in rooms and areas within healthcare facilities is necessary to fully understand the potential impact.

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Chapter 5
Biocidal Hard and Soft Surfaces Containing Copper Oxide Particles for the Reduction of Healthcare-Acquired Pathogens

Gadi Borkow

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Abstract Potentially overlooked and neglected sources of healthcare-acquired pathogens are non-intrusive soft and hard surfaces located in clinical settings. Microbes can survive on bedding, uniforms, trays, bed rails and other such surfaces for days to months. Furthermore, on some of these surfaces, such as patient bedding, the microorganisms proliferate as textiles are an excellent substrate for bacterial and fungal growth. Additionally the temperature and humidity conditions present between the patients and these textiles are appropriate for microorganism multiplication. Bed making in hospitals can release large quantities of microorganisms into the air, which contaminate the surroundings. Thus soft and hard surfaces that are in direct or indirect contact with the patients can serve as a source of healthcare-acquired pathogens.

Copper oxide impregnated materials have potent intrinsic biocidal properties. This manuscript reviews the laboratory and clinical studies that demonstrate that soft and hard surfaces containing copper oxide particles reduce bioburden and healthcare-acquired infection rates.

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List of Abbreviations

AATCC	American Association of Textile Chemists and Colorists
cfu	Colony forming units
EPA	USA Environmental Protection Agency
GLP	Good laboratory practices
HAI	Healthcare-acquired infections
HD	Hospitalization days
MRSA	Methicillin-resistant *Staphylococcus aureus*
SEM	Scanning electronic microscope
TM	Test Method
VRE	Vancomycin-resistant enterococcus

5.1 Hospital Textiles as a Source of Healthcare-Acquired Pathogens

Textile products are widely used in the hospital environment. They range from simple cleaning wipes to advanced barrier fabrics used in operating rooms. Some of the products are in direct contact with the patients, such as blankets, sheets, pyjamas, towels, gowns, and pillowcases. Others are used by the healthcare personnel, such as uniforms, surgical gowns, face masks, and head and shoe covers. Some products are present in the patient wards, such as drapes, table covers and privacy curtains.

Textiles in general are an excellent substrate for microbial proliferation when in contact with the human body. The very large surface area, the capacity to retain oxygen, and the moisture and temperature conditions present between the skin and the textiles provide ideal environment for microbial proliferation. Humans shed bacteria directly from their skin, nasal cavities, genitalia area, and sweat onto the textiles they use [1]. Bacterial shedding is greater in patients than in healthy individuals [2, 3]. In addition, hospital textiles come in contact with spillages and body exudates, such as blood, stool, urine, nasopharyngeal secretions and vomit, all of which can contain large amounts of bacteria and serve as a bacterial nutrient source. Heavy microbial colonization of sheets, patient pajamas, healthcare worker uniforms, and privacy curtains, including by antibiotic resistant bacteria, has been reported [4–29]. Contamination of clean laundry occurs shortly after use [14]. Without washing, bacteria, fungi and viruses can remain viable on textiles, under ambient temperature and humidity, for very prolonged periods of time; even months [19, 29–36] (See also Chap. 2). The higher the bacterial titer spiked onto the fabrics, the longer the bacteria can survive [31]. Unfortunately, some microorganisms remain viable even after industrial laundry [7, 37–39], and contaminated laundry can lead to cross-contamination of clean laundry [40].

While proliferating on the textiles, some microorganisms secrete unpleasant volatile molecules creating foul odors [41]. But, more importantly, some

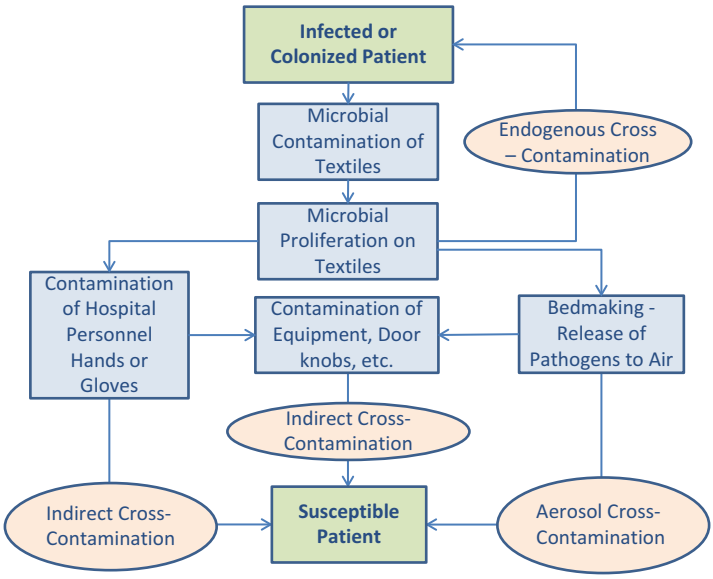

Fig. 5.1 Potential transmission routes of pathogens from a colonized or infected patient to a susceptible patient via hospital textiles

microorganisms that multiply or remain on the textiles can be a source of healthcare-acquired pathogens [7, 42] (Fig. 5.1). These pathogens can be transmitted from one part of the host's body to another [43]. They can also be the source of direct or indirect infection of patients and hospital personnel, as discussed below.

Contamination and healthcare-acquired infections (HAI) of patients and hospital personnel via contaminated towels, gowns, sheets, cleaning wipes and other hospital textiles with Methicillin-resistant *Staphylococcus aureus* (MRSA) [11, 24, 28, 44–47], Vancomycin-resistant *enterococcus* (VRE) [19, 24, 48], Carbapenem-resistant *Acinetobacter* [20], multidrug-resistant *Acinetobacter baumannii* [25, 28, 49], multidrug-resistant *Pseudomonas aeruginosa* [24, 50], *Bacillus cereus* [51–55], *Cryptosporidium* [56], *Microsporum canis* [57], *Norwalk gastroenteritis* [58], *Klebsiella pneumonia* [28], *Rhizopus* [59], *Salmonella gastroenteritis* [60], *Salmonella typhimurium* [61], *Sarcoptes scabiei* [62], or *Streptococcus pyogenes* [37, 63] have previously been reported (Table 5.1). Viruses can also survive on textiles for days and thus be a source of contamination ([33, 64–67] and Chap. 2).

When handling contaminated textiles hospital, personnel can contaminate their gloves with micro-organisms and then contaminate other surfaces, such as door knobs, and even patients directly [3, 11, 20–22, 24, 25, 49, 68, 69]. Furthermore, studies have shown that when the personnel change the bed linens or patients garments, large quantities of micro-organisms are released into the air, which then contaminate the immediate and non-immediate surroundings in the same room as well as throughout the building via the air conditioning system [3, 46, 70–72]. Healthcare workers who touch the aerosol contaminated surfaces can then

Table 5.1 Reports on textiles as a possible source of healthcare-acquired infections (HAI)

Bacteria	Textile	HAI of	Reference
Bacillus cereus	Linens, towels, sheets	Patients	[51–55]
Cryptosporidium	Uniform	Personnel relative	[56]
Hepatitis A virus	Laundry	Personnel	[66]
M. canis	laundry	Personnel	[57]
MRSA	Linens, gowns, sheets	Patients	[11, 45, 46]
N. gastroenteritis	Uniforms	Personnel	[58]
P. aeruginosa	Patients' clothes, linens	Patients	[50]
Rhizopus (fungi)	Linens, pillowcases, coats	Patients	[59]
S. gastroenteritis	Linen	Personnel	[60]
S. typhimurium	Sheets	Personnel	[61]
S. scabiei	Linens	Personnel	[62]
S. pyogenes	Babies' vests, vinyl sheet	Patients	[37, 63]
VRE	Drawsheet, seat cushions	Patients	[19, 48]

contaminate patients [3, 73]. Airborne transmission of pathogens such as *Mycobacterium tuberculosis* and *Aspergillus niger* is well documented [3]. It has also been implicated in healthcare-acquired outbreaks of *A. baumannii*, *P. aeruginosa*, *Scedosporium prolificans*, *S. aureus*, MRSA, and other *Staphylococci spp* [74–82].

5.2 Biocidal Textiles as a Tool to Fight Healthcare-Acquired Infections

Based on the above, it has been hypothesized that endowing the hospital textiles, especially those that come in contact with the patients, such as patient's sheets, pillowcases, robes, and pyjamas, with biocidal properties, would help reduce HAI by reducing an important source of microbes contributing to endogenous, indirect-contact, and aerosol transmission of healthcare-acquired pathogens [42]. Nicas and Sun [83] by using an integrated mathematical model of the infection risk in a health-care environment, also concluded that biocidal textiles have the potential to substantially reduce HAI.

Biocidal textiles should have potent broad spectrum antimicrobial, antifungal and antiviral efficacies. They should be highly effective against antibiotic resistant micro-organisms, especially those already circulating in the hospital environment causing HAI outbreaks, such as MRSA, VRE, and Carbapenem-resistant *K. pneumoniae* (CRKP). Additionally they should not enable the development of resistant micro-organisms to the biocidal compound. They should not be affected by washings and continue to be efficacious for the life of the product. Obviously, they should be safe to humans following continuous dermal exposure [42] (Table 5.2).

Table 5.2 Key properties that biocidal textiles should have

Wide spectrum antimicrobial, antifungal and antiviral properties
Effective against the already existent antibiotic resistant micro-organisms involved in healthcare-acquired infections
Not allow development of resistance to the active component in the textiles by micro-organisms;
Withstand multiple industrial washings without losing biocidal potency;
Not cause skin irritation or sensitization;
Be safe to humans

In the last 20 years, the development of biocidal textiles in general and specifically for the use in the hospital environment has gained momentum, and different biocidal compounds are being explored for this purpose [84–87]. Biocides can by chemically or physically attached to the natural or synthetic fibres from which the textile products are made or to the surface of the finished textile products. Surface applications usually have a lower persistence over time, as the active ingredient is lost due to friction and washing. Other biocides are introduced earlier during the production stage by impregnating them in the polymeric fibres used in the textile industry. Some of the biocidal active ingredients being studied are Cliniweave®, organofunctional silane, citric acid, copper, silver, zinc, triclosan, quaternary ammonium compounds, chitosan and zeolite. Some of the above active ingredients have been found not to be appropriate for use in hospital related applications (e.g. [88, 89]).

Only a few clinical trials have been performed to determine the efficacy of biocidal textiles in clinical settings. Most of the studies examined the capacity of the biocidal textiles to reduce microbial contamination [4, 90–94]. These studies, which included personnel uniforms, patient linens, scrubs, blankets, privacy curtains, cloths and mops, found statistically significant lower bioburden levels than those found in the matched non-biocidal controls. One study, performed with only 10 workers that used silver containing jackets and pants, did not find any significant difference in the extent of microbial contamination between the silver containing textiles and control textiles [95]. It may be that a larger sample size was required to prove the silver containing fabric's efficacy. Also, a randomized controlled study that compared the bacterial contamination of uniforms of healthcare workers when using a regular textile and two textile containing antimicrobial finishes, did not find any decrease in the bioburden levels in the antimicrobial textiles [96]. Unfortunately, the identity and nature of the antimicrobial components in the scrubs tested is not clear. Interestingly, in a recent study in which copper-coated films (21×39.7 cm) were attached to bed sheets used by a heavily MRSA-colonized patient found 20–130 MRSA colony forming units (cfu) in these films as opposed to 6,600–11,000 cfu on the surface of the non-film-coated control sheet areas [97].

The capacity of antimicrobial cleaning cloths to reduce bioburden and HAI was demonstrated. For example, a recent study examined the capacity of copper treated cleaning cloths in neutralizing the bacterial virus MS2. This virus serves as a non-pathogenic surrogate virus to clinically relevant viruses such as hepatitis A, enteroviruses, poliovirus or novovirus, due to its structure and environmental stability. Ninety percent of the absorbed virus in the cloths were killed, reducing

significantly potential cross contamination during cleaning [98]. More dramatically, by introducing peracetic acid sporicidal wipes, the *Clostridium difficile* infections rates in an acute London trust were reduced by 72 % during the monitored 18 months as compared to the previous period [99].

In contrary to lab conditions, during *in vivo* use, continual re-inoculation with pathogens occurs. Since the killing of the microorganisms is not instant, the expectation is not to obtain a sterile fabric, but a fabric that prevents microbial proliferation and reduces the bioburden levels significantly. The concept that such textiles can reduce HAI, to the best of my knowledge, has been demonstrated in only one clinical trial (to be discussed below) and obviously more trials are needed to clearly establish the capacity of biocidal textiles to help in the fight against HAI.

Furthermore, as HAI are spreading into the community (e.g. [100]), the use of biocidal textiles and biocidal hard surfaces may not only significantly contribute to the reduction of HAI, but may also confer protection in other environments where at-risk individuals run the risk of contracting infections such as in long term care facilities.

5.3 Biocidal Textiles Containing Copper Oxide

Copper is one of the several materials that are being explored as a potent wide spectrum biocide to be used in hard and soft surfaces in clinical settings for the reduction of HAI. The biocidal mechanisms of copper are discussed in Chap. 6 of this Book. Different copper compounds that were applied to different textile fibres or polymers via different techniques, demonstrated potent *in vitro* biocidal efficacy including against antibiotic resistant bacteria [4, 101–116]. Most of these studies were conducted in the academia and the only technology that has generated textile products widely used commercially is the technology based on the impregnation of copper oxide particles into products [4, 108–110, 117–123].

Copper oxide has been chosen as the active copper form to be introduced into textiles due to two main reasons: it is a non-soluble form of copper and it is highly reactive with potent wide spectrum biocidal properties [124]. As can be seen in Fig. 5.2, the copper oxide particles are an integral part of the polymeric fibers, as they are homogenously distributed throughout the polymeric matrix. This is very important for biocidal textiles as even when some of the external polymeric fiber material disintegrates due to friction, repeated use and laundry resulting in loss of the surface copper oxide particles, there are always “new” copper oxide particles that “reach” the surface of the fiber, endowing the fiber with biocidal properties for the life of the fiber.

The biocidal efficacy is not affected by repeated use, home or industrial washings [109] (Fig. 5.3). This is in contrast to coating technologies in which the active material is only bound to the external layer of the fiber. Once this externally bound active material is removed from the surface of the fiber due to friction or laundry, the fiber losses its bioactive characteristics.

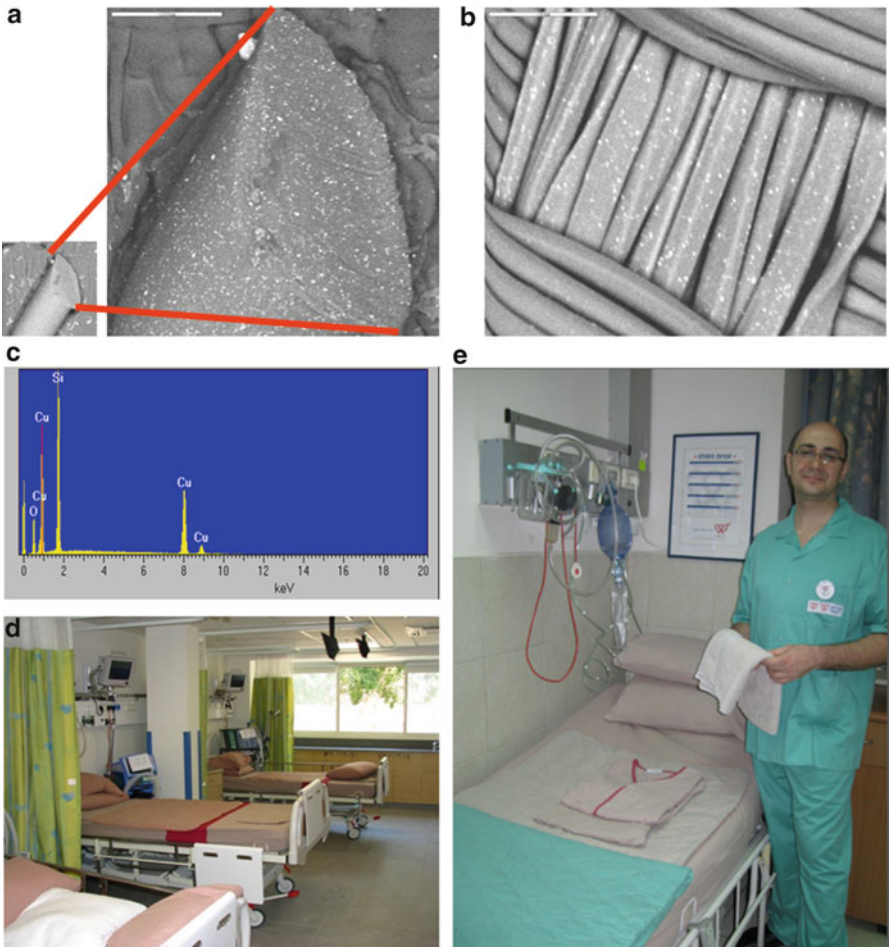

Fig. 5.2 Copper oxide impregnated textiles. (**a**) Scanning electronic microscope (SEM) picture of a cross section of a synthetic fiber impregnated with copper oxide particles (*white dots*). (**b**) SEM picture of a woven fabric in which the copper oxide impregnated polyester yarn is found only in the weft of the textile. (**c**) An X-ray photoelectron spectrum analysis of a *white dot* shown in (b), demonstrating that it is copper. (**d** and **e**) Pictures showing hospital textiles containing copper oxide in use in clinical settings (e.g. the beige and *brown linens* and the *green* uniform and blanket)

The biocidal potency of a hospital linen containing 1 % copper oxide particles (w/w) was tested by an independent laboratory (AminoLabs, Rehovot, Israel) by using the American Association of Textile Chemists and Colorists (AATCC) test method (TM) 100. The linen was washed following the AATCC TM 150. *Candida albicans* and *Trichophyton mentagrophytes* were exposed to the fabric for 24 h. *Staphylococcus aureus* and *Escherichia coli* were exposed to the fabric for 4 h. The results shown are the mean of duplicate samples. The titers of each microorganism before being exposed to the fabric samples are shown by the arrow.

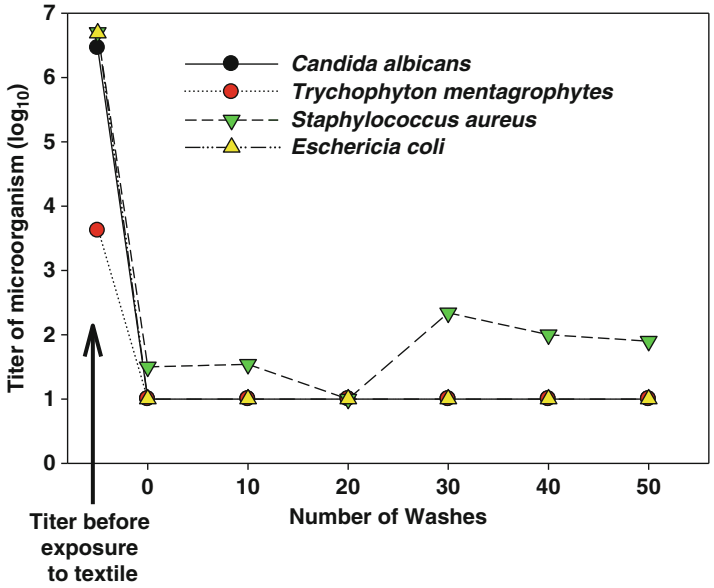

Fig. 5.3 No loss of biocidal efficacy with laundry

The copper oxide impregnated products possess broad-spectrum antimicrobial properties, including against antibiotic resistant bacteria [4, 108–110, 117, 119, 125, 126]. These products include biocidal fabrics [4, 108, 109, 117], anti-fungal socks [108, 118, 121, 123, 127], anti-viral masks and filters [119, 125, 126, 128], anti-dust mite mattress-covers [108, 129], and non-porous biocidal countertops (see next section).

A preliminary pilot study with 30 patients, who slept overnight on regular sheets and then overnight on sheets containing copper-oxide, demonstrated a statistically significant lower (~50 %) bacterial colonization on the copper-oxide containing sheets than on regular-sheets [4]. Similar statistically significant lower titers of gram positive and gram negative bacteria were recovered from copper oxide containing sheets than regular sheets ($n = 40$), immediately after 7 h of patient's use [130].

Importantly, in a clinical trial in the Reuth Medical Center (Tel Aviv, Israel), in which the regular non-biocidal linens in a chronic care head injury ward were replaced with the biocidal copper oxide impregnated linens, the rates of HAI per 1,000 hospitalization days (HD) were reduced by 24 % ($P < 0.05$). Accordingly there was a 32.8 % reduction in total number of days of antibiotics administration per 1,000 HD ($P < 0.0001$) and there was a 47 % reduction in the number of fever days (>38.5 °C) per 1,000 HD ($P < 0.01$) [130]. The study was conducted in a chronic care head injury ward as most of the patients hospitalized in this ward are high risk patients typically immunocompromised. Unfortunately the most common medical complication which afflicts them is a HAI [131–133]. Based on

the successful result of this trial, the Reuth Medical Center has now changed all their linens in all wards to the copper oxide impregnated linens.

There is no reason to believe that reducing bioburden in other wards or clinical settings by using biocidal linens would not reduce bioburden and HAI. The use of biocidal textiles should be a complementary approach to fight HAI in medical institutions as well as long term care facilities, where the risks of acquiring an infection are high. However, additional studies with other patient populations and different wards should further test this notion. Currently, a study is being conducted at Sentara Norfolk General Hospital (Norfolk, Va.), where a critical care unit that shares the same nursing staff will test the biocidal fabrics in one side of the unit for 6 months before switching them to the other unit for another 6 months.

5.4 Non-porous Solid Biocidal Surfaces Containing Copper Oxide

Elemental copper and copper alloys have been registered by the USA Environmental Protection Agency (EPA) as antimicrobial substances with approval to make public health claims that the copper-oxide impregnated surfaces kill greater than 99.9 % of gram negative and gram positive bacteria within 2 h of exposure. The approvals were obtained after demonstrating in independent laboratories potent biocidal efficacy following Good Laboratory Practices (GLP) testing. The significant contribution of metallic copper surfaces to the reduction of bioburden in clinical settings [134–140] and to reduction of HAI [141] has recently been demonstrated and is discussed in Chap. 4.

Recently, similarly to the elemental copper and copper alloys, also non-porous hard surfaces containing copper oxide particles (Fig. 5.4) have been registered by the EPA as antimicrobial surfaces and allowed to make public health claims (EPA Registration number 84542–7). The approval is based on GLP testing demonstrating the ability to kill specific disease-causing bacteria: MRSA, *Staphylococcus aureus, Enterobacter aerogenes, P. aeruginosa and Escherichia coli* (O157:H7). The product is approved for use in a wide range of applications, including health care. The samples were tested in various environmental conditions, cleaning protocols, and for efficacy after repeated exposure. The non-porous copper infused surfaces also underwent extensive American Society for Testing and Materials (ASTM) standard testing to support mechanical performance claims, allowing making efficacy claims that the copper-oxide impregnated surfaces kill greater than 99.9 % of gram negative and gram positive bacteria within 2 h of exposure between routine cleaning and sanitizing steps and even after repeated exposure [142].

Currently seven hospitals have already installed the non-porous copper infused surfaces – six in the USA and one in Israel, with the aim of further reducing the rates of HAI. The Sentara Leigh Hospital, which is one of the 11 acute care Sentara

Fig. 5.4 Copper oxide impregnated non-porous surfaces. (**a**) A nurse top station in which the non-porous countertops are impregnated with copper oxide particles. (**b**) A sink made with non-porous copper oxide impregnated material. (**c**) SEM picture of the non-porous material shown in (b), demonstrating the homogenous distribution of the copper oxide particles on the material surface. (**d**) An X-ray photoelectron spectrum analysis of a *white dot* particle shown in (c), demonstrating the presence of copper

Hospitals in Virginia, has outfitted the copper-infused countertops, bed rails, and over the bed tables in their new 129 bed-tower in all patient care areas, including nursing units, visitor lounges, and patient rooms. In early 2014, the hospital also introduced copper-infused hospital gowns, pillowcases, and towels in the new tower. They will compare the rates of HAI, such as urinary catheter-associated and central line blood stream infections, with the infection rates in a similar existing 129-bed tower, in which no copper containing products will be utilized.

In conclusion, the introduction of soft and hard surfaces containing biocidal copper oxide particles in clinical settings may be an important adjunct for the reduction of bioburden and HAI. Furthermore, as HAI are now spreading out from the hospital environment into the community, the use of biocidal textiles, such as those impregnated with copper oxide, and hard surfaces containing a high percentage of copper, may not only significantly contribute to the reduction of HAI, but may also confer protection when used in homes for the elderly and in other environments where immune compromised individuals are at high risk of contracting infections.

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Chapter 6
Biocidal Mechanisms of Metallic Copper Surfaces

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Abstract Hospital acquired infections (HAI), also known as nosocomial infections, have a vast impact on patient and staff health and affect survival chances of patients with compromised immune system, elderly, and young children. Moreover, hospital environments are favoring the development of drug-resistant strains of bacteria, making treatment of such HAI more challenging. The Center of Disease Control estimates that one of the deadliest types of antibiotic-resistant bacteria, MRSA (methicillin-resistant *Staphylococcus aureus*), causes 19,000 death cases per year, whereas another superbug, *Clostridium difficile*, causes 500,000 incidents per year.

The natural medicinal and sanitizing properties of copper and its minerals were used throughout the ages by many civilizations. However, only recently have we started understanding the mechanisms of such bactericidal effects of copper. One of the latest research developments in this area is concerned with showing that metallic copper surfaces strongly reduce microbial surface-burden, both in laboratory settings and healthcare environments. Microbiologists and hygiene specialists are increasingly recognizing this unique antimicrobial property of metallic copper as a very promising novel tool for reducing HAI, which are known to spread through touching contaminated surfaces. Copper surfaces have universal microbe-inactivating properties against a wide variety of Gram-positive and Gram-negative microbes under moist (droplets of cell suspensions, mimicking splash-contamination) or dry (direct contact between cells and surfaces, mimicking touch surfaces) conditions.

This chapter reviews the molecular mechanisms underlying bactericidal properties of solid copper surfaces and factors that influence such processes: copper surface oxidation and corrosion, copper cell accumulation, copper alloy content and roughness, temperature, moisture, presence of chelators, osmotic stress, reactive oxygen species, cellular characteristics, cell wall structure, spores, genetic traits for copper resistance systems, anaerobiosis, viable but not culturable state (VBNC). Additionally, primary targets for metallic copper toxicity, DNA and lipids, are also included in discussion in this chapter.

Our understanding of the antimicrobial properties of metallic copper surfaces have made great strides in the last 5 years both under laboratories and healthcare conditions, highlighting safe, economical and sustainable application of metallic copper surfaces in hospital or any public settings for prevention of HAI.

Keywords Metallic copper surface • Antimicrobial • Biocidal • Toxicity • Killing mechanism • Membrane damage • Genotoxicity

List of Abbreviations

BCS	Bathocuproine disulfonate
BTA	Benzotriazole
C=C–C$^{\bullet}$	Allylic radicals
CFU	Colony forming units

ComC	Copper-induced outer membrane component
ComR	Copper-induced repressor
CopA	Copper exporter P-type ATPase
CopB	Cytoplasmic copper and delivers it to the P_{1B}-type ATPase
CopY	Copper-responsive repressor
CopZ	Cytoplasmic copper binding chaperone
CueP	Periplasmic copper binding protein
CueR	Copper response cytoplasmic MerR-family activator/repressor
CusCFBA	Copper/Silver transporting efflux system
CusRS	Periplasmic copper two-component system sensor
CycA	D-cycloserine uptake permease
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
FabR	Repressor for unsaturated fatty acids biosynthesis
FAME	Fatty acid methyl esters
GSH	Glutathione
GSSG	Glutathione disulfide
HAI	Healthcare-acquired infections
ICP-MS	Inductively coupled plasma mass spectrometry
L	Lipid
$L^{\bullet}$	Lipid radical
$LO^{\bullet}$	Lipid alkoyl radicals
$LOO^{\bullet}$	Peroxyl radical
MDA	Malondialdehyde
MerR	Mercury resistance repressor
Pco	Plasmid-borne copper resistance
PMF	Proton motive force
ROS	Reactive oxygen species
TBARS	Thiobarbituric acid-reactive substances
TetR	Tetracycline repressor protein
Tris	Tris(hydroxymethyl)aminomethane
VBNC	Viable-But-Not-Culturable

6.1 The Biocidal History of Copper

The word "copper" comes from the Late Latin word *cuprum*, which in turn originates from the Latin word *cyprium aes* (Cyprus metal) – name of the Mediterranean island of Cyprus, known to have one of the largest copper mines. Nevertheless, copper mining activity is more ancient than the origin of the name: copper was one of the first metals used by human civilizations, probably because it was easily extracted, available in great quantities and very malleable [74]. Copper belongs to the seven Metals of Antiquity: Gold, Copper, Silver, Lead, Tin, Iron (smelted), and Mercury. The discovery and usage of each of these metals, along with the respective alloys, promoting the development of more

sophisticated instruments allowing civilizations to evolve from the Stone Age [74]. Whereas gold was mainly used for luxury purposes, such as jewelry, use of copper was of greater practical significance. Indeed, the first metal tools, implements and weapons were made from this metal. The rise of Eastern civilizations (Egypt and Middle Eastern) flourished at the same time as they expanded the knowledge on copper extraction and annealing [74]. During this times it was noted that hammering and grounding of extracted copper pieces resulted in much harder metal that can be used in production of many tools, an event that marked the start of the Copper Age. Such treated copper was used for fabrication of many utensils, including weapons, however their strength was not yet sufficient against harder materials, such as bones [74]. This issue was solved by mixing two metals, copper and tin, to produce a copper alloy which was stronger than either of each individual metal. This alloy improved already existing tools and it enabled the creation of new utensils, moving civilizations to the new era of the Bronze Age [74]. Besides playing such distinguishing role in tools production, copper was also known to prevent and treat infectious diseases, and disinfect fluids and solids [19, 91]. The Smith papyrus, an Egyptian medical document, (circa 2400 B.C.) states that copper was used to sanitize drinking water and wounds. Copper oxide and malachite, a copper carbonate mineral, was used in Mesoamerica by the Aztecs to treat skin conditions. In ancient Greece, Hippocrates (400 B.C.), the "father" of medicine, prescribed copper for pulmonary diseases and to disinfect drinking water [19]. The Roman Empire used copper piping to improve public hygiene. Great traders, like the early Phoenicians, in order to clean ship hulls for faster travel, fixed copper strips on the ship bodies to inhibit biofouling. Furthermore, different cultures throughout many continents dropped copper coins in water vessels to prevent diseases like dysentery [19]. Until the nineteenth century, all these civilizations were using copper without knowledge of the existence of microorganisms. Only when Antonie van Leeuwenhoek observed microscopic shapes in his newly invented microscope, and Louis Pasteur in his Germ Theory of Disease emphasized the notion that microscopic germs may lead to disease, copper usage gained a more specific meaning: copper as a biocide. At the same time it was noted that copper workers were not affected during a raging cholera epidemic in Paris. The employment of the metal and its salts in the subsequent century became widespread in medicine: a variety of copper compounds were used to treat diseases such as eczema, tubercular infections, and "The Great Pox", syphilis. Nonetheless, with the discovery of antibiotics pharmaceutical companies started to commercialize these new drugs heavily forcing them into becoming the prevailing form of infection treatment for humans and animals. Thus, the exploitation of copper as an antimicrobial material was all but forgotten [17]. Nowadays, human healthcare is confronted with the widespread occurrence of antibiotic resistant bacteria, and it is, therefore, of great importance to revisit old methods, including the use of copper, to improve and develop alternative therapeutic ways to treat and prevent diseases.

6.2 Copper the "Modern" Bioelement

6.2.1 General Chemistry Properties

Along with the transition metals silver and gold, copper belongs to the group 11 of the periodic table and is referred to as a coinage metal due to the characteristic color, corrosion resistance and value. The two stable isotopic forms present in the Earth's crust is constituted primarily of form 63 (69.1 %), and form 65 (30.9 %). The atomic number of copper is 29 with an electronic distribution of $1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^6\ 4s^2\ 3d^9$, yet, this distribution does not represent a low energy state. Naturally, copper has one electron from the 4s orbital shifted to the 3d orbital ($4s^1\ 3d^{10}$). The inner electronic layers (1s, 2s, 2p, 3s and 3p) are closer to the positively charged nucleus permitting the 4s electron to "escape" to the 3d orbital, characterizing a low energy state [40]. Copper can lose up to two electrons in one-electron step transfers resulting in cuprous (Cu(I)), and cupric (Cu(II)). Altogether, these physical-chemical features are of extreme importance for living organisms: copper can be used in different types of reactions by controlling the mechanism and rate of copper-catalysis. Copper importance and bioavailability was greatly enhanced with the shift from an anaerobic to an aerobic atmosphere during the Great Oxidation event, making it an essential micronutrient and multicellular life possible [21]. Further details will be discussed in the next section.

6.2.2 How Organisms Use Copper

The primordial Earth atmosphere was anaerobic and early life forms used iron given its bioavailability as water-soluble ferrous iron [Fe (II)] [15, 32, 68]. Redox properties of iron were in the range of biological reduction potentials under anaerobic conditions, making its presence essential for survival of early organisms. Conversely, copper was not accessible for biological processes due to its existence mostly in the form of water-insoluble cuprous sulfides [Cu(I)]. Soluble copper was only present in acidic waters near hydrothermal vents, which are extreme environments that have not been representative of life on earth. In other words, copper was not readily bioavailable under anaerobic conditions [15, 32, 68]. The aerobic atmosphere started to be established about 10^9 years ago with the production of oxygen as a by-product of prokaryotic (cyanobacterial) metabolism [15, 32, 68]. This fundamental change in the atmosphere resulted in one of the major alterations (or pollution) of Earth's life conditions. The arrival of dioxygen was dramatic for most living organisms because of its toxicity and its effect on the bioavailability of metals such as iron and copper. The new atmosphere oxidized iron to the water-insoluble ferric iron (III) state and, as a result, bioavailability of iron was lost. Instead, copper became bioavailable due to the oxidation of insoluble Cu(I) to soluble Cu(II). Metabolism under anaerobic conditions was designed to use

proteins and enzymes with low redox potential, whereas life under the new atmosphere required proteins and enzymes with higher redox potentials. The later ones were ideal for life adaptation to the new oxidizing atmosphere, therefore becoming useful for living organisms. A new era had started, the copper era [15, 32, 68].

Organisms are able to take advantage of copper due to one major property: alternate oxidation states by one electron transfer, between Cu(I) and Cu(II). This allows handling a variety of oxidation-reduction processes [43]. Copper can function as a cofactor for a variety of enzymes involved in processes of respiration (cytochrome c oxidase), photosynthesis (plastocyanin), reactive oxygen species (ROS) turnover (copper-zinc superoxide dismutase), nitrite and nitrous oxide reductases and oxygen transport (hemocyanin) [56]. In oxidases, hydroxylases, and reductases copper acts as an electron donor/acceptor [56]. Additionally, it can also act as an electron carrier for instance in azurin and plastocyanin [56].

In order to control the intracellular copper pool within optimal levels, organisms developed systems regulating copper homeostasis, which will be briefly described in the next section.

6.2.3 Copper Homeostasis

Copper is an important micro-element essential for life under an oxygenated atmosphere, but, when in excess, becomes very toxic to cells. In order to control intracellular copper concentration, cells developed systems that can either remove copper from cell, sequester excess of copper or, additionally, may oxidize Cu(I) to the less toxic Cu(II). Among Gram-negative bacteria, *Escherichia coli* is the best studied microorganism regarding genes and mechanisms responsible for copper homeostasis and resistance. *E. coli* possess multiple systems that confer resistance against rising concentrations of copper ions. Surprisingly, it is not yet clear how copper enters bacterial cells (Fig. 6.1). There are multiple possible ways in theory: by diffusion across membranes, through porines across the outer membrane, or by an unknown specific or unspecific transporter across the cytoplasmic membrane. When copper is able to pass through the cytoplasmic membrane into cytoplasm most of it is getting reduced to Cu(I), toxic for bacteria cells. Binding of the excess of Cu(I) ions and activation of the expression of copper-detoxifying genes occurs by means of CueR and CusRS, where CueR is a cytoplasmic MerR-family activator/repressor that activates expression of *cueO* and *copA* genes upon binding to Cu(I) [83], CusRS is a periplasmic two-component system inducing the expression of the *cusCFBA* operon [62, 71], and CopA is a P-type ATPase (Fig. 6.1), which transports cytoplasmic Cu(I) into the periplasm via ATP hydrolysis [75]. Removal of the Cu(I) ions also occurs through its oxidation to Cu(II) by CueO – multicopper oxidase [34], another mechanism of protection of the periplasmic space from Cu (I) toxicity (Fig. 6.1) [79]. The removal of periplasmic copper from the bacterial cell is known to proceed through the Cus efflux system consisting of four proteins (CusCFBA) and energized by the proton motive force (PMF) (Fig. 6.1). A copper

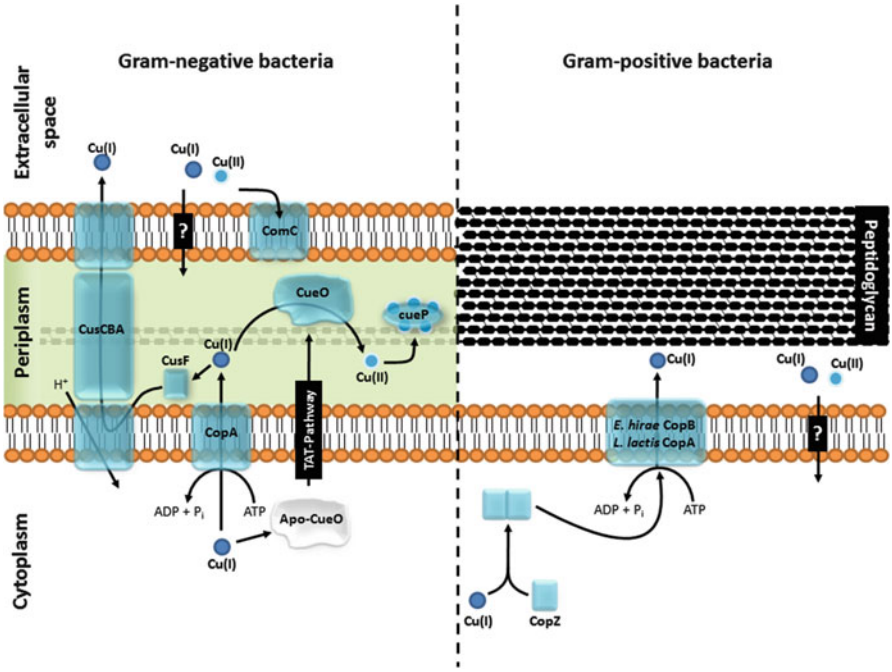

Fig. 6.1 Copper homeostasis and resistance mechanisms from Gram-negative and -positive bacteria. In Gram-negative bacteria (from the *left* to the *right*): CusCBA extrudes Cu(I) from the periplasm, CusA is a member of the resistance-nodulation-division (RND) protein superfamily of proton-driven cation antiporters, CusC is an outer membrane factor (OMF), and CusB belongs to the family of membrane fusion proteins (MFP), CusF is a copper chaperone that directs the copper to the CusCBA efflux system [30]; CopA is a P_{1B}-type ATPase that expels Cu(I) to the periplasm with ATP hydrolysis [75]; ComC is an outer membrane protein that reduces copper permeability by copper scaffolding; CueO is a multicopper which oxidizes periplasmic Cu(I) to Cu(II) [34]; CueP functions as a periplasmic-copper-pool. In Gram-positive (from the left to the right): CopZ is a copper chaperone that binds to cytoplasmic copper and delivers it to the P_{1B}-type ATPase (*L. lactis* CopA/*E. hirae* CopB) [69], which in turn, extrudes Cu(I) outside of the cell [80]

chaperon CusF binds Cu(I) and delivers it to the CusCBA complex [30]. In *Salmonella enterica* sv. *Typhimurium*, a copper binding protein (CueP) (Fig. 6.1), under control of CueR, functions as a copper-pool, protecting the periplasm from free-copper toxicity [70]. In a recent study it was shown that periplasmic and cytoplasmic concentrations of copper in methylotrophic bacteria were higher in the absence of ComC (copper-induced outer membrane component) protein, highlighting its involvement in copper permeability (Fig. 6.1). When this protein was not present, copper concentrations were higher inside the periplasm and cytoplasm, functioning as scaffolding or tethering protein in *E. coli* outer membrane. The expression of this protein was shown to be controlled by ComR, a novel TetR-like copper-responsive repressor [55].

In addition to these chromosome-encoded genes, Gram-negative bacteria possess plasmid-encoded copper resistances. The best studied is the plasmid-encoded copper resistance determinant, Pco system of plasmid pRJ1004, isolated from *E. coli* present in the gut flora of pigs fed with a diet supplemented with copper sulphate as a growth promotant [5]. This plasmid encodes seven genes, *pcoABCDRSE*, whose expression is dependent on copper and is accomplished by PcoRS [5]: PcoA is a multicopper oxidase related to CueO, PcoC and PcoE are two periplasmic copper chaperones, and PcoB and PcoD have unknown functions.

Among Gram-positive bacteria, *Lactococcus lactis*, *Bacillus subtilis*, and *Enterococcus hirae* are the best studied organisms on copper homeostasis with *E. hirae* being the model organism for metal handling [81]. In this organism an operon of four genes, *copYZAB*, is responsive to copper stress. Free cytoplasmic Cu(I) binds to CopY (a copper-responsive repressor) resulting in derepression of the *cop* operon. Start of transcription results in increased production of CopY, copper chaperone CopZ, and the Cu(I)-translocating P_{1B}-ATPases, CopA and CopB (Fig. 6.1) [69], followed by Cop B-promoted extrusion of excess of copper and silver from the cytoplasm [80]. On the other hand, the function of CopA is still unclear, but it might function as an efflux system for copper incorporation into enzymes such as cytochrome oxidases [2, 73].

The ability of *L. lactis* to withstand copper released from traditional Swiss cheese copper vats ignited renewed interest in studying copper homeostasis in this bacterium. The mechanism of coping with high copper concentrations in *L. lactis* is similar to *E. hirae* and includes a copper-inducible operon, *copRZA*, where CopR is a CopY-type repressor, CopZ is a copper chaperone, and CopA is a copper export ATPase. CopB is encoded separately and repressed by CopR, but its copper export function has not been determined yet [82].

The inability of bacterial cells to expel copper when its concentration rises above a certain threshold leads to accumulation of free copper in cells, which, in turn, causes damage to multiple biomolecules. The next section will discuss mechanisms of copper toxicity.

6.2.4 Ionic Copper Toxicity

Organisms did not develop copper specific resistance mechanisms in vain; excess of this metal is very toxic to cells. Copper is ranked fifth among the most toxic of seventeen metals to soil bacteria, preceded by silver, mercury, chromium, and cadmium [20]. Furthermore, copper was found to be one of the most toxic metals to heterotrophic bacteria in aquatic environments [1], where metal-salt sensitivity of aqueous microflora was higher when exposed to: $Ag >> Cu$, $Ni > Ba$, Cr, $Hg > Zn$, Pb, Na, Cd. Copper is capable of forming stable complexes with a wide variety of ligands regardless of its valence state, thus, it binds easily to biomolecules such as proteins, lipids, and nucleic acids [32].

$$H_2O_2 + O_2^{-\bullet} \longrightarrow O_2 + OH^- + OH^{\bullet} \quad (1)$$

$$Cu^{2+} + O_2^{-\bullet} \longrightarrow Cu^{+} + O_2 \quad (2)$$

$$Cu^{+} + H_2O_2 \longrightarrow Cu^{2+} + O_2 + OH^- + OH^{\bullet} \quad (3)$$

Fig. 6.2 Reactive oxygen species produced by copper-mediated catalysis

$$2\,Cu^{2+} + 2\,RSH \longrightarrow 2\,Cu^{+} + RSSR + 2\,H^{+} \quad (4)$$

$$2\,Cu^{+} + 2\,H^{+} + O_2 \longrightarrow 2\,Cu^{2+} + H_2O_2 \quad (5)$$

Fig. 6.3 Copper-mediated sulfhydryl group depletion

Copper-induced toxicity can be very diverse depending on the environment surrounding this metal, generating damage by multiple mechanisms. Therefore, measuring copper toxicity in vivo can be a challenge. One of the most important modes of toxicity is based on the redox properties of copper, where under aerobic conditions copper can alternate oxidation state and exchange electrons with acceptor or donor groups, such as oxygen and sulfur residues. Copper can exert its toxicity by reacting directly with biomolecules or indirectly through activation of oxygen species.

In case of indirect toxicity, copper catalyzes production of reactive oxygen species (ROS) via Fenton-like Haber-Weiss reactions (Fig. 6.2) [49]. ROS, per se are extremely reactive when formed and quickly cause damage to the surrounding biomolecules: lipids, proteins and DNA [95].

The low constant rate of hydrogen peroxide reaction with superoxide, as described in the equation 1 (Fig. 6.2), is greatly enhanced by presence of copper ions. During one cycle cupric ion is first reduced to cuprous ion by superoxide anion (Fig. 6.2, equation 2), then Cu(II) is oxidized back to Cu(I) by hydrogen peroxide (Fig. 6.2, equation 3), and the cycle repeats itself producing more hydroxyl radicals that induce cell damage. Additionally, in the low valence state Cu(I) can form active complexes with oxygen capable of attacking surrounding molecules. These types of reactions will be further discussed in the membrane damage section (or lipid oxidation chemistry).

Direct copper toxicity to biomolecules has been linked to the depletion of sulfhydryl groups (Fig. 6.3), where copper reacts with glutathione (GSH) producing glutathione disulfide (GSSG) or with the amino-acid cysteine generating cystine groups (Fig. 6.3, equation 4).

Cuprous ions generated by sulfhydryl group oxidation are recycled back to cupric ion producing hydrogen peroxide. This hydrogen peroxide can be converted to the more highly reactive ROS, hydroxyl radical and superoxide, by the equations 2 and 3 described above [50].

Damage to nucleic acids, lipids, and proteins by previously described mechanisms has been demonstrated in vitro in many studies (e.g. [4, 7, 11, 18, 42, 45, 95]).

Some recent findings suggest an alternative mechanism responsible for the primary toxic effects of copper in vivo. A first evidence of such is that the majority of copper inside the cell is bound to biomolecules, while free copper is at extremely low levels or even nonexistent, thus making the Fenton chemistry and sulfhydryl depletion very unlikely mechanisms [10]. Another study by [51] showed that *E. coli* cells grown without copper are more sensitive to killing by hydrogen peroxide than *E. coli* pretreated with copper. In addition, copper decreases the rate of DNA damage induced by hydrogen peroxide. The authors suggested that copper exerts its toxicity by mechanisms other than oxidative stress. Furthermore, [50] showed in vivo as well as in vitro that a rise of intracellular copper concentrations is associated with the displacement of iron from iron-sulfur clusters. For example, it was shown that copper specifically damaged the iron–sulfur clusters of various dehydratases involved in branched amino acid biosynthesis from *E. coli* cells. Further investigation in this field is needed, in order to have a clear conception on the mechanism of copper-induced toxicity in cells.

Without copper-detoxifying mechanisms, cells suffer copper-induced toxicity that might compromise survival. Moreover, another challenge presents a severe stress for bacterial survival – toxicity caused by contact to metallic copper. Knowledge related to this so-called "contact-killing" [35] by metallic copper surfaces, is reported in the following section.

6.3 Metallic Copper Surfaces as a Biocidal Tool

6.3.1 Quick Cell Inactivation by Metallic Copper Surfaces

The effectiveness of copper surfaces in bacteria killing was investigated both in laboratory and hospital conditions. Generally, two major inoculation techniques were employed to study metallic copper's antimicrobial properties: the wet and dry methods, imitating different environments of bacteria surface contamination. The first ever described method explores the antimicrobial activity of metallic copper surfaces against cells suspended in a buffer solution (wet method). This inoculation technique was first developed by [29] and then further optimized by [93] to the method that is widely used today. In the presence of a buffer solution cells are not directly in contact with the surfaces but instead suspended away from the metallic copper (e.g. [29, 65, 66, 93, 94]). The wet method mimics moist environments, such as food processing, public baths, water conservation, pipelines, and bathrooms, where droplets containing germs fall on top of surfaces and can be picked up by a person. In the early studies the bactericidal property of copper surfaces was screened against panel of microorganisms. In 2004 Faúndez [29] demonstrated that copper surfaces are able to reduce bacterial counts of *S. enterica* and *Campylobacter jejuni* – two notorious human pathogens mainly transmitted by food ingestion. This study was the first peer-reviewed publication

confirming the antimicrobial efficacy of copper surfaces versus steel control surfaces. In addition, earlier that year, conference papers by Harold Michels and colleagues [58, 59] reported the killing kinetics of various copper alloys against *E. coli* O157:H7, an enterohemorrhagic strain often found in ground beef. Moreover, this was the first study pointing out that temperature played a role in the killing process: inactivation of cells on 99 % pure copper occurred within 1.5 h at 20 °C, whereas lowering temperature to 4 °C prolonged the killing time up to 3 h. Non-copper containing surfaces failed to inactivate *E. coli* O157:H7. Another parameter, copper concentration in different alloys, was shown to have direct correlation with killing rate of studied surfaces: diminishing the copper content in the alloys was accompanied by a reduction of the killing rate. These results were published by Wilks and co-workers [93] and were in accordance with the ancient-knowledge that early civilizations had applied but not understood, such as using copper vats to store drinking water.

Later on many studies followed that focused on establishing killing efficiencies of copper surfaces versus control surfaces against a variety of microorganisms (Table 6.1). Typically, under wet conditions, microorganisms are killed within hours on metallic copper surfaces (Table 6.1). These studies resulted in registration of almost 300 different copper alloys as antimicrobial by the Environmental Protection Agency (EPA) in 2008 (http://www.epa.gov/pesticides/factsheets/copper-alloy-products.htm).

Although a substantial amount of data concerning the antimicrobial efficacy of copper against multiple microbes was obtained prior to 2008, not much attention was paid to the elucidation of the bacterial killing mechanism underlying such process. Only in 2008 an initial step was made by Espírito Santo et al. [25], towards understanding the mechanisms of metallic copper surface-mediated killing of bacteria. For the first time, an alternative method was developed to mimic touch to dry surfaces, where cells in a minimal buffer volume are applied directly on the surface. Evaporation of the liquid occurs very rapidly, within seconds, mediating immediate contact between bacterial cells and surface. This method may be applied as a laboratory model to simulate bacteria spread through touch to surfaces, air particles in hospitals or other public places, and air conducts. The bactericidal properties of copper surfaces using dry copper exposure model were tested against the panel of microorganisms (Table 6.1). Typically organisms are all inactivated within minutes, highlighting much faster killing kinetics using this inoculation technique [25].

Data obtained from studies using wet and dry methods suggest that they employ different toxicity mechanisms resulting in unique killing kinetics as observed by, e.g., [22, 25, 61, 93]. As a result of wet inoculation, there is no direct contact between bacterial cell and copper surface. In order to achieve the desired antimicrobial effect, copper ions have to be released directly into the buffer suspension [61], where their concentration has to reach a certain level to be toxic [23, 61]. Consequently, cells are inactivated as a result of deadly concentrations of copper ions and copper-induced stress [61], typically within hours [22, 54, 61, 65, 67, 87, 88, 90, 93, 94].

Table 6.1 Killing of microorganisms by wet and dry exposure on metallic copper surfaces

Method	Bacterial species	Reference
Wet	*Aspergillus niger*, *A. flavus*, *A. fumigatus*, *Fusarium culmonium*, *F. oxysporium*, *F. solani*, *Penicillium chrysogenum*	[89]
	E. hirae	[61]
	E. faecium	[23]
	E. coli W3110	[27]
	C. jejuni	[29]
	Candida albicans	[54, 89]
	Clostridium difficile	[88, 92]
	Cronobacter sakazakii	[24]
	Influenza A Virus	[67]
	Listeria monocytogenes Scott A	[94]
	methicillin-resistant *Staphylococcus aureus* (MRSA)	[33, 54, 57, 66]
	Methicillin-sensitive *S. aureus* (MSSA)	[33]
	Mycobacterium tuberculosis, Klebsiella pneumonia, Acinetobacter baumannii	[54]
	Pseudomonas aeruginosa	[22, 33, 54]
	S. enterica	[29, 96]
	Vancomycin resistant *Enterococci* (VRE)	[33, 86]
Dry	*Acinetobacter johnsonii*, *Pantoea stewartii*, *Pseudomonas oleovorans*, *Staphylococcus warnerii*, *Brachybacterium conglomeratum*	[26]
	E. coli W3110	[25, 27]
	C. albicans, *Saccharomyces cerevisiae*	[72]
	C. sakazakii	[24]
	Francisella tularensis, *Bacillus cereus*, *B. anthracis*, *Brucella melitensis*, *Burkholderia mallei*, *B. pseudomallei*, *Yersinia pestis*	(Bleichert, Espírito Santo & Grass, unpublished results)
	S. enterica	[96]
	Staphylococcus haemolyticus	[28]

Instead, the dry method entails that cells are directly in contact with the metallic copper surfaces providing faster and sharper toxicity. Characteristically, cells exposed by this method are inactivated within minutes [25–27, 72].

In a study conducted by [53], the role of the direct contact of bacteria with metal was investigated by using contact arrays consisting of microstructure polymer grid on top of a copper surface. The copper release from such arrays was not affected when compared to control copper coupons, nevertheless, no significant contact killing was observed even after 3.5 h. This finding highlights the importance of the direct contact for efficient killing [53].

This sharp and fast killing observed by metallic copper surfaces exposure and the mechanism by which bacteria are inactivated will be detailed in the next sections.

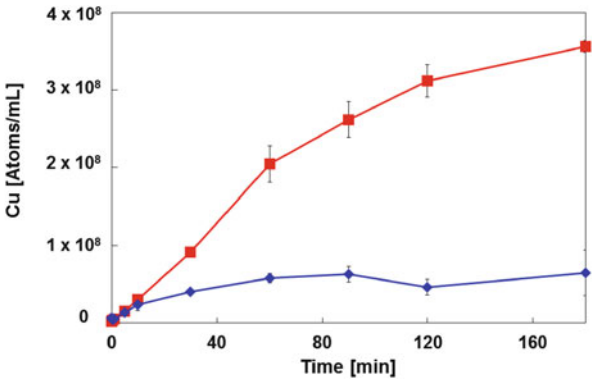

Fig. 6.4 Copper release from metallic copper surfaces. The *red line* represents copper release in a droplet which contains buffer and *E. coli* cells; the *blue line* corresponds to copper release in buffer alone (Espírito Santo and Grass, unpublished results)

6.3.1.1 Copper Surface Oxidation and Cell Accumulation

Both under wet and dry exposure to metallic copper surfaces, microbes, when in contact with the surface, enhance the release (or "solubilization") of copper ions from the surface [23, 27, 61].

Copper Release and Accumulation Under Wet Conditions

When a droplet gets in contact to a copper surface, oxidation of the metal surface occurs which leads to increase concentrations of dissolved copper. Moreover, when the droplet contains bacterial cells copper concentration increases significantly over time compared with a buffer-only-containing droplet (Fig. 6.4). Cell inactivation occurs due to the increase of copper ion concentrations within the droplet [23, 27, 61], and after hours of exposure, when copper concentration reaches a critical level, cells are unable to sustain survival accumulating toxic amount of copper.

Combined data for copper accumulation and killing kinetics have clearly demonstrated direct correlation between release of copper from the coupon surface and its accumulation by the cells, with lethal consequences (Fig. 6.5). Therefore, cells are killed by result of deadly concentrations of copper ions and copper-induced stress (Fig. 6.5) [27, 61].

Copper Release and Accumulation Under Dry Conditions

Under dry exposure, cells are exposed with the smallest amount of buffer possible, which dries very quickly. Cells are exposed directly to the surface, which undergoes oxidation and cells accumulate copper almost instantaneously and are killed within minutes of exposure (Fig. 6.5). At the shortest time (few seconds) of exposure, cells accumulate large amounts of copper ions (10^9 atoms/cell) from dry metallic copper surfaces (Fig. 6.5) [27, 28, 72]. As a result, cells are unable to keep up copper homeostasis and struggle to survive such high copper concentrations.

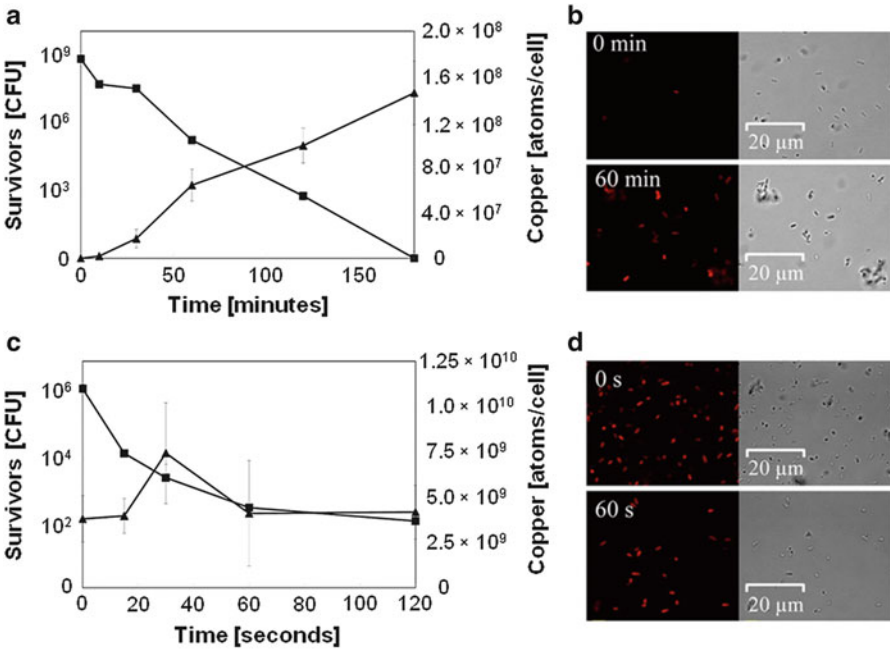

Fig. 6.5 Example of copper uptake on moist (**a** and **b**) and dry (**c** and **d**) by *E. coli* cells. Cells were exposed to metallic copper surfaces for the indicated times, removed, washed, and plated on solidified growth media. Survival was assessed by counting colony forming units (CFU) (squares in **a** and **c**). In parallel, samples were mineralized and subjected to ICP-MS analysis to determine cellular copper content (triangles in **a** and **c**) or were stained with the Cu(I)-specific fluorescent dye Coppersensor-1 and subjected to fluorescence microscopy (**b** and **d**). Shown are averages and standard deviations (*error bars*) from triplicate experiments (**a** and **c**) and representative phase-contrast (*right*) and fluorescence (*left*) microscopy images (**b** and **d**) [27]

Cells experience a short sharp shock by contact with copper surfaces and a few minutes are sufficient to completely inactivate all cells [35].

Under dry conditions, it was also noted that buffer composition or presence of protectants influence the survival rate of bacteria. Cells applied with ROS protectants (catalase, superoxide dismutase, manitol, etc.), chelators (EDTA), and osmotic stress protectans (sucrose), increased survival on copper surfaces. This topic will be a focus of the next section.

Survival Depends on Buffer Composition and Surface Corrosion

Under wet exposure, cells are suspended in a buffer that is in contact with the surface. Composition of this buffer is important for copper ion release and, consequently, killing efficacy differs in different buffer systems [61] (Fig. 6.6). Tris-buffer provokes higher copper ion solubilization [61], hence cells become more sensitive to copper

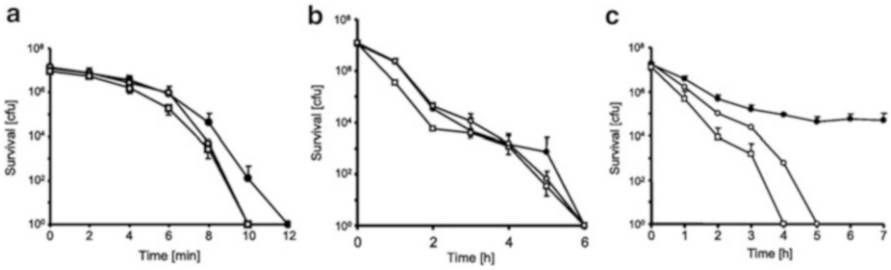

Fig. 6.6 Effect of different media on the survival of *E. Hirae* wild-type and mutant strains on metallic copper surfaces coupons. Cells were washed and applied to copper coupons in 0.1 M Tris-Cl, pH 7 (**a**), water (**b**), or 100 mM NaPi, pH 7 (**c**), wild type (*filled circles*); *ΔcopB* mutant (*open circles*); *ΔcopAB* mutant (*open squares*), incubated at room temperature for the times indicated, and washed off with phosphate-buffered-saline. Survivors were counted as CFU. Shown are averages and standard deviation from triplicate experiments [61]

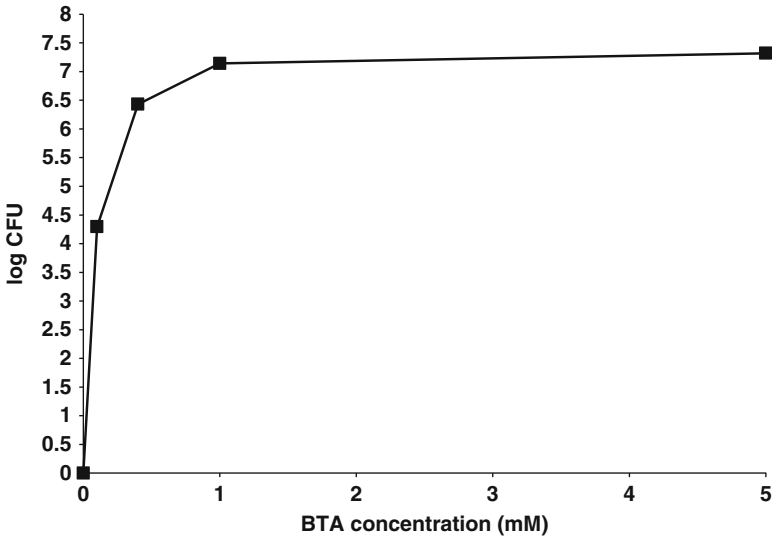

Fig. 6.7 Effect of benzotriazole (BTA)-coating electroplated copper surfaces on the survival of *E. coli*. Cells are applied, incubated for 30 min, washed and survivors were counted by CFU [23]

surface toxicity under this condition compared to when water or phosphate-based buffer was used. Complementary results were obtained with inhibition of surface corrosion, where Elguindi and colleagues [23] were the first to show that metallic copper surfaces need to be naturally oxidized in order to display antimicrobial properties. The presence of corrosion inhibitors, such as benzotriazole (BTA), enhances bacterial survival on wet copper surfaces by lowering the copper ion release from copper surfaces (Fig. 6.7) [23]. Considering these results, new disinfection and cleaning materials need to be developed to aid maintaining the antimicrobial activity of copper surfaces.

6.3.2 *Additional Physical and Physiological Factors Modulating the Contact-Killing Process*

There are several factors which influence killing rates by metallic copper surfaces: alloy copper content, temperature, moisture, copper chelators, osmotic stress, reactive oxygen species, cellular physiology, copper detoxifying systems, and pre-adaptation to copper, but not anaerobiosis (oxygen-free environment).

6.3.2.1 Copper Alloy Content and Roughness

Copper alloy content influences cell survival exposed to these alloys. This seems to be a logic feature of copper alloy surfaces, where the higher the copper content of the alloy the higher the killing efficiency. For the majority of the alloys this is true, but there are exceptions. The metallurgy industry mixes copper with other metals in order to modify intrinsic alloy properties for a defined purpose. Nordic Gold is a gold-colored alloy used in coinage which contains 89 % copper, 5 % aluminium, 5 % zinc, and 1 % tin; it was developed to be of low allergenic without compromising the resistance to tarnishing. This is not as efficient in bacterial killing as other alloys [72]. For example, a lower copper containing alloy, 18 % Nickel Silver alloy, which contains 65 % copper, 18 % nickel, 17 % zinc, and does not contain silver, has a higher killing efficiency than Nordic Gold [72]. These results can be explained by different copper release rates or to the presence of other metals that can aid in copper toxicity, however there is no experimental data to support such claim. A great challenge for metallurgic industry would be the development of an alloy that compromises a fast killing efficiency and tarnish resistance (with good esthetics), together with the capacity to remain "bioactive" in the long term.

In the experimental studies pure copper (99 % Cu) is often chosen to investigate effect of copper on contact killing with different bacteria. Additionally, this has also facilitated the comparison of results between experiments and groups.

The degree of surface homogeneity was also shown to modulate the killing efficiency [37]. A rougher surface is characterized by larger area of contact with bacteria cells; hence cells are exposed to more corrosion products, thus more toxicity. As described before, contact is required for killing by metallic copper surfaces, which corroborates these findings [53].

6.3.2.2 Temperature and Moisture

Temperature was one of the first identified factors influencing the killing by metallic copper surfaces. Typical metallic copper experiments were performed at controlled room temperature, between 18 and 24 °C. The rate of killing is in direct correlation with temperature changes, where higher temperature corresponds to

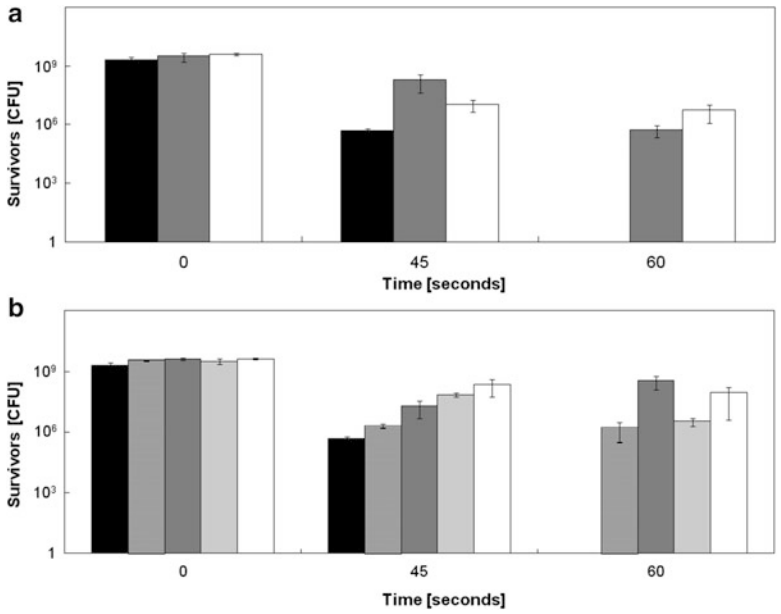

Fig. 6.8 Protective effects of metal chelators (**a**) and reactive oxygen species quenchers or sucrose (**b**) on the survival of *E. coli* on copper surfaces. Cells were washed, mix with Cu (II) chelator EDTA ((**a**), *gray bars*), Cu(I) chelator BCS ((**a**), *white bars*), ROS quenchers mannitol ((**b**) *horizontally striped bars*), catalase ((**b**), *dark gray bars*), superoxide dismutase ((**b**), *light gray bars*) sucrose ((**b**), *white bars*), or no additive ((**a**, **b**), *black bars*), and applied on the metallic copper surface. After 0, 45, and 60 s, samples were withdrawn and CFU were counted. Shown are averages with standard deviations (*error bars*) from three independent experiments [25]

faster killing, and conversely, lower temperature corresponds to slower killing (Fig. 6.9) [25, 57, 93].

At the same time reversed correlation was observed between air moisture content and killing rate with higher humidity of the air corresponding to the higher survival rates on metallic copper [23, 57].

6.3.2.3 Copper Chelators

As described before, copper is actively released from the surfaces and is either dissolved in the buffer suspension or accumulated by the cells. Presence of chelators, such as EDTA, increases the chance of survival on metallic copper surfaces (Fig. 6.8) [25, 86]. Cell survival rates vary depending on the amount of chelator [25]. However, it is noteworthy to mention that presence of chelators does not prevent cell death but only delays it. Indeed, the released copper that has been chelated is no longer available to cause toxic damage. However, more copper is released and copper-saturated chelators are no longer available to avoid the toxic effects of free

Cu(I) cations towards cells. During this process, killing efficiency depends on the amount of copper that has been released and accumulated by cells.

6.3.2.4 Osmotic Stress

Cells that are applied directly onto the surface without a buffer intermediate (dry method) suffer osmotic stress. Upon exposure to dry surfaces (copper and non-copper containing surfaces), survival decreases due to the additional stress of adaptation to a new environment. Osmotic protectants, such as sucrose, are able to ease the adaptation to the surfaces and increase the survival counts at the beginning of exposure. Nevertheless, protection is not permanent; cells will eventually succumb to metallic copper toxicity (Fig. 6.8).

6.3.2.5 Reactive Oxygen Species

Upon exposure cells provoke surface oxidation with subsequent copper release by both wet and dry methods, and this causes the production of ROS [27, 72, 86], as was discussed in the previous section "Ionic copper toxicity". ROS formed by surface oxidation and copper release are very harmful to cells, therefore introducing ROS quenchers in the experiment increases survival on metallic copper. Upon both wet and dry exposure, simultaneous events, where catalase catalyzes the decomposition of hydrogen peroxide (H_2O_2); superoxide dismutase catalyzes superoxide ($O_2^{-\bullet}$) dismutation; and finally, mannitol acts as hydroxyl radical scavenger, are capable of effectively protecting the cells and prolonging their survival (Fig. 6.8) [27, 72, 86]. Ultimately, due to the surface oxidation, ROS are produced continuously, thus depleting the protective effect and eventually cells succumb to metallic copper toxicity.

6.3.2.6 Cellular Physiology

Intrinsic physiological characteristics and structures of cells are able to affect survival on metallic copper surfaces. Here, we will focus only on the best studied ones.

Cell Wall Structure

Prokaryotes can be divided into two groups based on their cell wall organization: Gram-positive and Gram-negative bacteria. Gram-positive bacteria have a thicker peptidoglycan layer and a cytoplasmic membrane. Instead, Gram-negative bacteria have two membranes, the outer and the cytoplasmic membrane, and a thinner peptidoglycan layer in between the membranes. In general, Gram-positive bacteria are able to survive longer on metallic copper surfaces than Gram-negative bacteria with both methods (wet and dry) [23, 26, 28, 61,

65, 66]. Interestingly, both Gram-positive and -negative bacteria show a similarly high copper accumulation [27, 28]. Differences in survival rate coupled with equally high copper accumulation might be due to an intrinsic feature of the Gram-positive bacteria: a thicker cell-wall peptidoglycan layer which functions both as a buffer and diffusion barrier for copper ions. Additionally, these cells may also have an innate ability to resist higher degrees of desiccation (in the case of dry-exposure) aiding the survival on copper surfaces.

Spores

Sporulation is a process that some bacteria use to escape unfavorable growth conditions and ultimately survive. Spores are able to resist very high and very low temperatures and extreme dehydration. These structures are able, in some cases, to endure metallic copper toxicity [26, 92]. However not all bacterial spores are able to germinate after metallic copper exposure [26]. Further studies are required to understand why some spores have the ability to escape the toxic effects of metallic copper and others have not.

Copper Detoxifying Systems and Pre-adaptation to Copper

As discussed before, cells are able to control copper concentrations by using copper homeostasis systems. It is expected that strains with genetically deleted copper detoxification systems are more sensitive to metallic copper stress than their parental strain. Surprisingly, mutated strains of *E. coli* were only slightly more sensitive than their wild-type parental strain (Fig. 6.9) [25]. Similar results were obtained with bacterial strains from *E. hirae* [61], *P. aeruginosa* [22] and yeast strains from *C. albicans* and *S. cerevisiae* [72]. However, when pre-incubation with non-toxic copper concentrations was applied to parental strains, cells were able to show enhanced survival on metallic copper (Fig. 6.10), but this did not prevent killing [25]. Pre-adaptation to copper permitted cells to fully activate and produce copper detoxifying systems, which strengthen their ability to sustain metallic copper toxicity longer [25].

Anaerobiosis

Aerobic conditions signify the presence of oxygen, which in turn is partially responsible for ROS production by redox active metals, such as copper and iron. Accordingly, it is predicted that when oxygen is not present (anaerobiosis) copper stress by ROS is limited. However, under anaerobiosis the most predominant copper ion is Cu(I), which is the most toxic of the two ions since it is a much stronger soft metal. When cells are exposed to metallic copper surfaces under microaerophilic conditions (very low oxygen content), their survival is not much higher compared to survival under aerobic conditions [25].

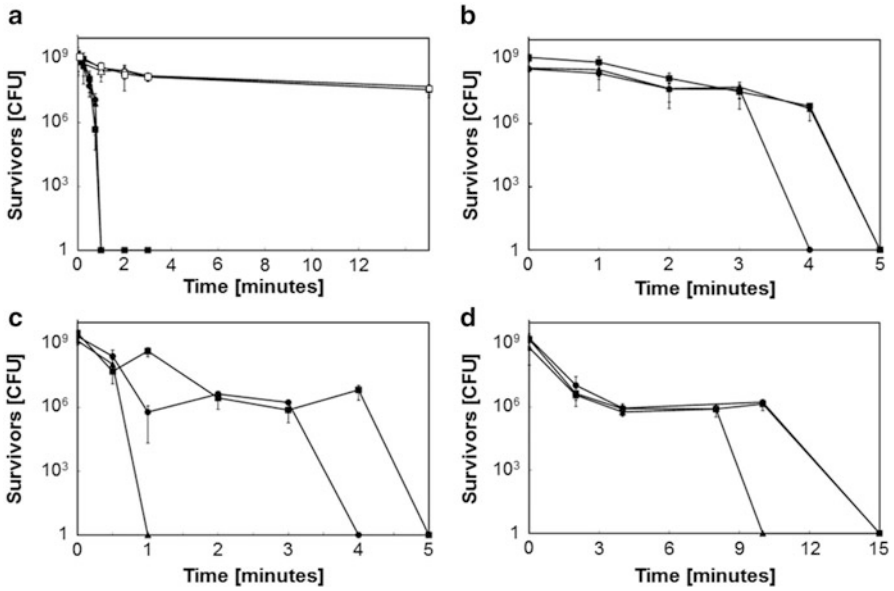

Fig. 6.9 Effect of copper resistance mechanisms on the survival *E. coli* on copper alloy surfaces and stainless steel. *E. coli* wild-type strain W3110 (*squares*), its copper-sensitive derivative Δ*copA* Δ*cus* Δ*cueO* (*triangles*) or W3110 harboring the high-level copper resistance system Pco (*circles*) were applied on dry copper alloy surfaces (*filled symbols*) or stainless steel (*open symbols*). After the indicated time periods at ambient conditions (23 °C (**a**, **c**, and **d**) or 5.5 °C (**b**)), after the indicated times, cells were removed and CFU counted. Surviving cells were counted as CFU. The alloys were pure copper (99.9 % Cu) (**a** and **b**), "nickel-silver" (maximum of 62 % Cu) (**c**), Muntz metal (maximum of 62 % Cu) (**d**), and stainless steel (AISI 304) (**a**). All experiments were measured in triplicates and standard deviations are indicated as error bars [25]

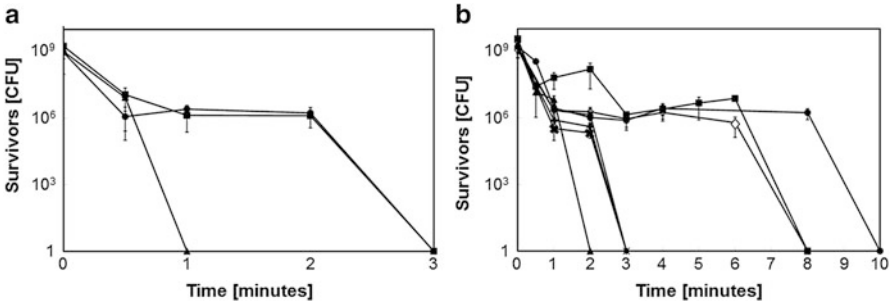

Fig. 6.10 Effect of copper preadaptation by *E. coli* strains on copper alloy surfaces. Copper detoxifying systems were induced by growing *E. coli* cultures in the presence of nontoxic concentrations of $CuCl_2$. Washed cells of *E. coli* wild-type strain W3110 (*squares*) or its copper-sensitive Δ*copA* Δ*cus* Δ*cueO* (*triangles*), Δ*cus* Δ*cueO* (*filled diamond*), Δ*copA* (*plus*), Δ*cueO* (X), Δ*cus* (*open diamond*), or W3110 harboring the high-level copper resistance system Pco (*circle*) were streaked on 99.9 % copper (**a**) or "nickel silver" alloy (maximum of 62 % Cu) (**b**) surfaces. Average and standard deviations (*bars*) were calculated from tree independent CFU counts [25]

Viable-But-Not-Culturable (VBNC)

In response to adverse conditions that would otherwise harm growing cells, some bacteria are thought to be able to enter the so-called Viable-But-Not-Culturable (VBNC) state. This state is characterized, as the name indicates, by the absence of observable cell growth, cell division and the ability to form colonies on standard solid media [64]. Thus, such cells appear all but dead with very low metabolic activity. This property, however, might become handy, e.g., when cells are starving, exposed to sub-optimal physical growth conditions or challenged by antimicrobial substances. Only a fraction of the stressed cultures are able to enter the VBNC state and the transition is governed by mostly unknown regulatory mechanisms. Unexpectedly, some reports suggest that VBNC cells may still remain infectious [3, 36]. Remarkably, upon reversal of the initial stress that caused the VBNC state, these differentiated cells are able to resuscitate, meaning revert to the actively growing, multiplying state that defines live bacteria [3, 36]. Previously, it has been shown that for *E. coli* or *Ralstonia solanacearum* copper ion stress was able to induce the VBNC state. Upon reversal of the copper stress by adding a metal ion-chelator the VBNC cells resuscitated and resumed growth [3, 36].

In contrast, no resuscitation occurred after *E. coli* cells have been exposed to metallic copper surfaces (Bleichert and Grass, unpublished). To test this, cells were first challenged on the copper surfaces just long enough to allow for complete inactivation (i.e. failure to form colonies upon plating on solid growth media). Cells were then washed to remove traces of copper ions and the resuscitation process was initiated by incubating the cells in solutions containing metal chelator. While in well-studied resuscitation models (cold-shock, copper ions) cell growth can be observed after days or weeks [3, 36], no growth was observed up to 1 month when cells were challenged with metallic copper surfaces and resuscitated. Thus, metallic copper, in contrast to copper ions, is unlikely to induce the VBNC state in *E. coli*.

6.3.3 Cellular Targets of Metallic Copper Toxicity

6.3.3.1 DNA Mutations and Degradation

There are many possibilities for cellular targets metallic copper toxicity. For example, DNA was suggested to be the primary target for metallic copper toxicity by some studies [57]. This subject was taken under careful examination, and metallic copper toxicity was tested for inducing mutations and degradation. Results obtained with the bacterium *E. coli*, *S. haemolyticus*, and the yeast *S. cerevisiae*, show that exposure to metallic copper does not cause DNA mutations (Fig. 6.11) [27, 28, 72]. Furthermore, *Deinococcus radiodurans* was tested on metallic copper under wet and dry exposure. This bacterium isolated from a nuclear power plant is highly resistant to γ-radiation. This type radiation is capable of breaking down DNA bonds causing DNA fragmentation. *D. radiodurans* harbors sophisticated and

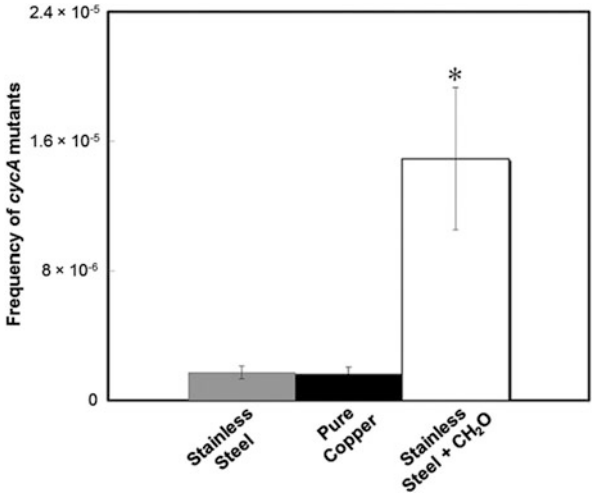

Fig. 6.11 Mutations assessment after *E. coli* exposure to surfaces. A number of 10^8 cells were exposed for 5 s to copper surfaces, stainless steel surfaces, or surfaces containing 0.25 % (wt/vol) of the mutagen formaldehyde (CH_2O) plus stainless steel, removed, concentrated, and spread on solid medium containing 20 μg/mL of the bacteriostatic compound d-cycloserine. CFU were counted as originating from mutation events leading to resistance via inactivation of CycA, a d-cycloserine uptake permease. Triplicates were performed. The asterisk denotes significantly different values ($P \leq 0.05$) for formaldehyde-challenged cells and standard deviations indicated as error bars

very effective DNA repair systems enabling cells to recover from highly fragmented genomes [14]. If the primary target of metallic copper toxicity is DNA fragmentation, then *D. radiodurans* should be resistant to metallic copper exposure. Yet, stationary-growth-phase cells of *D. radiodurans* are quickly inactivated by metallic copper surfaces (Fig. 6.12). Even when *D. radiodurans* cells have their maximum DNA repair capacities [84], in exponential-growth-phase, *D. radiodurans* is unable to survive metallic copper stress. Furthermore, DNA degradation was measured by the comet assay technique, which permits observing DNA degradation at the individual cell level. In this experiment DNA degradation was not observed before cell death but only after cell death, indicating that the primary target is not DNA but instead another biomolecule.

6.3.3.2 Membrane Permeability

As DNA was ruled out as the primary target for metallic copper toxicity, the bacterial cell envelope was considered to be the first structure to encounter metallic copper-induced damage. Indeed this was proven by using the Live/Dead® staining technique that entails two nucleic acid stains: a green-fluorescent SYTO® 9 and a red-fluorescent propidium iodide stain [27, 28, 72]. On one hand, SYTO® 9 stain

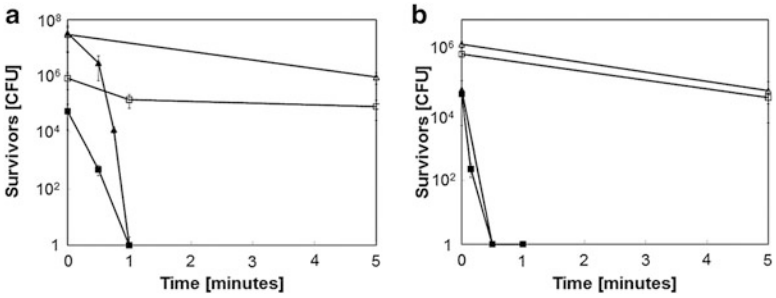

Fig. 6.12 Survival of stationary-phase (**a**) and exponential-phase (**b**) cultures of *D. radiodurans* (*squares*) or *E. coli* (*triangles*) on stainless steel (*open symbols*) or copper (*filled symbols*) surfaces. Shown are averages and standard deviations (*error bars*) from three independent experiments [27]

labels DNA of intact membrane (live) and compromised membrane (dead) bacteria. On the other hand, propidium iodide stains only cells which have impaired membranes. Thus, bacteria with intact membranes fluoresce green, while bacteria with damaged membranes fluoresce red. Upon exposure to metallic copper, most of the cells fluoresce red, indicating that they are all inactivated. This was the first clue suggesting that metallic copper stress induces damage to membranes. Furthermore, experiments performed with *S. cerevisiae* yeast cells have shown that inactivation of cells was caused by damage inflicted on the membranes, followed by the loss of membrane potential [72, 86] and likely release of cytoplasmic contents. Furthermore, it was also observed that intracellular vesicles disappeared during exposure to metallic copper [72].

Loss of respiration observed during metallic copper exposure might be another event that supports the idea of membrane damage by metallic copper toxicity [65, 66, 86–88, 94]. Such an effect can be due to loss of the proton motive force (PMF) initiated through escape of protons through the damaged membrane. When damage is inflicted to the membrane making it permeable, the respiratory chain becomes uncoupled [86].

The investigation of this membrane damage hypothesis was further continued by studying effect of metallic copper exposure on membrane lipid oxidation employing thiobarbituric acid-reactive substances (TBARS) assay [39]. This study delineated rapid lipid peroxidation upon cell exposure to copper, followed by a sharp killing effect with the loss of membrane integrity at the peak level of peroxidation. It was noted that DNA degradation only occurred after this point [39] as another evidence that DNA is not the primary target of metallic copper toxicity. Additionally, mutant strains with increased levels of unsaturated fatty acids (Δ*fabR*), were more sensitive to copper toxicity causing TBARS levels to peak earlier. Such observations are indicative of unsaturated fatty acids being targeted by metallic copper toxicity. To understand how metallic copper toxicity affects

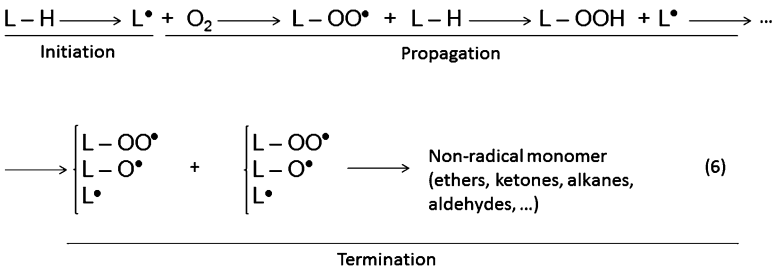

Fig. 6.13 Schematic representing the three steps that characterize lipid oxidation

membrane lipids leading to their oxidation (lipid peroxidation) and degradation (products measured by the TBARS assay), we have to look at how lipid oxidation events occur.

6.3.3.3 Lipid Oxidation Chemistry

Lipid oxidation is a series of oxidative chain reactions that leads to the degradation of lipids and starts with electron transfer from lipids to radicals by free radical chain reaction mechanism. These events are known to happen ubiquitously in biological systems [78], however mechanisms underlying such processes in vivo are not fully understood. Lipid (L) oxidation was shown to follow in three steps: initiation, propagation, and termination (Fig. 6.13) [31, 78].

Polyunsaturated fatty acids are more prone to the electron transfer process than saturated fatty acids due to the presence of the double bond, which stabilize allylic (C=C–C•) radicals formed in oxidation process. Initiation of lipid oxidation starts by removal of a hydrogen from a lipid (unsaturated/saturated), leaving an unpaired electron on the carbon (lipid radical (L•)), which can then react with molecular oxygen to form a peroxyl radical (LOO•) (Fig. 6.13). After that, the peroxyl radical is capable of removing a hydrogen from another lipid, thus propagating the lipid oxidation (Fig. 6.13) [38]. Oxidized lipids rapidly oxidize other lipids leading to further lipid oxidation until non-radical monomers are formed (termination) (Fig. 6.13). Once started, this process is self-propagating and self-accelerating, being designated as autocatalytic. A single initiating-event can lead to about 200–300 chain reactions, showing how effective one initiation -event is [13, 41]. Nevertheless, biological systems developed counter measures antioxidants, capable to control lipid oxidation. When the initiation of lipid oxidation is at higher than normal rates, lipid oxidation can result in irreversible membrane damage and, consequently, cell death.

Lipid oxidation is not a spontaneous reaction but can be triggered very easily. Thermodynamically, direct damage to biomolecules by molecular oxygen is not possible due to different electron spin states, however in the presence of catalysts

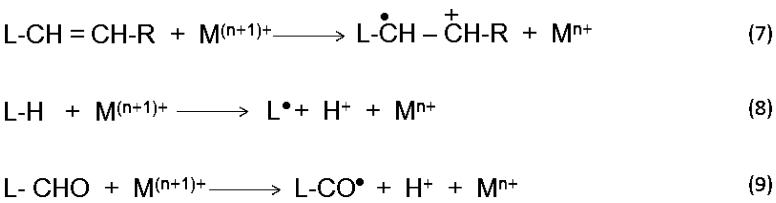

Fig. 6.14 Direct initiation of lipid oxidation by the high valence transitional metals, such as Cu (II). Reaction 7 illustrates an electron abstraction from a double bond; reaction 8 represents a labile hydrogen removal. Reaction 9 is an oxidation of aldehyde group. The aliphatic chain is represented by R

such electron spin barrier can be overcome. Copper, with its one-electron transfer reactions, is considered an active catalyst [85]. Redox active metals, like copper, are able to initiate lipid oxidation by producing lipid alkoyl radicals (LO•) and lipid peroxyl radicals (LOO•) (Fig. 6.13) [78]. In vitro studies were able to show that trace metal amounts sufficed to initiate lipid oxidation [78, 85], and only metals that undergo one-electron transfers appear to be active catalysts; these include cobalt, iron, copper, manganese, magnesium, and vanadium [78]. Initiation of oxidation by redox active metals can occur indirectly by ROS or directly reaction with the lipid. Indirect oxidation by ROS were described previously by Fenton-like reactions (Fig. 6.2), where the hydroxyl radical is the main responsible for hydrogen abstraction [76].

Mechanism and rate of the direct reaction between metal and lipids depend on a multitude of factors: type of formed complex, chelator/complexing agent, redox potential, solvent, phase localization and availability of oxygen or hydroperoxides. Below, we will show the multiplicity of mechanisms that are possible.

The simplest mechanisms for metal catalysis is direct initiation by higher valence metals, such as Cu(II). This implicates electron transfer from the lipid bond to the metal. Lipid radicals (L•) are formed directly by removing an electron from the double bond (Fig. 6.14, reaction 7), or by abstraction of labile hydrogen from lipid molecules (Fig. 6.14, reactions 8 and 9) [78].

Reactions 7 and 8 are the primarily mode of catalysis for cobalt, manganese and chromium [78]. However, other metals, such as copper, can induce these reactions when bound to a chelating agent that shifts the redox potential or in the presence of solvents that alter acid/base properties and electron transfer efficiency. Non-polar environments are also known to allow extremely rapid electron transfers that generate oxidize lipids [8, 9]. Oxidation of aldehydes can also occur (reaction 9) and is strongly catalyzed by Cu(II), as well as Co(II) and Mn(II), and this reaction occurs primarily in non-polar solvents and is inhibited by water competition [78].

In the case of lower valence metal ions, such as Cu(I), activation of oxygen is required to start lipid oxidation. Cu(I) can form complexes with oxygen, thus forming an active complex capable of attacking lipids and form lipid radicals (Fig. 6.15). These reactions are facilitated in hydrophobic environments [12]. Figure 6.15 shows multitude of ways by which Cu(I) can oxidize lipids leading to

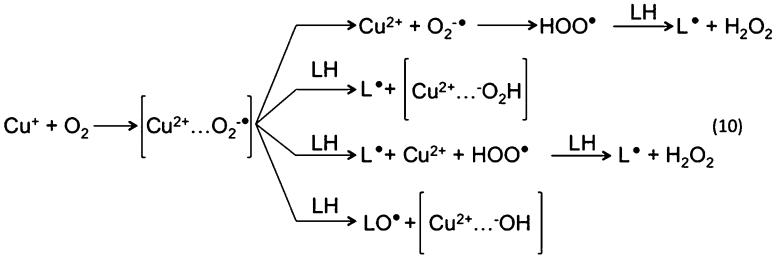

Fig. 6.15 Direct initiation of lipid oxidation by the low valence transitional metal, Cu(I). Oxygen forms a complex with copper, permitting hydrogen abstraction and producing lipid radicals

$$Cu^{+} + LOOH \longrightarrow Cu^{2+} + LO^{\bullet} + OH^{-} \quad (11)$$

$$Cu^{2+} + LOOH \longrightarrow Cu^{+} + LOO^{\bullet} + H^{+} \quad (12)$$

Fig. 6.16 Hydroperoxides can be directly decomposed by copper ions, producing alkoyl radicals (LO•) and lipid peroxyl radicals (LOO•), thus, propagating the lipid oxidation

lipid radicals and ROS. Resulting Cu(II) and ROS (like hydrogen peroxide) can be "re-cycled" back to oxygen and Cu(I).

Furthermore, Cu (I) and (II) are able to propagate lipid oxidation by enhancing the chain reaction to produce more lipid radicals [46, 78]. Copper can form complexes with lipid hydroperoxides decomposing them to lipid alkoyl radicals (LO•) and lipid peroxyl radicals (LOO•) (Fig. 6.16) [76, 78].

The validity of the demonstrated reactions was confirmed in in vitro studies, however in vivo studies still lack to show the extent of each of these reactions. Due to the multitude of lipid oxidation reactions (Figs. 6.14, 6.15 and 6.16), many intermediates can be formed capable of reacting further with formation of other products, making these events very hard to measure and characterize in vivo.

The simple strategy used by [39], was to measure the concentration of a stable lipid oxidation byproduct, such as malondialdehyde (MDA) that reacts with thiobarbituric acid, giving an indirect quantification of lipid oxidation. Thus, authors were able to show that lipid peroxidation occurs before cells are dead, reaching peak in its intensity when membrane integrity is already lost [39].

This study demonstrated that lipid oxidation occurs by metallic copper exposure but the exact mechanism by which copper is able to induce membrane damage still waits to be discovered. Whether toxicity occurs indirectly through ROS or by direct reaction between copper and lipid is still unknown. Additionally, unsaturated fatty acids are more prone to initiation of lipid oxidation, although in bacterial membranes they represent the minority of the membrane lipids. Future studies need to address this specific mechanism of oxidation by demonstrating which lipid is preferably targeted and if saturated fatty acids can be affected by the metallic copper toxicity.

6.4 Holistic "Systems View" of Biocidal Effect of Metallic Copper

There are multiple interrelated molecular factors that play a role during bacterial killing by dry exposure to copper surfaces. When cells get in contact with copper surfaces, copper ions are dissolved from the surface leading to the first steps of cell damage [25, 27, 61, 72]. The presence of copper ions and ROS stress induces toxicity to the membranes, leading to loss of membrane structure (Espírito Santo, Bleichert and Grass, unpublished results, [27, 28, 39, 72]). In a recent study by [53], it was suggested that the major factor that causes cellular damage is the surface ability to generate Cu(I). Further cell damage is induced by copper ions and ROS generation affecting other cellular biomolecules, such as proteins [63]. After cell death, genomic and plasmid DNA become degraded [27, 39]. This mechanism supports the view of the chain of events that lead to cell inactivation by copper surfaces proposed by Grass, Rensing and Solioz [35] (Fig. 6.17).

When cells are applied on a dry metallic copper surface, copper ions are rapidly released and high quantities are quickly accumulated by cells, as outlined by the copper quantification assays in the data published by [27, 28, 72]. Simultaneously, generation of ROS occurs, as evidenced by protective effects of ROS quenchers [25, 86] and by ROS fluorescent indicators [72]. As a consequence, these two related events (copper and ROS generation) induce toxicity and damage cellular components. Indeed, membranes are the first component to be damaged by copper surface toxicity as observed by the Live/Dead™ experiments [27, 28, 72]. Present evidences indicate that membranes are damaged due to lipid peroxidation [39]. Consequently, when lipid oxidation reaches an overwhelming level, the process becomes lethal for the cells. Thus, cells become inactivated by the damage inflicted on the membranes, which then leads to loss of membrane potential [72, 86] and likely release of cytoplasmic contents. Finally, continuous presence of copper leads to further ROS production which induces further damage to various biomolecules,

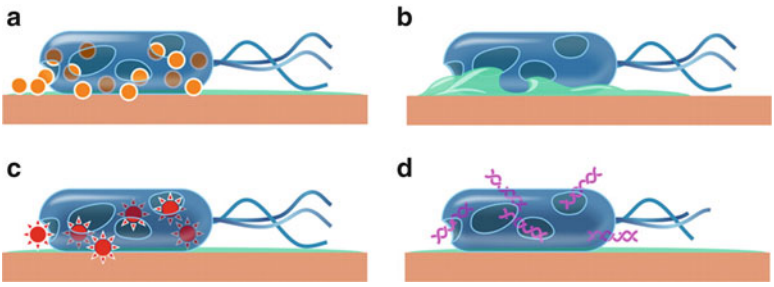

Fig. 6.17 Representation of the chain of events in contact killing. (**a**) Cells enter in contact with the surface; copper is released causing cellular damage. (**b**) Cell membrane becomes permeable due to copper and other stress, leading to loss of membrane potential and cytoplasmic content. (**c**) Generation of reactive oxygen species is provoked by copper ions, which cause further cell damage. (**d**) cellular DNA becomes degraded [35]

including proteins and cellular DNA [27, 39]. Although major steps of this process have been identified, the exact mechanism of this process is still under discussion.

At the same time, Keevil and co-workers [86, 87] proposed an alternative chain of events, suggesting that Cu(I), Cu(II) and superoxide are responsible for killing under wet and dry exposure; and the first event that leads to cell death is DNA damage followed by cessation of bacterial respiration and membrane depolarization, with no observed membrane damage [86]. However, killing experiments performed with *D. radiodurans* and mutation rate experiment with *E. coli* [27], *S. haemolyticus* [28] and *S. cerevisiae* [72] confirmed that DNA is not the first target of copper surface-induced toxicity. Eventually, when cells are dead, DNA becomes degraded, as demonstrated by the comet assay [27]. Additionally, one can assume that freshly surface-released copper and ROS would induce toxicity to the closest biomolecules available – the lipids. Indeed, recent experimental data suggest that lipids are damaged first [39] followed by protein oxidation [63] and copper and ROS are indicated to be contributors for initiation of lipid oxidation processes [78]. In fact, cells accumulate such a high quantity of copper ions that copper-induced lipid peroxidation seems more than likely. Considering the presence of ROS, in particular the highly reactive hydroxyl radical (HO•), lipid peroxidation appears to be unavoidable and leads to further damage through autocatalytic and self-propagating mechanism [78] and is then boosted by the continuous presence of high copper-levels and further ROS production. Additionally, oxidation is rapid, and propagates into many different reactions, which further initiates other reactions leading to deeper lipid degradation. These findings correlate well with the observed fast killing kinetics of cells exposed to copper surfaces. Preliminary data from fatty acid methyl esters (FAME) analysis revealed that the most predominant fatty acids were affected by metallic copper exposure when compared with stainless steel surfaces (Espírito Santo unpublished observations). Further analysis is needed to determine which lipids are mainly targeted by the toxicity and by which reactions occur during oxidation.

Damage to the membranes also can explain the loss of respiration observed by Keevil and co-workers [66, 86–88, 94] likely via loss of the proton motive force. Also, respiration can be inactivated by protein oxidation [63]. Alternatively, as suggested by Warnes & Keevil [86], some cytochromes are inhibited by copper binding through a change in their conformation. However, this alternative seems untimely: the first damage that causes lethality, occurs on the membranes, making the membrane permeable [27, 28, 39] and uncoupling the respiratory chain. Additionally, due to the fast killing kinetics, and given the high copper accumulation and high ROS generation [25, 27, 28, 72], toxicity should not be focused just on cytochromes but on all components of the membrane (including the complete respiratory chain).

6.5 Metallic Copper Under Healthcare Environments

The bacteriostatic effect of copper in hospital settings was reported as early as 1983 by Dr. Phyllis J Kuhn [48]. During a microbiology training for housekeeping and maintenance personnel at the Hamot Medical Center in Pennsylvania, students were given

blood agar plates to perform sampling of diverse sources: toilet bowl water (remarkably clean), salad from the employees' cafeteria (heavily colonized), and doorknobs. Brass (67 % copper and 33 % zinc) doorknob cultures showed scarce staphylococcal and streptococcal growth while stainless steel (about 88 % iron and 12 % chromium) doorknob cultures showed heavy growth of Gram-positive organisms and an array of Gram-negative organisms. Under laboratory conditions, antimicrobial properties of copper surfaces have been well established as outlined in the previous section. However, antimicrobial copper surfaces must also show efficacy as an additional barrier against microbes in healthcare settings. As an important caveat, it should be mentioned that metallic copper surfaces cannot replace strict hygienic conditions but instead act as an additional approach that can help further reduce microbial surface burden and consequently be used to diminish infection rates in patients. It is known that regular cleaning and proper hygiene conditions help to lower transmission-rates of infectious diseases, but complete elimination of germs appears to be unrealistic [16]. Hospital surfaces are highly contaminated with microorganisms, such as *C. difficile*, *Acinetobacter spp.*, *Enterococcus spp* and *S. aureus*, capable to persist on regular surfaces for months [47]. Therefore, the usage of a self-sanitizing antimicrobial surface might strongly diminish transmission of microbes to humans by reducing fomite contamination (Fig. 6.2). Worldwide hospital trials confirmed the suitability of use of metallic copper as an antimicrobial surface [6, 44, 52, 60]. These trials were able to validate that metallic copper surfaces effectively reduced surface burden compared to control surfaces (such as stainless steel, aluminum and plastic). During the 2010 trial in the Selly Oak Hospital in Birmigham, United Kingdom [6], recovery of microbes was between 90 and 100 % lower from copper surfaces compared to control surfaces. Copper surfaces remained active even when these surfaces were oxidized ("aged") over time. Similar positive results were obtained by [44], where copper alloys (greater than or equal to 58 % copper) reduced microbial quantity on the surface compared with control surfaces, as well as by [52], in a South African trial reporting reduction of bacteria survival rate by 71 % on copper. The German trial also reported a surface burden reduction in the magnitude of 63 % [60]. Furthermore, the repopulation rate of copper surfaces was less than half compared to that of control surfaces.

There are still ongoing trials worldwide. Promising results were obtained in a trial that involves three hospitals: the Memorial Sloan-Kettering Cancer Center in New York City, the Medical University of South Carolina, and the Ralph H. Johnson VA Medical Center, both in Charleston [77], where application of metallic copper lowered infection-rates for patients in rooms with copper objects compared with the ones without copper objects [77]. See Chap. 4 for more details.

6.6 Closing Remarks

Despite all the differences regarding the mechanism of copper surface–induced inactivation of bacteria cells, it is clear that the use of metallic copper is an important concept in the area of reducing healthcare acquired infections (HAI).

Hospitals and other public places are expected to benefit from these natural antimicrobial properties of metallic copper which quickly inactivate microbes. Moreover, use of such materials is expected to have only low probability of emergence of resistance due to DNA degradation in the killing process. From the practical point of view, these surfaces are easy to apply and can also be adjusted to any targeted environment. Finally, the history of copper usage for thousands of years with little toxicity observed proves that metallic copper surfaces are quite safe for human usage. Overall, all these qualities make use of copper a valuable complimentary tool for hygiene in preventing spread of HAI.

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Chapter 7
An Overview of the Options for Antimicrobial Hard Surfaces in Hospitals

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Abstract Contaminated surfaces make an important contribution to the transmission of several important pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA), *Clostridium difficile* and a number of resistant Gram-negative

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rods, including *Acinetobacter baumannii*. Several different approaches are available for improving hospital hygiene, including improving the effectiveness of existing methods and a range of new approaches, including novel disinfectants. A complimentary approach is the introduction of antimicrobial surfaces (AMS), which exert a continuous reduction on the level of microbial contamination on hospitals surfaces. There are several approaches to making a hospital surface 'antimicrobial': permanently 'manufacture in' an agent with antimicrobial activity; periodically apply an agent with antimicrobial activity; or physically alter the properties of a surface to make it less able to support microbial contamination and/or easier to clean. Promising options for AMS in healthcare settings include metals (principally copper or silver), chemicals (organosilanes, quaternary ammonium compounds, light-activated antimicrobials, and polycationic polymers) and physical alteration of the surface to reduce microbial attachment or improve cleanability. Before widespread adoption of AMS, promising candidates require rigorous *in vitro* and *in situ* assessment, including an evaluation of their clinical impact and cost effectiveness. Copper alloy surfaces are the most closely evaluated option for AMS, and have demonstrated *in vitro* activity against a range of pathogens (although their sporicidal capacity remains equivocal), evidence of efficacy in *in situ* studies and their introduction has been associated with a reduction in healthcare-associated infections (HAI). However, their long-term durability, acceptability and cost-effectiveness have not been evaluated formally. Finding and evaluating the optimal AMS will require a multidisciplinary approach, involving industrial partners, materials scientists, healthcare scientists and epidemiologists to refine and test the available options.

List of Abbreviations

AMS	Antimicrobial surfaces
CFU	Colony forming units
DLC	Diamond-like carbon
EPA	Environmental Protection Agency
HAI	Healthcare-associated infections
HPV	Hydrogen peroxide vapour
ICU	Intensive care unit
MDRO	Multidrug-resistant organisms (MDROs)
MRSA	Methicillin-resistant *Staphylococcus aureus*
PEG	Polyethylene glycol
PHMB	Polyhexamethylene biguanide
QAC	Quaternary ammonium compound
R-GNR	Resistant Gram-negative rods (R-GNR)
TAC	Total aerobic count
VRE	Vancomycin-resistant enterococci

7.1 Role of the Environment in Transmission

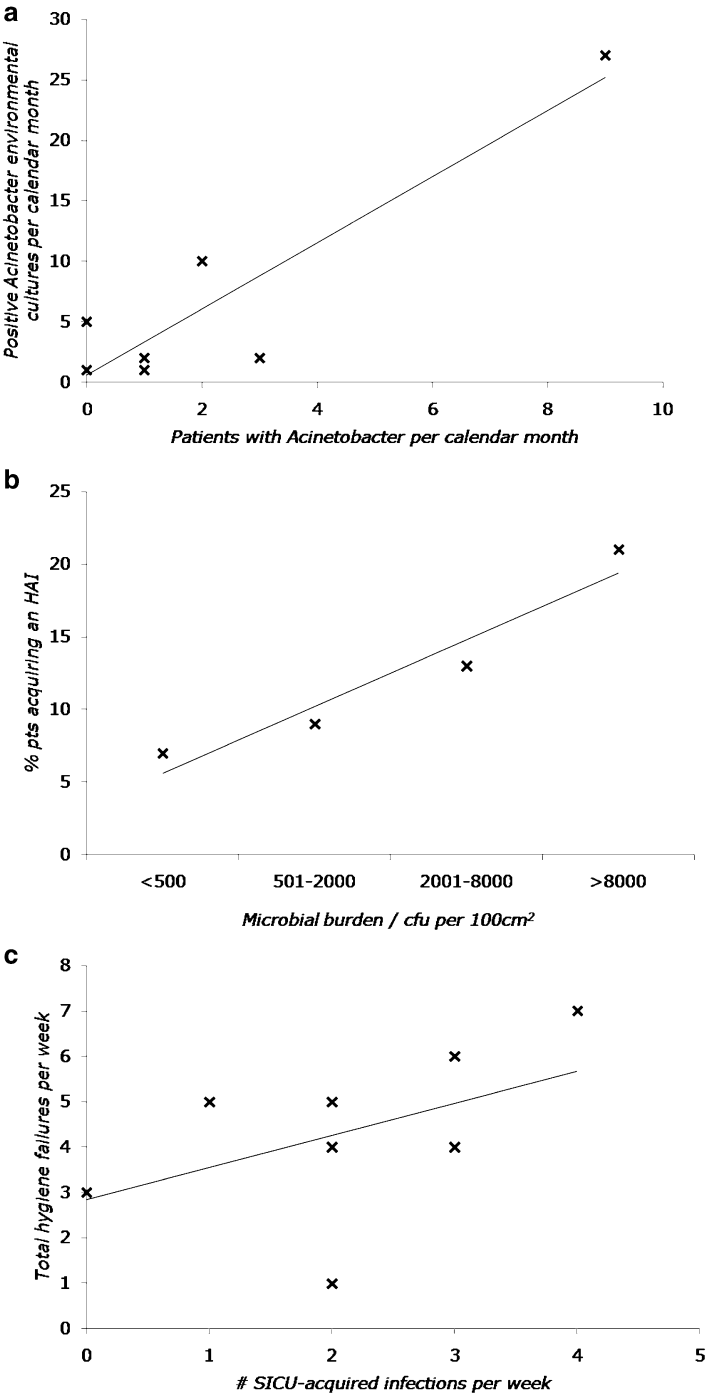
7.1.1 Evidence That Contaminated Surfaces Contribute to Transmission

The contaminated environment has historically been considered to play a negligible role in the transmission of most hospital pathogens [1, 2]. However, the healthcare environment has been shown to become contaminated with multidrug-resistant organisms (MDROs) such as methicillin-resistant *Staphylococcus aureus* (MRSA), *Clostridium difficile* and some resistant Gram-negative rods (R-GNR) including *Acinetobacter baumannii* [3–6]. Transmission routes of pathogens are complicated and difficult to investigate so studies focused on the role of surfaces in transmission have been rare until relatively recently [2]. Data suggesting that contaminated surfaces play an important role in transmission come from studies modeling transmission [7–9], microbiological studies *in vitro* and *in situ* [3, 10–12], observational epidemiological studies [13–18], intervention studies aimed at improving the efficacy of cleaning and disinfection [5, 19–24] and outbreak reports [25–27].

Recent epidemiological evidence suggests that patients admitted to rooms previously occupied by a patient with environmentally-associated pathogens increases the chances of acquiring the same pathogen [14–16, 28]. The most likely explanation is residual contamination from the prior room occupant. The epidemiological association is strengthened by the finding that improving terminal disinfection mitigates the increased risk from the prior room occupant [19, 21].

7.1.2 The Relationship Between Contamination Burden and Transmission Risk

The relationship between the level of surface contamination and the risk of transmission has not been studied in detail. It depends on various factors, including the characteristics of the organism involved, patient susceptibility, and staff compliance with infection control policies (for example hand hygiene following contact with environmental surfaces) [11, 29, 30]. A number of studies have identified a correlation between a quantitative or semi-quantitative measure of the level of environmental contamination and the risk of pathogen acquisition (Fig. 7.1) [25, 31, 32]. However, since none of these studies demonstrate that an intervention to reduce the level of contamination reduces the risk of transmission, the correlation could be explained by the fact that patients who are already infected or colonized shed more contamination into the environment, which is plausible. Whilst one of the three studies (Salgado et al.) did evaluate an intervention, the data correlating contamination burden with HAI was not stratified by the intervention, which would have been one way to assess likely causation [31].


a
Positive Acinetobacter environmental cultures per calendar month
Patients with Acinetobacter per calendar month
b
% pts acquiring an HAI
<500
501-2000
2001-8000
>8000
Microbial burden / cfu per 100cm²
c
Total hygiene failures per week
SICU-acquired infections per week

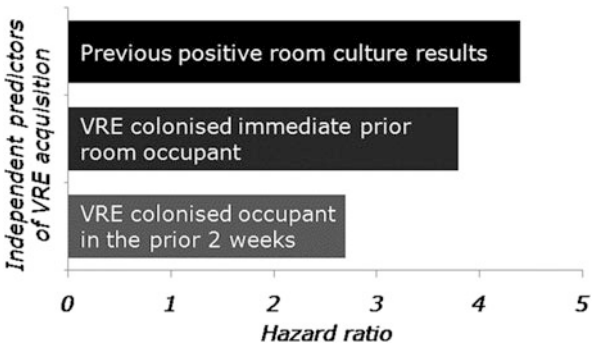


Fig. 7.2 How the increased risk of acquiring VRE from the prior room occupant changes due to patient and environmental factors (Data extracted from Drees et al. [13])

The fact that subsequent occupants of a room vacated by a previously colonised or infected patient are at an increased risk of infection indicates that conventional terminal disinfection does not reduce contamination sufficiently to prevent transmission in these cases [2, 13–16]. Further, one of the studies demonstrating that admission to a room previously occupied by a patient with vancomycin resistant enterococci (VRE) increases the chances of VRE acquisition identified something amounting to a 'dose response' [13]. The greatest increased risk was for patients admitted to a room with an environmental culture positive for VRE, and being admitted to a room where the immediate prior room occupant was colonized with VRE carried a greater increased risk than being admitted to a room where any patient in the 2 weeks prior to admission was VRE colonized (Fig. 7.2).

Another strand of evidence suggesting a causal relationship between contamination burden and the risk of transmission comes from the finding that improving the level of terminal disinfection mitigates or eliminates the increased risk from the prior room occupant [19, 21]. Improving terminal disinfection by modifying conventional methods (the use of UV markers, immersing the cloth in the disinfectant and education of cleaners) resulted in a significant mitigation of the increased risk for MRSA but not for VRE [21]. Whereas in another study, the introduction of hydrogen peroxide vapour (HPV) 'no-touch' automated room disinfection resulted in an elimination of the increased risk, to the extent that patients admitted to rooms disinfected using HPV were less likely to acquire an MDRO even than patients admitted to rooms where the prior occupant was not known to be infected or colonized with an MDRO [19]. Both studies demonstrate that interventions to enhance the level of reduction in environmental burden result in reduced patient acquisition, and thus suggest a causal relationship between the level of contamination and transmission. Furthermore, since HPV is associated with an elimination of

Fig. 7.1 Studies correlating environmental contamination burden and transmission. (**a**) Correlation between the number of patients infected with *Acinetobacter* spp. and the number of positive *Acinetobacter* spp. environmental cultures per calendar month during an outbreak on a neurosurgical intensive care unit (ICU) [25]. (**b**) Correlation between microbial burden and the number of patients who acquired a hospital acquired infection in ICUs [31]. (**c**) Correlation between the number of hygiene failures and the number of patients who acquired an infection on a surgical ICU each week [32]

pathogens from surfaces whereas enhanced conventional disinfection is associated with a reduction but not elimination of pathogens [3, 33], it seems likely that the improved environmental reduction achieved by HPV explains the improved clinical outcomes due to a greater reduction on the environmental burden. However, further studies are necessary to confirm this point.

There is *in vitro* evidence that the extent to which transmission is interrupted is proportional to the level of surface contamination. Lawley et al. used an *in vitro* mouse model to show that the degree to which transmission of *C. difficile* was blocked correlated with the log-reduction of the various disinfectants tested [8].

Despite the evidence that there is a causal relationship between the concentration of contamination and the risk of transmission, the level of contamination on a surface that is a risk for onward transmission is not known. The degree of shedding and the infective dose can be used to guide the appropriate target for hospital cleaning and disinfection. Certain pathogens such as *C. difficile* and norovirus can be shed into the environment in high numbers and have a low infectious dose [2, 34, 35]. For example, stool concentrations of norovirus can reach more than 1×10^{12} particles per gram [2] and up to 10^5 virus norovirus particles per 30 cm^2 have been identified on hospital surfaces [36], whereas the infectious dose is 1–100 particles [35]. Therefore, the presence of a pathogen on a surface at any concentration may be a risk for transmission. This is reflected in proposed guidelines for microbiological hygiene standards [37] and recent discussion surrounding the intended target for hospital disinfection [38, 39]. Dancer [37] proposed an absence of specific indicator organisms (*S. aureus*, including methicillin-resistant *S. aureus*, *C. difficile*, VRE and various Gram-negative bacilli), and a quantitative aerobic colony count of <5 cfu/cm^2. The <5 cfu/cm^2 was selected based on its use in some sectors of the food industry. A lower standard of <2.5 cfu/cm^2 has been adopted in recent years [32, 40–42]. Whilst it has not been demonstrated that contamination below this level is ‘safe’ and above this level is ‘unsafe’, the number of hygiene failures has been correlated with the acquisition of pathogens (Fig. 7.1) [32, 43].

7.1.3 Potential Role for Antimicrobial Surfaces

A number of different interventions aimed at improving environmental hygiene have been evaluated. Approaches to improve the efficacy of existing methods include increased staff training, more hours for cleaning staff, and the use of fluorescent markers or ATP analysis of surfaces to performance-manage the cleaning process [21, 22, 40, 44, 45]. Novel methods include new disinfectants with superior efficacy, the advent of ‘no-touch’ automated room disinfection systems that do not rely of the operator to assure adequate distribution and contact time of the chemical agent, and new cleaning materials such as microfiber [24, 33, 46].

Switching from one disinfectant to a product with superior microbiological efficacy in particular has been shown to reduce transmission [5, 20, 23, 24, 47, 48]. However, one of the problems with available disinfectants is the

lack of residual effect, meaning that recontamination occurs quickly [49, 50]. Another problem is that a high proportion of surfaces in a room are missed during cleaning and disinfection [51]. Antimicrobial surfaces (AMS) offer the potential for providing a continuous reduction on microbes deposited on surfaces, which provides a complimentary approach to improving hospital surface hygiene. AMS have a potential role in supplementing, to an extent, deficiencies in conventional cleaning and disinfection. AMS also have a potential role even when conventional cleaning and disinfection is functioning perfectly, since surfaces cannot feasibly be continuously cleaned and disinfected using standard methods.

AMS have a number of inherent limitations. Firstly, in general, contamination is reduced but not eliminated by AMS. It would be possible to create a surface that would eliminate pathogens on contact, but this surface would not be safe for human contact. Continuous sub-lethal exposure of microbes to any antimicrobial agent is likely to result in some level of microbial reduced susceptibility, which is therefore a potential concern for all AMS. Also, it is not clear what level of environmental contamination burden reduction is necessary to block transmission, as discussed above. Secondly, it is not feasible to make all surfaces in a room antimicrobial with most AMS technologies. Also, sometimes it may not be feasible to make the highest-risk touch surfaces antimicrobial with some technologies. Thirdly, the introduction of AMS will be associated with some cost, which requires evidence-based justification. Fourth, the long-term durability of AMS in the busy healthcare environment has not been established for many technologies. Finally, there may be problems with patient and staff acceptability of AMS for some technologies.

7.2 Current Options and the ‘Ideal’ Candidate for Antimicrobial Surfaces

A number of review articles provide an overview of AMS [52–55], and others have reviewed the literature related specifically to copper [56, 57] or silver surfaces [58]. A number of these reviews have used the term ‘self-disinfecting surfaces’. This term suggests an active disinfection process, which does not properly capture the continuous disinfection activity. Thus, I have used the term ‘antimicrobial surfaces’ (AMS) throughout this review.

There are several approaches to making a hospital surface ‘antimicrobial’ (Table 7.1):

- Permanently ‘manufacture in’ an agent with antimicrobial activity (e.g. copper or a chemical).
- Periodically apply an agent with antimicrobial activity (e.g. copper containing liquid agents, or chemical disinfectants with residual activity).
- Physically alter the properties of a surfaces to make it less able to support microbial contamination and/or easier to clean (e.g. a coating that make a surface ‘superhydrophobic’).

Table 7.1 An overview of the options for antimicrobial hard surfaces in hospitals

Candidate	Application	Mode of action	Pros	Cons
Metals				
Copper	Manufactured in [31]/ retrofitted [70, 124]/ liquid disinfectant [68]	Redox activity of copper resulting in reactive oxygen species [57]	Rapidly microbicidal; [125] large evidence-base; [57] evidence of reduced acquisition [31]	Sporicidal activity equivocal; [60] cost, acceptability and durability may be questionable [53]
Silver	Manufactured in [77]/liquid disinfectant [97]	Microbes accumulate silver until toxicity threshold exceeded [52].	Broadly microbicidal [55, 81]	? sporicidal; tolerance development; relies on leaching so surface loses efficacy over time [55, 81]
Chemicals				
Organosilane	Periodic application [72, 103]	Microscopic physical damage to microbe through direct contact [72]	Easy to apply [72, 103]	Limited microbicidal activity; questionable "real-world" efficacy [72, 103]
Light-activated (e.g. titanium dioxide or photosensitisers)	Manufactured in/periodic application [75, 109]	Microbicidal reactive oxygen species generated when irradiated with light of a suitable wavelength [75, 109]	Broadly microbicidal; can be activated by natural light [75, 109]	? sporicidal; requires light source for photoactivation (some require UV light); may lose activity over time [75, 109]
Quaternary ammonium compound based agents with residual activity	Liquid disinfectant [105]	Disruption of cell membrane [105]	Easy to apply, well-adopted [105]	Limited microbicidal activity (formulation dependent); limited evidence for residual activity; environmental toxicity [105]

Triclosan	Manufactured-in/ liquid disinfectant [52]	Multiple cytoplasmic and membrane targets [112]	Already adopted in some consumer markets [52]	Resistance/tolerance development; relies on leaching so surface loses efficacy over time [52, 112]
Polycationic e.g. polyhexamethylene biguanide, PHMB	Liquid disinfectant [69]	Active polymer immobilised microbes; PHMB disturbance of cell membrane lipid bilayer [69]	Easy to apply [69]	Limited microbicidal activity; questionable "real-world" efficacy and durability [69]
Physical alteration of surface properties				
"Liquid glass" (silicon dioxide)	Liquid application [126]	–	Reduces deposition; improves 'cleanability' [126]	Not microbicidal; some evidence of reduced contamination; unknown required frequency of application [126]
Sharklet pattern	Manufactured-in [113, 114]	–	Reduces deposition; reduced. biofilms [113, 114]	Not microbicidal; not feasible to retrofit [113, 114]
Advanced polymer coatings (e.g. polyethylene glycol PEG, superhydrophobic/ philic, zwitterionic)	Manufactured-in [115, 118]	–	Reduces deposition; some can be 'doped' with copper or silver [115, 116, 118]	Not microbicidal; may be expensive; scale up to large surfaces questionable; not feasible to retrofit [115, 118]
Diamond-like carbon (DLC) films	Manufactured-in [119]	–	Reduces deposition; can be 'doped' with copper or silver [119]	Not microbicidal; likely to be expensive; feasibility of scale up to large surfaces questionable; not feasible to retrofit [119]

The introduction of hospital textiles with antimicrobial activity is another option, which is reviewed elsewhere [55, 59]. The optimal deployment mode for AMS is unclear: should AMS be manufactured-in or periodically applied, or are ways to make the surface physically less able to support contamination or easier to clean preferable? Furthermore, it may be possible to make a surface physically less able to support contamination, easier to clean and exert AMS properties.

To permanently manufacture-in an AMS is advantageous for a number of reasons. There is no concern with the adhesion and durability of a coating, and the various issues associated with application are not a concern, for example, frequency, cost and thoroughness. However, it is probably not feasible to manufacture-in the same AMS for all items in a room, limiting this mode of deployment to a fairly small number of high-touch items in real terms. Another approach is to periodically apply an agent that offers residual AMS properties. If periodic application is selected, the frequency and durability of application are key concerns. An effective disinfectant with residual activity that does not compromise staff or patient safety or promote the development of reduced susceptibility is desirable, and could be delivered through pre-existing cleaning and disinfection arrangements at little or no extra cost.

7.2.1 Considering the 'Ideal' Antimicrobial Surface

Although not achievable with currently available candidate technologies, a consideration of the properties of an 'ideal' antimicrobial hospital surface is helpful:

- Versatile application. Ideally, the active ingredient would have the capacity to be manufactured-in or applied as liquid agent.
- Non-leaching. This will mean that the surface remains antimicrobial for its lifetime. Furthermore, the efficacy of AMS that rely on leaching an active agent from a surface may be considerably less effective in a dry environment and should be tested accordingly.
- Rapid antimicrobial activity. The deposition of contamination and potential acquisition of contamination through contact with surfaces often occurs in quick succession, so antimicrobial surfaces with a contact time measure in hours (rather than minutes or seconds) may be too slow to be useful.
- Sporicidal. *C. difficile* spores represent a real challenge to antimicrobial surfaces. Copper seems to get closest to demonstrating inactivation, but even here data are somewhat equivocal [60]. There is a legitimate concern that introducing an AMS that is not effective against *C. difficile* could provide a selective advantage to *C. difficile*.
- Retains activity with low-level soiling. Surfaces in hospitals are often dirty; it's not clear how much the presence of organic matter would interfere with the activity of AMS. Clearly, AMS do not obviate the need for careful attention to hospital cleaning and disinfection. In fact, their continued effectiveness depends on it.

- Does not promote clinically-significant resistance or reduced-susceptibility. There is a risk that continuous sub-lethal exposure to microbes could occur on AMS, and that this may lead to the development of resistance or reduced susceptibility.
- Prevents biofilm formation. The ability to prevent the formation of biofilms, or disrupt biofilms that have been formed, is a property of some oxidizing disinfectants [61]. This property may be shared by AMS that exert antimicrobial activity through oxidization. Also, modification of the physical structure of surfaces may reduce biofilm formation.
- Compatible with current cleaning and disinfection products. The chemicals that are used for regular cleaning and disinfection of the AMS should not interfere with the antimicrobial activity of the surface, either in the short- or long-term.
- Safe. AMS must remain safe for regular and intimate contact with patients, staff and visitors. AMS will sometimes come into contact with sensitive areas and broken skin, and contact with the mouth and other mucous membranes should be assumed possible.
- Low-cost. All AMS will be associated with a cost of some kind, which may be absorbed into the manufacturing process for some products and coatings.

7.3 Assessing Antimicrobial Surfaces

7.3.1 In Vitro *Activity*

The first test of an AMS is an *in vitro* laboratory assessment of antimicrobial activity. A number of test methodologies have been proposed. Historically, an ISO standard test has been used (ISO22196), but test method is not appropriate for testing surfaces specified for dry hospital surfaces since it is performed at high humidity (100 %) and temperature (37 °C) [62]. A standardized test has been proposed that better reflects in-use conditions for dry hospital surfaces, but not yet adopted widely [62]. Importantly, this methodology specifies an aerosol deposition of microbes whereas other proposed methodologies specify the deposition of microbes in a liquid suspension. Testing the 'wet' deposition of microbes may overestimate the antimicrobial potential of the surfaces, which would usually be challenged with dry deposition in the real world. Another option is a United States Environmental Protection Agency (EPA) test method for chemical agents with residual activity [63], which has been modified and applied to metallic copper (including alloys) [64] and copper oxide impregnated surfaces [65]. This method also includes a test for the impact of abrasion on the activity of the surface.

Since standard testing methods have not been available, *in vitro* evaluations of AMS have been performed using a wide range of parameters, principally, the test organisms, concentration of the inoculum, method of deposition (including wet and dry inocula), microbial recovery, contact times, temperature and relative humidity

conditions [57]. These differences make comparison between studies very difficult. Thus, since few studies have evaluated more than one AMS under the same conditions, evaluating the relative *in vitro* activity of different AMS technologies is problematic.

Activity against bacterial endospores, and *C. difficile* in particular, is particularly problematic for AMS. The application of a 'germination solution' through the usual cleaning and disinfection channels may provide a useful angle for further research [66].

7.3.2 In Situ *Activity*

An important step to assess an AMS specified for dry hospital surfaces is to evaluate its ability to exert an antimicrobial activity when applied in the clinical setting. The development of meaningful *in situ* evaluations of AMS is challenging for a number of reasons. First, microbes in the environment will be determined to a great degree by microbes shed by individual patients [2, 67]. Also, considerable variation in the level of contamination occurs on various objects within the same room [43, 68]. Further, the method chosen to sample the environment introduces still further variability, with some methods having a great sampling efficiency than others [69]. Therefore, *in situ* studies of AMS should be carefully designed and large enough to statistically control for these variables. Most study designs have been prospective observational studies, although a number have introduced a degree of randomization or cross-over to strengthen the study design (Table 7.2).

Second, defining the target in terms of environmental burden reduction, and the method to assess the level of contamination and the impact of AMS are challenging. A number of different microbiological and non-microbiological methods have been applied to assess the impact of AMS (Table 7.2). A commonly used method is a total aerobic count (TAC) determined using either swabs or contact plates. Several studies have also performed quantitative or semi-quantitative culture for indicator organisms, such as *S. aureus*. Few studies have evaluated the presence of anaerobes or *C. difficile* spores. A handful of studies have used ATP analysis to evaluate the impact of AMS on surface hygiene. Most studies have evaluated the impact of the AMS by quantitative comparison of the TAC. Meanwhile, a number of studies have evaluated the impact of AMS on the proportion of 'hygiene fails' using the standards defined above. A small number of studies have evaluated both TAC and hygiene fails, with similar results [70–72].

Third, since microbes are likely to be continuously deposited on surfaces in the healthcare environment, it is not reasonable to expect that a surface would have no contamination at any point in time. But the continual deposition of microbes at an undefined rate makes it difficult to determine the speed of activity of an AMS *in situ*. However, this can be inferred from some studies. For example, Casey et al. compared the level of contamination on copper and non-copper nurses' pens immediately after a shift and again after 11 h of storage [73]. They found

Table 7.2 *In situ* evaluations of antimicrobial surfaces in healthcare settings

Author/ year	Location	Setting	Antimicrobial surface	Design	Sampling schedule	Findings	Comment
Schmidt 2013 [70]	Charlson, South Carolina, USA	ICU	Copper caps on bedrails (99.9 %)	Prospective observational study. The rails on beds in 3/17 rooms were fitted with copper surface caps	Copper and control bedrails on seven beds per group were sampled before cleaning then 0.5, 2.5, 4.5 and 6.5 h after cleaning	The cfu count on the copper bedrails was significantly lower than on the controls at all time points, excepting 30 min after cleaning	Copper bedrails were associated with significantly less 'hygiene fails' than controls (34/75 for copper vs. 58/75 for controls). Co-authored by the CDA
Schmidt 2012 [43]	Three hospitals, USA	ICUs	Copper alloy (75–99.99 %)	Forty-three month multicentre intervention study; the intervention, commencing at month 23, was fitting six high-touch objects with copper alloy in half of the 16 study rooms	Surfaces were sampled weekly throughout the study phases, resulting in a 23 month pre-intervention phase, and a 21 month intervention phase	Significant 83 % reduction in the mean cfu count on copper surfaces (mean 465 cfu/100 cm^2 on copper vs. 2,674 cfu/ 100 cm^2 for control); significant reduction on 5/6 individual copper surfaces; frequency of isolating indicator organisms was also reduced	There was a striking 64 % reduction in contamination of the control objects between pre- and intervention phases. Marked differences in the cfu count of the six objects; bed rails consistently most heavily contaminated
Karpanen 2012 [127]	Birmingham, UK	Nineteen bed acute medical ward	Copper alloys (58–99.95 %)	Cross-over; 14 copper alloy hand touch sites vs. control items made from other materials	Swabs used to sample copper items and matched controls in duplicate weekly or fortnightly. Items crossed over after 12 weeks of sampling, for a further 12 weeks of sampling following a 16 week "wash out" period	Bacterial growth was lower on all copper items, and significantly lower on 8/14. Indicator organisms were grown from significantly fewer copper items (18 % of 542 control items vs. 8 % of 559 copper items)	Items installed for a least 3 months prior to study start. Hand hygiene compliance, staffing levels and bed occupancy were not significantly different in the study phases. Contamination with *C. difficile* was not significantly different on copper items. No indicator organisms exhibited reduced copper susceptibility. Funded by CDA

(continued)

Table 7.2 (continued)

Author/ year	Location	Setting	Antimicrobial surface	Design	Sampling schedule	Findings	Comment
Casey 2011 [73]	Birmingham, UK	Two critical care units	Copper alloy (85 %)	Prospective randomized observational study. Contamination of randomly assigned copper or stainless steel control pens during a 12.5 h shift	Twenty-five copper and 25 control pens sampled using swabs immediately after a 12.5 h shift; a second set of 50 pens sampled after storage at room temperature for 11 h following the end of the shift	Contamination rate and median cfu count significant lower for copper pens after 11 h storage; cfu count only significantly lower for copper pens immediately after shift	None of the pathogens infecting or colonizing the patient who was being cared for were cultured from the pens
Casey 2010 [71]	Birmingham, UK	Acute medical ward	Copper alloys (60–70 % Cu)	Cross-over; three copper alloy items (toilet seat, tap handles, push plate) vs. control items made from other materials	Swabs used to sample copper items and matched controls in duplicate once weekly at 0700 or 1,700 for 10 weeks. Items crossed-over after 5 weeks, at the study mid-point	Significant 90–100 % reduction in bacterial contamination in 9/10 paired control/copper items; indicator pathogens isolates from control but not copper items; less 'hygiene fails' on copper items	Items installed 6 months prior to study start so that staff became accustomed to them. No MRSA or *C. difficile* was identified throughout the study. Funded by CDA
Marais 2010 [124]	Grabouw, South Africa	Walk-in primary care clinic	Copper (99.9 %)	Prospective observational study. One of two similar consulting rooms was fitted with copper sheets on the desk, two trolleys, cupboard and windowsill	Swabs used to sample 12 sites on the five surfaces in both rooms daily from Mon-Fri every sixth week over 6 months. Three samples collected each day: pre-clean, post-clean and post-consultation	Significant 71 % reduction in mean bacterial count on copper surfaces (mean count 5.9×10^4 cfu/cm^2 for copper vs. 2.0×10^5 cfu/cm^2 for control). Colony counts similar over the weekend when clinic unoccupied	Residual contamination on copper and control surfaces differed up to twofold after cleaning. Substantial seasonal temperature changes (indoor range 15–33 °C) did not affect efficacy. Funded by CDA
Mikolay 2010 [76]	Hamburg, Germany	Respiratory and geriatric wards	Copper alloys (concentration not specified)	Prospective observational study. Forty-eight push plates, 48 door handles and 48 light switches replaced with copper alloys	Direct agar contact performed once or twice per week for 16 weeks in the summer and 16 weeks in the winter; total aerobic count and presence of ciprofloxacin-resistant *Staphylococcus* (CRS) determined	Significant 22 % reduction in bacterial count overall (total 27,467 cfu on copper vs. 35,249 on controls); counts on push plates and light switches not significantly lower. Counts of CRS were reduced, but not significantly	Increase in mean cfu after cleaning was 12–14 cfu/h on copper vs. 22–33 cfu/h on controls. Funded by the German Copper Institute

Hamilton 2010 [68]	Dumfries and Galloway, Scotland, UK	Three wards plus A&E	Copper-containing liquid disinfectant	Seven week cross-over; UMF mops wetted with water (control) or copper-containing disinfectant. Two wards began with control (weeks 1–3), followed by copper (weeks 4–7), vice versa for the other two wards	Ten standardised sites per ward sampled using contact plates on Mon, Weds and Fri 1 h before cleaning then 1 and 4 h after cleaning	UMF + copper median counts significantly lower than control at all time points. The cross-over yielded the anticipated findings, with lower median counts associated with UMF + copper across both study arms	Wide variation in the level of contamination across the 13 sites included in the study. No sampling for indicator organisms was performed. The residual effect of UMF + copper took a number of weeks to become established, perhaps due to accumulation. Co-authored by the copper disinfectant manufacturer
Varghese 2013 [98]	Salford, UK	Toilet cubicle	Silver-silicia CVD coating	Prospective observational study. Coated tiles were mounted on wood and placed adjacent to a toilet in a cubicle	Coated and control tiles were swabbed after 2 weeks, and 2, 3 and 4 months	The coated tiles had a 95 % lower level of contamination after 2 weeks, and 99.8 % lower contamination after 4 months compared with controls	The coating head is limited to 10 cm wide substrates. The concentration on the control tile exceeded 10^3 cfu/cm^2 in study month 4
Taylor 2009 [77]	Not specified	Two comparable outpatient units	Silver ion treated items	Prospective observational study. A range of silver ion items were included in unit A; matched items of similar material without silver were used as controls in unit B	The silver and control ideas were sampled at baseline (12 months after installation), 2, 6 and 12 weeks	Bacterial counts were 62–98 % less contaminated on silver items compared with controls. Effectiveness varied with the type of surface finish from 99 % (laminate) to 70 % (fabrics)	Items were installed for 12 months prior to the start of swabbing. Counts from wet silver surfaces were lower than from dry surfaces. Counts on untreated products in unit A (silver) were lower than in unit B (control)
Keward 2013 [105]	Liverpool, UK	Cardiology ward and HDU	QAC	Prospective 2-week observational study; first week chlorine dioxide, second week QAC	ATP used to assess cleanliness of 18 high-touch sites on day 1, 4 and 7 of each week	17/18 sites had significantly lower ATP when cleaned with the QAC	QAC cheaper than chlorine dioxide. Staff surveys showed improved acceptability for the QAC over chlorine dioxide
Boyce 2014 [72]	New Haven, Connecticut, USA	Rehabilitation ward	Two organosilane products	Prospective randomized observational study. Three rooms randomized	Nine high-touch sites were sampled daily for 4 weeks using contact	Neither product yielded lower mean bacterial counts than those	Although no data are provided, the results would have looked

(continued)

Table 7.2 (continued)

Author/ year	Location	Setting	Antimicrobial surface	Design	Sampling schedule	Findings	Comment
				to each organosilane, and three control rooms	plates prior to daily cleaning	observed in control rooms; counts were significantly lower in control room for 3/9 sites	similar if a 'hygiene fail' approach had been taken
Thom 2014 [103]	Baltimore, Maryland, USA	Ten bed surgical ICU	Organosilane	Prospective intervention study. Five rooms treated with organosilane, five rooms left untreated	Standardised sites sampled twice weekly for all rooms (when the occupant had stayed >24 h)	No significant difference in the proportion of rooms contaminated with bacteria (90 % treated room vs. 83 % untreated rooms)	Sampling methods were not quantitative. No significant difference in the proportion of rooms contaminated with individual pathogens of interest
Hedin 2010 [69]	Falun, Sweden	Infectious diseases ward	PHMB + active polymer	Prospective observational study. Twelve bedside tables; half of each table treated with PHMB polymer, half left untreated	Treated and untreated sides of the tables sampled 1 day after application. Experiment repeated three times over three consecutive weeks	Two of three sampling methods showed a significant reduction associated with PHMB polymer; 50 % reduction in median counts	Tables were not cleaned during the study. Three different sampling methods were compared
Decraene 2008 [74]	London, UK	Dental clinic	Cellulose acetate coating + photosensitsers	Prospective observational study. Cellulose acetone coatings with and without photosensitisers (toluidine blue O and rose bengal)	Open agar plates and petri dishes with control and test coatings placed on a shelf adjacent to dental chairs and exposed to the air for 24 h	Significant median 64 % reduction in aerobic bacteria and 82 % reduction in anaerobic bacteria on the test coatings vs. control	Required illumination by a lamp. Similar concentration of aerobes cultured from agar plates and the treated surfaces

Ismail 2011 [75]	London, UK	Dental clinic	Silicon polymers + photosensitiser methylene blue (MB) +/− gold nanoparticles (Au)	Prospective observational study. Silicon polymer coatings supplemented with either MB only or MB + Au	Open agar plates and petri dishes with control and test coatings placed on a shelf adjacent to dental chairs and exposed to the air for 24 h	Significant mean 55 % reduction in aerobic bacteria and mean 71 % reduction in anaerobic bacteria on MB + Au coatings. MB only coatings had reduced counts vs. control, but not statistically significant	Required illumination by a lamp. In vitro studies using MRSA on the same surface types demonstrated substantially greater log reductions (2–4 log)
Leng 2013 [109]	Singapore	ICU and general ward	Titanium dioxide (TiO_2)	Prospective observational study. 2/8 ICU single rooms and four intermediate care beds treated with TiO_2; applied to surfaces and fixed furniture (such as beds and door handles)	Standardardised sampling performed 6, 9, 12, 18 and 24 months after application, combined with ad hoc sampling around MRSA-positive patients. 'Culture-positive' defined as identification of MRSA or Gram-negative rod	Overall, untreated samples were significantly more likely to be culture positive (12 % vs. 4 %) than treated surfaces. No significant different in culture positive rates from untreated vs. treated surfaces during ad hoc sampling (15 % vs. 12 %)	TiO_2 was sprayed on following thorough cleaning. Almost 10 % of samples grew MRSA. There was no evidence of reduced TiO_2 activity over time. TiO_2 treatment was not associated with reduced contamination in multiple logistic regression

A&E Accident and Emergency Department, *CDA* Copper Development Association, *CI* 95 % confidence interval, *CVD* chemical vapour deposited, *HDU* high dependency unit, *ICU* intensive care unit, *MDR-GNR* multidrug-resistant Gram-negative rods, *OR* odds ratio, *PHMB* polyhexamethylene biguanide, *QAC* quaternary ammonium compound

that copper pens were not significantly less contaminated immediately after the shift, but they were after storage. This suggests, as you would expect from *in vitro* studies, that even copper surfaces take some time to exert antimicrobial activity. However, since the pens were in frequent use, rapid and high-level deposition of microbes also seems likely in this study.

Whilst differences in the inherent capacity of the various AMS that have been tested by *in situ* studies combined with differences in study design make comparison of studies difficult, a number of common principles emerge (Table 7.2). AMS are typically associated with a 1–3 log reduction in TAC. The levels of reduction *in situ* are generally less than the levels achieved *in vitro*, most likely due to the presence of organic soiling [69, 74, 75]. For example, in a study of a polycationic AMS, inocula applied using a swab from a water suspension exhibited a 3-log reduction, whereas inocula applied using a swab from a wound or immersed in urine exhibited only a 1-log reduction [69].

The impact seems to be greater on more contaminated surfaces, suggesting that there may be an irreducible minimum level of contamination, which perhaps represents continual deposition of contamination [43, 76]. Longitudinal studies have identified evidence of reducing levels of contamination over a period of days and weeks, which may be due to the accumulation effects, which is plausible when an AMS is applied regularly [68, 70]. One interesting finding is that untreated objects adjacent or close to AMS objects have significantly lower counts than untreated objects that are not adjacent or close to AMS objects [43, 77]. This so-called 'halo' effect may be due to reduced transmission of microbes between surfaces via the hands of healthcare personnel.

7.3.3 Clinical Impact

Before AMS are adopted widely in healthcare, studies demonstrating that their introduction is associated with reduced acquisition of pathogens are necessary. To date, only one study evaluating the clinical impact of AMS (copper) has been published, and the methods of analysis have received criticism [31, 78, 79]. More studies evaluating the clinical impact of AMS are necessary.

7.3.4 Cost-Effectiveness

Even if clinical benefit associated with the introduction of AMS can be demonstrated, cost-effectiveness studies will be necessary before widespread adoption. The cost of AMS may be incrementally small, but the total cost of implementation could be substantial and would need to be justified. However, it may be that AMS could be introduced a no additional cost during the manufacturing process – or even with an associated saving for some items. For example, data from the 1980s

suggests that the cost of stainless steel door knobs was actually more than brass alternatives with antimicrobial activity ($117 v $108) [80]. If clinical impact can be demonstrated, then further studies evaluating the cost-effectiveness equation are required.

7.4 Appraising the Options

7.4.1 Metals

A number of heavy metals have antimicrobial properties, which have been known to mankind from antiquity [52, 57, 81]. The two metals that have been studied as candidates for AMS are copper and silver.

7.4.1.1 Copper

Whilst the precise antimicrobial mechanism of action for copper remains a topic for discussion, copper has redox potential, which generates reactive oxygen species [57]. The *in vitro* antimicrobial activity of hard surfaces made of copper and copper alloys has been evaluated in a number of studies, reviewed recently by Grass et al. [57]. A wide range of vegetative bacteria and fungi have been evaluated, with high-level log reductions possible, albeit sometimes with contact times that are perhaps not useful for the healthcare setting. The concentration of copper in the alloy being tested appears to be an important factor in determining efficacy. A number of studies have evaluated the impact of copper surfaces on *C. difficile* spores, with equivocal results [60, 82]. One advantage of copper surfaces is their ability to inactivate naked DNA [83], which may limit the spread of plasmids containing resistance genes via the healthcare environment [84, 85]. One *in vitro* study of note repeatedly soiled and cleaned copper and stainless steel surfaces with a suspension of *S. aureus* in bovine serum abumin over 5 days [86]. The result indicated that the stainless steel surfaces were less susceptible to the build-up of organic matter than the copper surfaces, which may influence antimicrobial activity over time.

A number of studies have evaluated the potential for resistance or reduced susceptibility to copper [87–90]. Whilst clinically-relevant resistance to copper has not been identified associated with the introduction of copper AMS in the healthcare setting, copper AMS do provide an environment that would select for microbes with reduced copper susceptibility and may result in resistance in the long-term.

Several *in situ* evaluations of the use of metallic copper surfaces (mainly copper alloys) have been published (Table 7.2). A range of study designs have been used, but these studies generally demonstrate a 1–2 log reduction in the level of contamination on copper-containing AMS. Perhaps the most thorough study of the *in situ*

activity of copper AMS was performed by Schmidt et al. [43]. The study was a 43-month prospective multicentre intervention study, which demonstrated that the introduction of six copper high-touch AMS was associated with a significant reduction in bacterial count through weekly sampling. The frequency of recovering indicator organisms was also reduced.

A number of studies have also been performed on copper-containing liquid biocides, demonstrating *in vitro* activity against MRSA and *C. difficile* spores [91, 92]. An *in situ* study showed that microfiber impregnated with a copper biocide were more effective at inactivating bacteria on hospital surfaces than microfiber impregnated with water [68]. However, a chemical disinfectant without residual activity would have been a more suitable control for this experiment.

A recently study is the only published study with a clinical outcome for any AMS [31]. The study design was a multicentre evaluation of the clinical impact of introducing six copper alloy high-touch sites into the rooms of patients on three ICUs. Patients (n = 614 following exclusions) were randomized to intervention 'copper' rooms and control 'non-copper' rooms in three USA ICUs over an 11 month period. The only difference between the rooms was the presence of six items made of copper alloy, comprising bedrails, overbed tables, IV poles and visitor chair arms in all rooms and the nurse call button, computer mouse, computer palm rest and rim of a touch-screen monitor in other rooms.

Patients admitted to copper rooms were significantly less likely to acquire HAI or colonization with MRSA/VRE. The authors also make an interesting association between the degree of contamination in patient rooms and the risk of acquisition (Fig. 7.1b). However, since sampling was performed weekly regardless of a patient's infection or colonization status, it is not possible to determine whether this association is causal or simply due to the fact that infected/colonized patients are likely to shed more bacteria into the hospital environment.

The study was designed carefully and executed with strong attention to detail. For example, they performed a daily census of the items in the study rooms to determine exactly who was exposed to copper surfaces, and for how long. This indicated that only half of the patients in 'copper' rooms were exposed to all six copper items for the duration of their stay, and 13 % of patients in the 'non-copper' arm were exposed to some copper items during their stay. It's important to note that the analysis was performed on an 'intention-to-treat' population, i.e. all patients randomized to the two groups, regardless of which items they were actually exposed to. It would have been interesting to see a sub-analysis on the 'per protocol' population (i.e. those patients admitted to 'copper' rooms and exposed to all six copper items vs. those patients admitted to 'non-copper' rooms and exposed to no copper items). Also, the authors reported the percentage of patients who acquired HAI or colonization, rather than a comparison of rates between the groups. Indeed, a letter published raised some questions over the validity of the analysis methods chosen, and the plausibility of the findings [78, 79]. However, it seems that the introduction of a handful of copper alloy high-touch sites had a profound impact on HAI rates. However, questions remain over the practicality and durability of the widespread adoption of copper alloy surfaces in healthcare.

The regulatory position for copper AMS is currently different in the USA and Europe. In the USA, the Copper Development Association obtained an EPA registration for copper containing alloys allowing claims that copper when used in accordance with the label 'kills 99.9 % of bacteria within two hours' in 2008 [93]. More recently, a manufacturer of copper-oxide impregnated surfaces received the same registration [65]. However, in Europe, copper is one of the active agents included on the list of items that should be phased out of use in private and public health for disinfection (product type, PT 2) [94]. This means that the use of copper for AMS is not permitted under current European Union legislation.

7.4.1.2 Silver

Silver is currently used as a component of some topical wound dressings, as an ingredient in combination with other chemical in some 'no-touch' automated room disinfection systems and as a coating on medical devices [58, 95]. The exact mechanism of antimicrobial activity for silver is controversial, but requires direct contact between the silver and the microbial cell wall [81]. Hence, microbes accumulate silver until the toxicity threshold is exceeded.

A number of options are available for producing silver impregnated AMS for hospitals. A silver-containing liquid disinfectant has been evaluated *in vitro* [96, 97]. An *in vitro* study demonstrated clear residual activity for a disinfectant containing 0.005 % silver, with a >4-log reduction on *P. aeruginosa* and *S. aureus* [97]. However, the test was performed at high humidity (>80 %) to maintain the viability of the test organisms, but may have over-estimated efficacy. In another study, the use of silver impregnated microfiber mops and containers appeared to limit microbial contamination of mops [96].

Also, there are a number of options for generating surface films and coatings containing silver [98, 99]. A study by Bright et al. found that stainless steel surfaces coated with an absorbent material coated with 2.5 % silver and 14 % zinc ions significantly reduced the survival of *S. aureus* within 1 h [99]. Another study of similar surfaces indicated a <1 to >5 log reduction of *S. aureus, E. coli, P. aeruginosa* and *L. monocytogenes* after 4 h, and >4-log reduction on all pathogens after 24 h [100]. However, the durability of this coating is questionable, since scrubbing the surface reduced efficacy considerably, whereas wiping it did not. Another option is chemical vapour deposition of silver coatings [98]. The *in vitro* activity of various formulations of the coating varied with pathogen; a 5-log reduction in MRSA was obtained after 24 h. This study found that silver concentration correlated with efficacy, but that coating hardness (and hence, durability) correlated negatively with silver concentration. An *in situ* study found that the concentration of contamination was significantly lower on silver coated surfaces placed in a toilet cubicle (Table 7.2).

An *in situ* study of items with silver ions manufactured in was performed in the UK (Table 7.2) [77]. This study, performed on two comparable outpatient units, demonstrated that treated items in one of the units were significantly less

contaminated than non-treated items. Interestingly, counts from silver impregnated surfaces in wet areas were lower than on silver impregnated surfaces in dry areas, which suggests the importance of water/relative humidity in the efficacy of silver AMS.

7.4.2 Chemical

7.4.2.1 Organosilane

Organosilane-based products are composed of silicon with a quaternary ammonium compound moiety that inactivate microbes through direct contact [72]. Organosilane products are not new, having been studied since the 1970s [101]. An *in vitro* study of an organosilane product demonstrated 1–3 log reductions of *S. aureus* (MRSA), *P. aeruginosa* and *E. coli* within 30 min [102].

Two recent *in situ* evaluations of organosilane products have demonstrated no impact in terms of reduced levels of contamination [72, 103]. These most likely illustrate the difficulties of achieving a suitable bond between the surface and the organosilane, rather than a fundamental problem with the efficacy of the chemical agent. Further studies are required to assess whether there is a useful application of organosilane products in healthcare facilities.

7.4.2.2 Quaternary Ammonium Compound

Quaternary ammonium compounds (QAC) are commonly used liquid agents for surfaces disinfection, particularly in the US. A 2006 study by Rutala et al. demonstrated that QACs have a residual activity when applied to keyboards [104]. More recently, a study from the UK demonstrated that a QAC was more effective for reducing ATP counts than a chlorine dioxide disinfectant [105]. Novel formulations based on QAC with residual claims have recently been launched but further studies are required to evaluate the potential of existing and novel QAC formulations for producing effective AMS.

7.4.2.3 Light-Activated

Light-activated antimicrobials generate microbicidal reactive oxygen species when irradiated with light of a suitable wavelength. A range of photosensiters have been evaluated, including methylene blue, toluidine blue and rose bengal [74, 106, 107]. Another option is titanium dioxide, which possesses photocatalytic properties [108, 109]. In addition, photocatalytic surfaces can be combined with metals to enhance their antimicrobial activity [75, 106].

The type of illumination required for photocatalytic activity depends on the surface. Historically, UV light has been required for photocatalytic activity, but recent developments mean that photocatalytic activity can be achieved with white light [75, 110]. However, photocatalytic surfaces may not provide 'round the clock' reduction in contamination because they would be less effective at night where light levels are lower, depending on the required frequency and duration of photoactivation.

A number of *in situ* studies have evaluated the impact of various photosensitisers in a London Dental clinic [74, 75]. Both cellulose acetate impregnated with the photosensitisers toluidine blue O and rose bengal, and silicon polymers impregnated with gold nanoparticles resulted in significant reductions in TAC. However, illumination with a lamp close by was used in the study, so efficacy under ambient light conditions was not assessed.

One study has evaluated the *in situ* activity of titanium dioxide, which was applied to surfaces and furniture in a Singaporean critical care unit [109]. Overall, untreated samples were significantly more likely to be contaminated with MRSA or a Gram-negative rods, but treatment with titanium dioxide was not associated with reduced contamination in multiple logistic regression analysis, suggesting that other factors are more important for influencing contamination rates. Further studies of titanium dioxide treated surfaces are required.

7.4.2.4 Polycationic Polymers

Polymers can be combined with antimicrobial agents to produce AMS [69]. The only polycationic polymer that has been studied in the healthcare setting is a combination between polyhexamethylene biguanide (PHMB) with an active polymer (A-200). The mechanism of action involves a combination of the active polymer, which immobilises the microbes and PHMB, which disturbs the cell membrane lipid bilayer. The product achieved a 3-log reduction on *S. aureus* when applied in water, but only a 1-log reduction when swabs from clinical specimens (wound and urine) were applied to surfaces; this difference is probably explained by organic soiling. The study also included an *in situ* evaluation of the product, demonstrating a significant reduction in the TAC on bedside tables. However, the tables were specifically not cleaned during the study, which was only performed for 24 h after each application, so further studies are required to assess durability.

7.4.2.5 Triclosan

Triclosan has been widely adopted in a range of consumer markets, but no relevant *in situ* studies have been performed in healthcare settings [111, 112]. Due to the high risk of resistance developing, triclosan alone is not an attractive candidate chemical for AMS.

7.4.3 Physical Alteration of Surface Properties

A number of options that are not directly antimicrobial but either reduce the deposition of microbes on surfaces, or improve their cleanability, or both (Table 7.1). These include "liquid glass" (silicon dioxide), Sharklet pattern [113, 114], advanced polymer coatings (such as polyethylene glycol (PEG), superhydrophobic/philic and zwitterionic) [115–118] and diamond-like carbon (DLC) films [119]. These technologies are currently at an early stage of development in terms of producing AMS, and no *in situ* studies have been performed. However, there is a potential that several of these methods to make surfaces less liable to microbial deposition, or easier to clean, could be combined with the addition of an antimicrobial agent [54]. This potential should be the focus of development activities.

7.4.4 Other Options

There are some other options not listed in the table, that could be considered candidates for antimicrobial surfaces, although they are currently at an early stage of development, including: negative air ionization to repel bacteria from surfaces [120], enzymes [121] or bacteriophages [122] immobilized on surfaces, or polyphenol-based AMS derived from foods such as green tea, red wine and dark chocolate [123]. These options are at an early stage of development, but offer potential for the future.

7.5 Summary

There's a plethora of potential options and approaches to make a hospital surface 'antimicrobial'. Copper is leading the way as a candidate, although other options are available. Making a surface less able to support contamination in the first place, and/or easier to clean is another tempting option, particularly if this can be combined with a level of antimicrobial activity. Finding and evaluating the optimal antimicrobial surface requires a multidisciplinary approach, involving industrial partners, materials scientists, healthcare scientists and epidemiologists to refine and test the available options. More studies in the clinical setting, including those with a clinical outcome and an evaluation of cost-effectiveness, are required.

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Chapter 8
Economics of Using Biocidal Surfaces

Panos A. Efstathiou

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Abstract

Aim Aim of this study was to evaluate the reduction on Intensive Care Unit (ICU) microbial flora after the antimicrobial copper alloy (Cu^{+}) implementation as well as the effect on financial – epidemiological operation parameters.

Methods Medical, epidemiological and financial data into two time periods, before and after the implementation of copper (Cu 63 % – Zn 37 %, Low Lead) were recorded and analyzed in a General ICU. The evaluated parameters were: the importance of patients' admission (Acute Physiology and Chronic Health Evaluation – APACHE II and Simplified Acute Physiology Score – SAPS), microbial flora's record in the ICU before and after the implementation of Cu^{+} as well as the impact on epidemiological and ICU's operation financial parameters.

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Results During December 2010 and March 2011 and respectively during December 2011 and March 2012 comparative results showed statistically significant reduction on the microbial flora (CFU/ml) by 95 % and the use of antimicrobial medicine (per day per patient) by 30 % ($p = 0.014$) as well as patients hospitalization time and cost.

Conclusions The innovative implementation of antimicrobial copper in ICUs contributed to their microbial flora significant reduction and antimicrobial drugs use reduction with the apparent positive effect (decrease) in both patients' hospitalization time and cost. Under the present circumstances of economic crisis, survey results are of highest importance and value.

List of Abbreviations

APACHE II	Acute physiology and chronic health evaluation
CHOC	Chocolate agar
DDP	Drugs (dose) per day per patient
EPA	Environmental Protection Agency
HCDI	Hellenic Copper Development Institute
ICU	Intensive care unit
NICU	Neonatal intensive care unit
PCR	Polymerase chain reaction
QALY	Quality-adjusted life year
SAPS	Simplified acute physiology score
SPSS 18	Statistical package for social science
WHO	World Health Organization

8.1 Introduction

Health Costs is currently one particular factor that is highly important for any economy of an organized society. National health systems in most countries of the world are funded by governments and assisted by private healthcare providers.

Operating costs of Intensive Care Units (ICUs) in hospitals have been subjected to approaches and analyzations of various types. Even though most of the patients hospitalized in ICUs have been treated (e.g. surgery) before entering the ICU and the cost of treatment should also include this stage, there have been efforts made by many scientists to isolate the cost effectiveness within the time limits of hospitalization in ICUs [12, 19, 20, 24].

Factors that either limit or minimize the operating cost of an ICU usually interact with each other and are associated with the use of pharmaceutical compositions, medical equipment and treatment protocols followed [1, 8, 16].

In the World Literature there have been described several types of economic analysis so as to estimate the Cost Effectiveness in Critical Care, such as Cost Minimization, Cost Benefit, Cost Effectiveness and Cost Quality [20]. Furthermore

estimation of Quality of Life after leaving the ICU adds more parameters to the effort of a total economic assessment [18].

The use of both technical materials and methods to minimize the parameters that increase the cost of hospitalization in ICU is presented and analyzed in this chapter.

8.2 Evaluation of ICU Cost Effectiveness

The ICUs host patients with multiple problems and medical interventions, thus their treatment is often supportive up to the final stage. Over 50 years in hospitals around the world these units are the main section dealing with the most difficult cases.

A key issue that emerges is the definition of an "ICU bed". American definitions reflect the intensity of physician staff (for example, nurse to patient ratio, intensity of physician staffing). In contrast, definitions of ICU beds in Belgium reflect the intensity of the illness and focus on the ability to care for patients with specific severities of illness (that is, organ dysfunction), [15, 23].

The variability of definition of intensive care (different staffing intensity, different patient type, or acuity) clearly impacts the ability to compare different types of care for critically ill patients. Even without universal definition of an ICU bed, however, the variation in availability of any type of ICU bed remains large [15].

Understanding of the different ways of economic analysis most often complicates the final assessment and creates difficulties in researchers communicating. The following table shows examples of financial formulas as recorded in Report from the Second American Thoracic Society Workshop on Outcome Research. (Table 8.1 – Report from the 2nd American Thoracic Society Workshop June 2001).

One of the questions that usually arise of the economic evaluation of the functioning of *Critical Care Units* is "What is the cost to achieve that effect?".

However, from our point of view, there are other questions that should be answered in the context of an ICU's economic assessment, such as:

- Is it patient's survival?
- Is it quality of life until death?
- Are there any technical terms such as hospitalization days' reduction?
- Is it the cost of pharmaceutical expenditure reduction?
- Is it the infections' reduction?
- Is it all the above?

It is also important to understand the difference between efficacy (can it work?), effectiveness (does it work in reality and clinical practice?), as well as and cost effectiveness (the consequences of the alternatives are measured in natural units, such as years of life gained. The consequences are not given a monetary value).

Table 8.1 Report from the Second American Thoracic Society Workshop on outcome research

Type of study	Numerator (costs)	Denominator (outcome or benefit)	Examples	Comment
Cost minimization	Dollars	None	Antibiotic therapy for ICU patients at low risk of nosocomial pneumonia Drug acquisition costs for a 3-d course of ciprofloxin are $9,520 less expensive than average acquisition costs for unregulated antibiotic prescription	No estimate of consequences on other health care costs. Clinical outcomes are assumed to be equivalent (i.e., no difference in subsequent pneumonia rate or mortality) even though formal equivalence study not conducted
Cost benefit	Dollars	Dollars	Use of an aminoglycoside dose-monitoring program for burn patients with gram-negative sepsis The dose-monitoring program led to $8.70 savings per dollar spent	A key advantage is that all costs and effects are expressed in monetary units (dollars), facilitating assessment of worth. However, the key concern is that converting clinical effects, such as lives lost (or gained), into dollar amounts is controversial, somewhat arbitrary, and biased toward saving the lives of those with greater earning capacity
Cost-effectiveness	Dollars	Specific Measure of effectiveness (lives saved)	Thrombolysis for acute myocardial infarction Tissue plasminogen activator costs an additional $32,678 per additional life saved when compared with streptokinase	Assesses change in both costs and effects but avoids controversy of converting clinical outcomes into dollar values. It is not clear whether "lives saved" are equivalent to other lives saved by other therapies in other diseases
Cost utility	Dollars	A common utility metric (quality-adjusted life – years)	Prophylaxis against recurrence of peptic esophageal strictures Omeprazole costs an additional $49,600 per additional QALY when compared with ranitidine	Cost per QALY allows comparison with other therapies used in other diseases. This is now the recommended approach

The value of life, as it was approached by the father of medicine Hippocrates, (460 B.C.–377 B.C.) is "priceless."

W.H.O. strongly reaffirms that health, which is a state of complete physical, mental and social wellbeing, and not merely the absence of disease or infirmity, is a fundamental human right and that the attainment of the highest possible level of health is a most important world-wide social goal whose realization requires the action of many other social and economic sectors in addition to the health sector. The existing gross inequality in the health status of the people particularly between developed and developing countries as well as within countries is politically, socially and economically unacceptable and is, therefore, of common concern in all countries.

Economic and social development, based on a New International Economic Order, is of basic importance to the fullest attainment of health for all and to the reduction of the gap between the health status of the developing and developed countries. The promotion and protection of the health of the people is essential to sustained economic and social development and contributes to a better quality of life and to world peace (*Declaration of Alma-Ata*).

Therefore, all references to ICU patients should have as a component that life does not have the opportunity to fit into economic analysis systems.

A parameter such as the quality of life in comparison with life expectancy, (Quality-adjusted life year – QUALY) may be a component of improving both health services' quality and the macroeconomic efficiency of the health system (cost effectiveness).

Technical evaluation of QUALY like the SF-36 and EuroQol have been described and can assist with their questionnaires on objective recordings [21, 22].

Recordings of the reduction on the consumption of pharmaceutical products, particularly of antibiotics, during the treatment of patients in ICU, under specific conditions of use of biocidal surfaces (Antimicrobial Copper Cu^{+} [see Sect. 8.3 and Chap. 4]) in them, have shown a statistically significant reduction on operating costs [5, 6, 7, 9, 11].

The following algorithm approximates total cost's evaluation by summing Depended and Independed Variables (see Sect. 8.5).

$$\Sigma_{\text{cost}} = \Sigma K_{\textit{depended variables}} + \Sigma K_{\textit{independed variables}}* \tag{8.1}$$

$$\Sigma K_{\textit{independed variables}} = \Sigma\pi_1 + \Sigma\pi_2 + \ldots + \Sigma\pi_\nu \tag{8.2}$$

e.g. $\Sigma\pi_1 =$ Consumption of antimicrobial agents per day per pt

$$\Sigma K_{\textit{depended variables}} = \mathrm{M}\pi_1 + \mathrm{M}\pi_2 + \ldots + \mathrm{M}\pi_\nu \tag{8.3}$$

e.g. $\mathrm{M}\pi_1 =$ Diagnostic Related Groups (DRGs)

* more than 250 variables

In other studies, as elements of the reference case, such as long-term costs and quality of life, may only be estimated using modeling and assumptions, it is recommended an inclusion of a "data-rich" case, where the cost – effectiveness

ratio is generated as closely as possible from data on actual patient outcomes and costs (e.g. hospital costs per hospital survivor). However, in order to approach the recording of fixed and non-fixed parameters, following the type above, we can estimate the total ICU's operating cost and have a comparable effect in two different time periods (before and after the use of agents to reduce the microbial flora). Recording time and comparison may vary, but it gives us the opportunity to realize economic efficiency in the operation of the selected unit [5, 6, 7, 9, 11].

A similar approach has been recorded for observation and assessment period as a "time horizon" and must be long enough to capture the important clinical and economic consequences of the therapy.

Generally speaking, economical evaluations are increasingly common in the critical care literature, although approaches to their conduct are not standardized because evidence for the effectiveness of critical care interventions is often lacking [20].

8.3 Antimicrobial Copper Cu^+ Implementation in NICU (Neonatal Intensive Care Unit)

The implementation of antimicrobial copper as a biocidal surface in ICUs' started since 2008 and today a large number of ICUs' worldwide has already been copperized. The following table lists the countries that have already installed antimicrobial copper in their respective ICUs (Table 8.2).

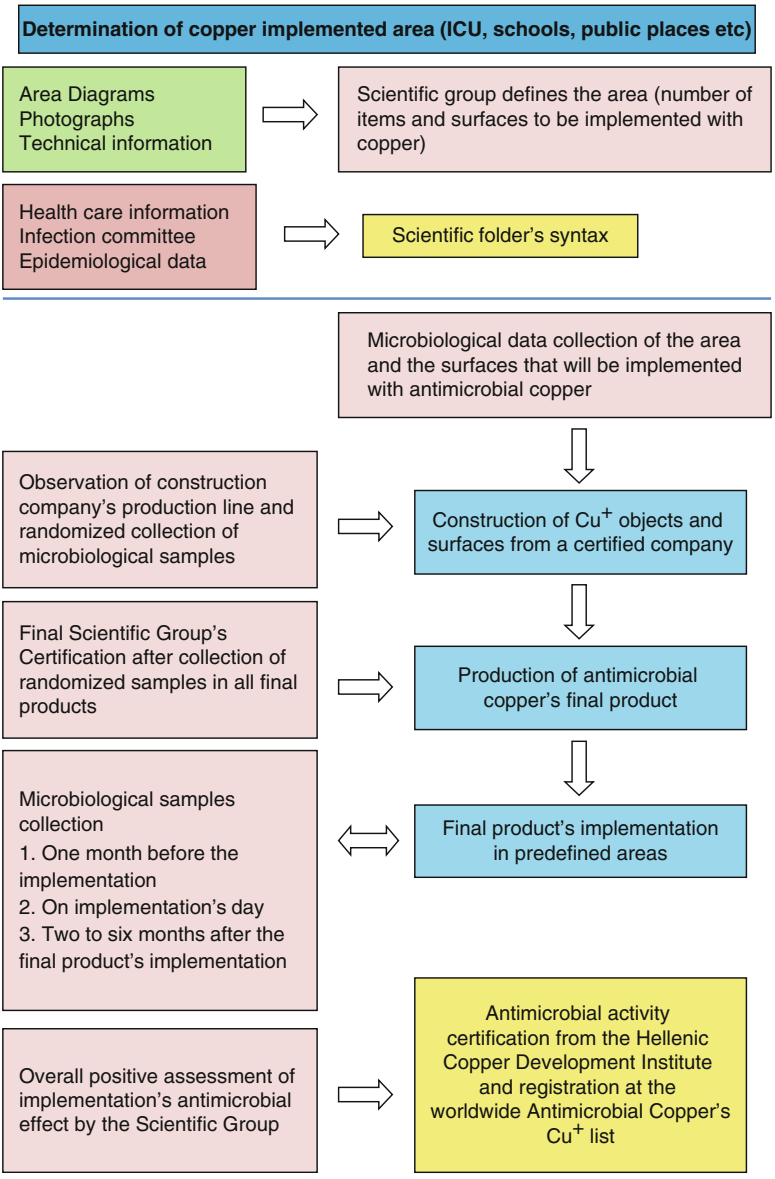
The implementation procedure as reported in literature [13] is described in Table 8.3, and thereby can ensure the final product regarding the effectiveness of its biocidal activity [4].

Up till now, studies related to microbial load reduction at the above implementations have clearly demonstrated the effectiveness of antimicrobial copper Cu^+ [2, 5, 6, 7, 9–11, 13]. It is important that microorganisms resistant to antibiotics present a 95 % reduction 2 h after their exposal to the specific biocidal surface and create the conditions that decrease the infections and the consumption

Table 8.2 "List of countries implemented antimicrobial copper Cu^+ in ICUs"

Bulgaria
France
Germany
Greece
USA
Japan
India
China
Cyprus
Great Britain
Chile

Table 8.3 Testing procedure of antimicrobial copper's final product



of antibiotics. In the following Fig. 8.9 we can see the microbial load reduction on antimicrobial copper surfaces compared to similar of stainless steel surfaces.

It is important that in NICU (Deane 1970) a similar reduction of microbial flora and infections was observed after certain period of recording time "before" and "after" antimicrobial copper Cu^{+} implementation (Figs. 8.1 and 8.2)

Fig. 8.1 Implemented trolley with antimicrobial copper in Neonatal Intensive Care Unit (Aghia Sophia Childrens' Hospital, Athens, Greece)

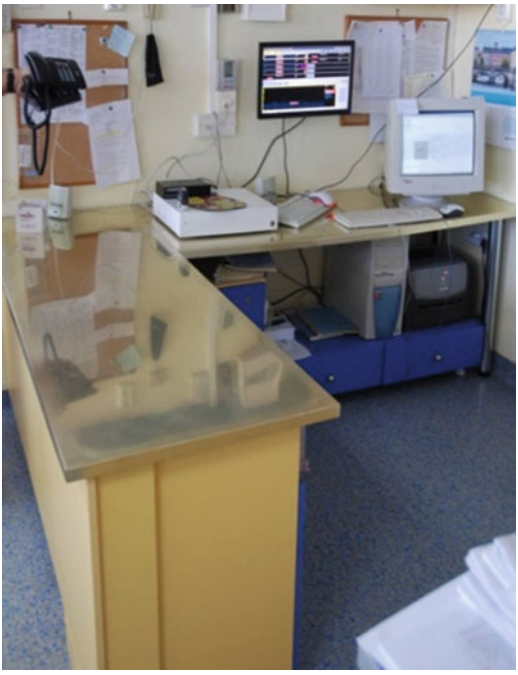


Fig. 8.2 Head nurse's desk implemented with copper in Neonatal Intensive Care Unit (Aghia Sophia Childrens' Hospital, Athens, Greece)

8.4 Economic Impacts on the Operational Costs of the ICU After Antimicrobial Copper Cu^+ Implementation

It is known that the pathogenic microorganisms accumulate at surfaces of the environment, multiply and form a reservoir of microbes transmitted by contact (hands or other objects). Commonly, microbial strains are found in hospitals and the most resistant of those, populate mainly in the ICU. This, combined with the excessive use of antibiotics, makes it difficult, tedious and expensive and sometimes impossible to address hospital infections [19].

In that sense, any attempt to restrict, or eliminate this "microbes reservoir" in hospitals, particularly in ICU's is welcomed, always accompanied by associated study and observation. Under this frame, the implementation of Antimicrobial Copper alloys Cu + (Cu63 %, Zn37 % Lead Free) on specific surfaces in ICU's and further study and result's analysis, of this implementation and its antimicrobial activity, is an innovative effort against microorganisms with beneficial effects to the public health [20].

Antimicrobial activity of copper and specific copper alloys has proved in several studies (Heslet 2007), highlighting potential positive impact in ICU & NICU (Nosocomial areas [1, 24]) together with the action of antimicrobial drugs which assist into reducing microbial flora in Nosocomial environment.

Simultaneously, the analysis of implementations of antimicrobial copper surfaces in public places (schools, hotels, public transportation facilities, subway, etc. [5, 6, 7, 9, 11]) has observed similar results.

Generally the results show a significantly reduced rate of microbial flora in antimicrobial copper surfaces and on a continuous basis up to 95 % [24] compared to common surfaces.

Results from a clinical trial in the U.S., funded by the Ministry of Defense, bring proved data to new levels, by evaluating the link between microbial growth on multi-touch surfaces and patients' infestations from HAI's. From these findings it appears that regarding patients receiving treatment in ICU, where objects of antimicrobial copper alloys were placed, the risk of infection was reduced to less than 58 % [17].

In a research carried out in Greece [5, 6, 7, 9, 11] in selected surfaces/objects in the ICU environment (mixed-type, four to six beds capacity) antimicrobial copper alloys were implemented.

In this ICU epidemiological, medical and financial data of operation were recorded and analyzed.

The preliminary (pilot) study section analyzed initially two periods, more specifically during the periods between December 2010 and March 2011 and between December 2011 and March 2012, i.e. before and after the implementation of Antimicrobial Copper (Cu^+) 63 % – Zn 37 %.

The antimicrobial copper alloy Cu^+, as the raw material for manufacturing surfaces or objects with antimicrobial properties is certified by the Environmental Protection Agency (EPA) in the USA and the Hellenic Copper Development Institute (H.C.D.I.) in Greece.

Fig. 8.3 Trolley implemented with antimicrobial copper (Peiraikon Therapeftirion, Piraeus, Greece)

Fig. 8.4 Nurse's station implemented with antimicrobial copper (Peiraikon Therapeftirion, Piraeus, Greece)

The objects and surfaces replaced with Antimicrobial Copper (Cu^{+}) were: all the door handles of the ICU (internal – external), all cabinet knobs, and all trolley's shelve knobs and surfaces, as well as the surfaces of the ICU nurse's station (Figs. 8.3 and 8.4).

Cultures were taken for detection of bacteria and viruses from the above surfaces, before and after the phase of Cu^{+} implementation or replacement of those, with a new of antimicrobial copper alloys. Samples taken from the surfaces were cultured in selective culture media for microbial growth and virus isolation by molecular techniques.

A total of 15 samples per period (before and after) were taken from door handles, cabinet knobs, trolleys surfaces and shelves, as well as from the nurse's station surfaces.

The sample taking was made with cotton swabs that were drawn on the surfaces and transported to the laboratory in special transport equipment "Stuart" within 30 min. Then, they were cultivated on selective culture media for the respective species of microbes *(Gram (-)* bacteria, *Gram (+)* grains, fungi and anaerobes). The samples were inoculated directly, into selective nutrient medium (*Vlood agar, Anaerobic Blood agar, MacConkey, Sabouraud with Gentamicin, Chocolate agar*

(CHOC), Chapman agar, SSA agar). Moreover, special swabs were used for isolating viruses (by molecular techniques – PCR).

After the inoculation, incubation followed at 38 °C for 24–48 h and respectively, under anaerobic conditions. A measuring of the growth (CFU/ml) was followed and identified with the Api system Biomereaux.

In the isolated stems, antibiograms were made, to assess the susceptibility to antibiotics. From the assessment of the susceptibility of microorganisms to antibiotics, we were able to identify the phenotypic similarity. All samples were examined by molecular techniques for virus identification.

The number of microorganisms isolated before Cu+ implementation from the respective surfaces was multiple to the number of microorganisms isolated after.

Cultures' results showed the following:

During **Phase I** (before Cu^{+} implementation) in 6 out of 15 samples taken, pathogens were isolated in concentration (CFU/ml):

- *Pseudomonas aeruginosa, (*two points*)* (>100.000 CFU/ml)
- *Acinetobacter baumannii,* (>100.000 CFU/ml)
- *Staphylococcus haemolyticus,* (>100.000 CFU/ml)
- *Staphylococcus capitis,* (>50.000 CFU/ml)
- *Stenotrophomonas maltophilia*. (>50.000 CFU/ml)

During **Phase II** (after Cu^{+} implementation) in only 1 out of 15 samples taken, a pathogen was isolated in low concentration. This was:

- *Staphylococcus Epidermidis. (<20.000 CFU*)

It is clearly obvious that the reduction of microbial flora on selected surfaces/objects was extremely significant (isolated in only 1 of the 15 samples Staphylococcus Epidermidis <20.000 CFU) (Table 8.4).

With the research underway, ICU epidemiological parameters were counted prospectively.

These parameters were age, gender, patients' severity admitting to the ICU scored by APACHE II (*Acute Physiology and Chronic Health Evaluation*), and SAPS II (*Simplified Acute Physiology Score*), the main cause for admitting in the ICU, time of hospitalization and patients' outcome.

The preliminary pilot results were recorded and analyzed initially for 3 months period between December 2010 to March 2011, (time period A) and between January 2012 to September 2012 (time period B).

Time period A is specified as the period that the specific ICU operated without having antimicrobial copper surfaces/objects, while maintaining the regular guidelines treatments and therapies of the patients based on international standards (generally characterized as period before).

Time period B is specified as the period that the specific ICU operated after the implementation or replacement of antimicrobial copper to chosen surfaces/objects, while maintaining the regular guidelines treatments and therapies of the patients based on international standards (generally characterized as period after).

Table 8.4 Microbial recording before and after Cu + implementation in 15 samples

	Before Cu^+ implementation			After Cu^+ implementation		
	Answer	Cfu/ml	Microorganism	Answer	Cfu/ml	Microorganism
1	Positive	100,000	*Pseudomonas aeruginosa*	Negative	0	
2	Negative	0		Positive	25,000	*Staph. epidermidis*
3	Positive	100,000	*Acinetobacter baumannii* *Staph. haemolyticus*	Negative	0	
4	Positive	100,000	*Pseudomonas aeruginosa* *Stenotrophomonas maltophilia*	Negative	0	
5	Positive	100,000	*Acinetobacter baumannii* *Staph. capitis*	Negative	0	
6	Negative	0		Negative	0	
7	Negative	0		Negative	0	
8	Negative	0		Negative	0	
9	Negative	0		Negative	0	
10	Negative	0		Negative	0	
11	Negative	0		Negative	0	
12	Negative	0		Negative	0	
13	Negative	0		Negative	0	
14	Positive	50,000	*Staph. epidermidis*	Negative	0	
15	Negative	0		Negative	0	

The analysis was conducted by using the statistical package **SPSS 18** (*Statistical package for Social Science*) before and after the antimicrobial copper implementation (Table 8.5).

There is no statistically significant difference for all epidemiological parameters recorded for the above sample (pilot) of the research i.e. the homogeneity of the sample in terms of age, patient's severity, gender distribution and also hospital stay is granted.

A statistically significant difference ($p<0.014$, SPSS18) was observed regarding the consumption of antimicrobials: ddp (dose – per day- per patient) (this is an adaptation of ddd, daily defined dose, which aims to show the actual consumption of antimicrobials in patients' treatment).

Specifically:

- The gender distribution between the two groups (male–female) does not show a statistically significant difference.
- The age distribution, as shown in Fig. 8.5 does not also show a statistically significant difference.
- The most important concerning the homogenicity of the sample regarding the patients severity admitting in the ICU is the "score" of the incidents according to APACHE II, and SAPS II, as it appears there is no statistically significant difference in both evaluation systems Figs. 8.6 and 8.7.

Table 8.5 Epidemiological data of the ICU patients

Preliminary pilot results for a time period of 3 months. Thirty-seven incidents relating to the period A (before) and 32 incidents relating to the period B (after) were studied	**Before**	**After**	
No. of patients	37	32	
Male	22	19	
Female	15	13	
Age	73.36	74.34	NS
APAII	27.17	27.15	NS
SAPSII	55.19	53.41	NS
Days of hospitalization	13	10	NS
Consumption of antibiotics (dose) per patient per day	7.28	5.27	
	P = 0.014 [SPSS18]		

NS: non statistically significant

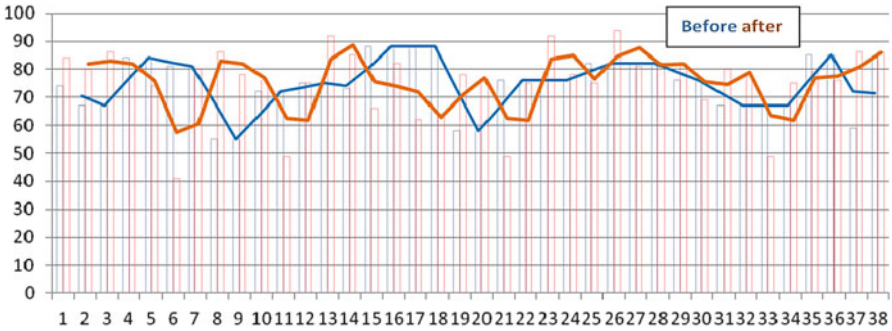


Fig. 8.5 "Age distribution"

8.4.1 APACHE II Score

Parameters recorded and severity score calculated, for patients treated in the ICU (during the first 24 h), using the APACHE II, showed no significant difference between patients hospitalized during the period A (before) and those hospitalized during the period B (after). Below is a graphic display of this observation.

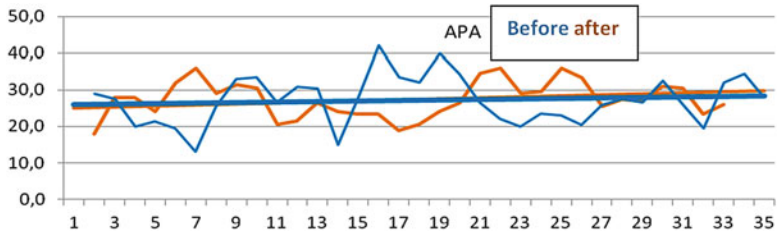


Fig. 8.6 APACHE II (before – after)

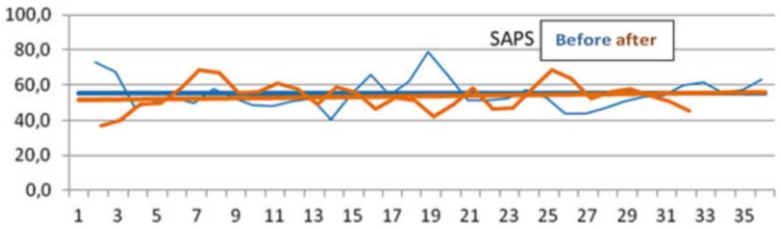


Fig 8.7 SAPS (before – after)

Table 8.6 Cause of admission (before – after)

Cause of admission	Before	After
Neoplastic disease	**3**	**0**
Acute respiratory failure	**22**	**11**
Acute renal failure	**4**	**2**
Surgical cause	**7**	**8**
Neurosurgery cause	**0**	**11**
Totals	**36**	**32**

8.4.2 SAPSII Score

Similarly, parameters recording the and severity score calculated for patients treated in the ICU (during the first 24 h), using SAPS II did not demonstrate, a statistically significant difference between patients hospitalized during the period A (before), and those hospitalized during the period B (after).

The distribution of ICU cause of admission before and after differentiated, without actually affecting the incidents' treatment before and after (Table 8.6).

Regarding to the patients' final outcome, in our research, preliminary pilot results showed deterioration associated with the different range cause of admission, Table 8.7.

The days of hospitalization, in this research, vary daily, but for the already recorded incidents it is shown that there is a decline to hospitalization days, without however a statistical significant reduction Figs. 8.8 and 8.9.

Table 8.7 Outcome (before – after)

Outcome	Before	After
Improvement	**20**	**11**
Death	**16**	**21**
Totals	**36**	**32**

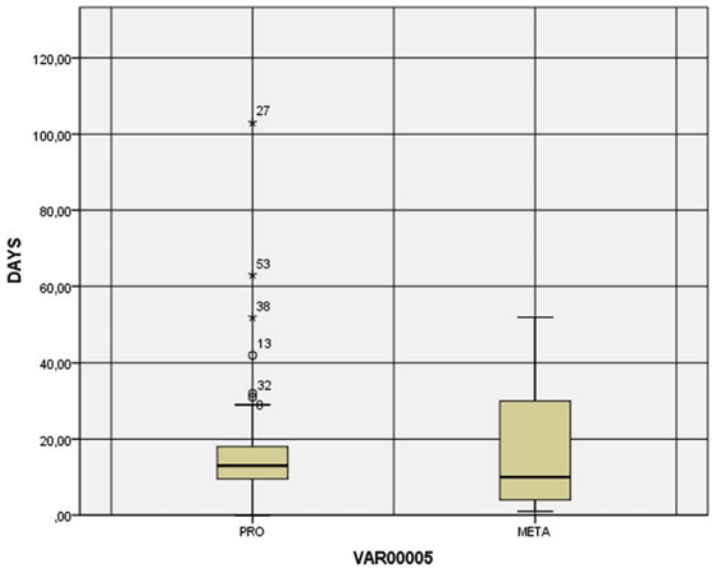


Fig. 8.8 Decline to hospitalization days

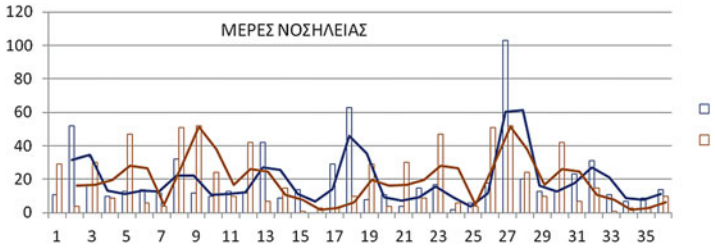


Fig. 8.9 Days of hospitalization

The pharmaceutical costs in general and specifically the antimicrobial drug costs, is known to be (perhaps) the main economic parameter to the function of ICU and generally of a hospital in every health system. The variation depends on parameters such as the severity of the patient, the management of the medical and nursing staff, but also from HAI (microbial flora ICU), and the development of antibiotic-resistant pathogenic strains. The overuse of antimicrobials in recent years has created major problems, both to health systems and to patients' treatment from the development of resistant pathogenic strains, even to the latest antimicrobials.

The total cost of hospitalization in ICU is a multifactorial result and it is very difficult to determine which factor is most important since exogenous factors can cause fluctuations at it.

But today, under the present circumstances of the implementation of "*Diagnosis related groups*", in the attempt to estimate the total cost of operation of an ICU, we must take for granted and in advance variables depended and independed.

$$\Sigma_{\text{cost}} = \Sigma K_{depended\ variables} + \Sigma K_{independed\ variables}$$

The recording of consumption of antimicrobial drugs per day per patient (ddp), in the two homogeneous groups of ICU patients, in two different time periods, is the technique that we implemented in order to identify cost reduction in ICU patients' treatment. The reduction of antimicrobial drugs consumption (ddp) is considered as the best and most objective scientific approach related in direct proportion to the statistical reduced microbial flora due to the implementation of antimicrobial copper Cu^+ (63 % – Zn 37 %).

The preliminary pilot results showed reduction in the consumption of antimicrobials after Cu^+ implementation.

The average reduction reaches the percentage of 30 % (27.61 %) and is considered to be the most reliable indicator of antimicrobial copper Cu^+ effectiveness in the ICU, as far as consumption of antimicrobials is concerned.

The difference in consumption of antimicrobials per patient per day is 7.28 ddp (before) and 5.27 ddp (after) showing a decline of 27 % which is considered to be statistically important. The prospect development of the study demonstrates that all the above parameters associated with the ddp research are evolving and strengthening.

Already the study period has reached, with data analysis, in timeouts of 9 months where ddp continues to improve and demonstrates that ddp reduction exceeds the percentage of 30 %.

Although the initial (pilot, 3 month period) analysis gave a difference of ddp by 27 % [7.28 ddp to 5.27 ddp] (with statistical importance $p = 0.014$, 95 % level of significance) the progress of the study (in a 9 month basis) demonstrates even greater difference ddp by 31 % [8.11 ddp to 5.55 ddp] (with statistical importance of $p<0.001$, 99 % level of significance).

In this study, the reduction of microbial flora in objects and surfaces in the specific ICU, due to the implementation of antimicrobial copper Cu+ influenced the 'consumption' of antimicrobial drugs, reduced and continues to reduce it (!) and it is statistically significant.

If one evaluates that after Cu+ implementation in the ICU rooms (where 75 % of the objects and surfaces are replaced by antimicrobial copper Cu^+), but also take into account the observed reduction of infections by 58 % as mentioned by M. Schmidt et al. [17] comes to the conclusion that economic parameters, i.e. the cost of operation of an ICU is reduced, with significant positive effects in the total operational costs of a hospital, especially under the current economic crisis adding further tribulation to our society.

8.5 Analytically

In assessing the economic impairment in operating expenses (total approximate) according to Efstathiou et al. [5, 6, 7, 9, 11], an estimation of doses of antibiotics per day and per patient was carried out, following the procedure per dose, per patient, per day. The estimation of the absolute number of ddp was made in two phases, before and after antimicrobial copper implementation in the Intensive Care Unit, as well as in two time periods, one pilot study of 3 months and an overall study of 9 months. The recording of such antibiotics are shown in Table 8.8. The patients who took part in the study had similar disease entities as well as similar input and outcome parameters' gravity in the Unit, which were statistically not significant (see Table 8.9).

The above relationship enables us to approach the impairment of daily doses in patients, initially in an absolute number. As mentioned in Efstathiou et al. [5, 6, 7, 9, 11] research, as an example, patients with VAPs (in patients' etiology and severity no statistically significant difference in SAPS and APACHE II), before Unit's copper implementation, consumed 281 total doses of the wide group of quinolones while in the same period of time, with a similar clinical frequency of disease, only 66 (weighted average reduction 76 %), while the group of carbapenems presented weighted average reduction of 19.4 %. There were groups that increased the use of glycopeptides, for instance, by 38 % totally and cumulatively, but the decrease in consumption level was 27 % for the initial period and more than 31 % for the 9-month study.

Table 8.8 Groups of antimicrobial agents

Groups of antimicrobial agents
1. Carbapenemes
2. Glycopeptides
3. Antifungals
4. Penicillines
5. Quinilones
6. Imidazole
7. Cephalosponnes
8. Aminoglycosides
9. Suifonamides
10. antiTBC
11. Anti b-lactamaces

Table 8.9 Difference in consumption of antimicrobial drugs (dose) per patient per day (3 month analysis – 9 month analysis)

	Before	After
Consumption of antimicrobial drugs (dose) per patient per day (3 month analysis)	7.28 $p = 0.014$	5.27
Consumption of antimicrobial drugs (dose) per patient per day (9 month analysis)	8.11 **$p < 0.001$ (99 %)**	**5.55**

For instance, per day per patient the consumption was 300 doses, while after copper implementation there was a total of 228 doses respectively (always in proportionate days of hospitalization). This reduction in consumption for specific groups of drugs in this patients' unit, corresponds to a reduction of antimicrobial treatment's cost and handling of those patients (calculated at constant prices of antimicrobials for hospital use in spring of 2012).

The research focuses on the consumption of antimicrobials in the ICU and not the cost of these, due to the fact that the cost is variable and cannot be estimated not only for this but also for the above example with fixed values for 2012. There is no doubt that reduction in consumption is expressed also by reducing the cost of antibiotics, which is a key to hospitalization's cost in the ICU patients [3, 14].

8.6 Conclusions

The use of biocidal surfaces in healthcare and especially of antimicrobial copper creates much better economic conditions in the operation of hospital units, particularly the Intensive Care Units. Antimicrobial copper under certified processes of implementation and control of the final product is undoubtedly the best material for the reduction of microbial flora [25].

On the contrary, the economic analysis of the positive impact of these implementations can be difficult and diverse, but are directly related to the economic policies of the National Health system. It is certain that following studies regarding the scientific and economic nature will further strengthen the already existing results.

Conclusively, the use of a biocidal material in the form of an object or surface, and in our case, antimicrobial copper gives the potential to reduce financial burden parameters on the functioning areas of critical care.

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Chapter 9
Alternative Room Disinfection Modalities – Pros and Cons

George Byrns

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Abstract The purpose of this Chapter is to review the efficacy, effectiveness, and efficiency of chemical fumigation and germicidal UVC irradiation use in healthcare and other related environments. The primary objective is to identify when the benefits associated with fumigation or irradiation outweigh the risks of human injury or other adverse effects. It is hypothesized that both fumigation and UV irradiation are capable of killing microorganisms; however, it is uncertain whether the benefits in terms of overall hospital patient infection rates outweigh the risks and costs associated with these methods.

Keywords Hospital acquired infections • Fumigation • UV irradiation • Environment • Contamination

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List of Abbreviations

CDC	Centers for Disease Control and Prevention
CRE	Carbapenem-resistant enterobacteriaceae
EPA	Environmental Protection Agency
HAIs	Healthcare-associated infections
HPV	Hydrogen peroxide vapor
MRSA	Methicillin-resistant *Staphylococcus areus*
OSHA	Occupational Safety and Health Administration
PEL	Permissible exposure limit
RH	Relative humidity
TLV™	Threshold Limit Value
UV	Ultraviolet
UVA	Ultraviolet A
UVB	Ultraviolet B
UVC	Ultraviolet C
VRE	Vancomycin-resistant *Enterococcus*

9.1 Introduction

There has been increased interest in the use of chemical fumigation and ultraviolet germicidal irradiation in healthcare facilities because of concerns about the role of the environment as a cause of healthcare-associated infections (HAIs) and a perception that current surface cleaning and disinfection methods are ineffective. Methicillin-resistant *Staphylococcus areus* (MRSA) and other gram-positive bacteria have become an increasingly common problem in healthcare environments [1–3]. One major concern has been the upswing in incidence of infections caused by *Clostridium difficile* (*C. difficile*) colitis [4]. This organism is now considered to be the most important cause of diarrheal HAI [5]. *Acinetobacter baumanni* is yet another microorganism involved in HAIs that has been linked to environmental contamination [6–8]. While norovirus has been primarily a food or waterborne disease, it is becoming a serious HAI problem in healthcare settings because it survives on surfaces and is highly infectious [9]. The latest microbes of concern are the carbapenem-resistant Enterobacteriaceae (CRE), especially involving *Klebsiella pneumoniae* [10, 11]. CRE is becoming more prevalent in U.S. hospitals because it is difficult to treat, and it also has a case fatality rate that may exceed 40 % [12]. On the positive side, CRE organisms will be less resistant to environmental disinfection than other organisms such as spore formers.

HAIs are a significant contributor to morbidity, mortality and cost in healthcare facilities [13–16]. Klevens et al. estimated there were 1.7 million HAIs in 2002 and 98,987 deaths. Scott estimated the cost of HAIs to range from $28.4 to $33.8 billion after adjusting to 2007 dollars. Research has demonstrated that the cost associated with drug resistant strains is $27,000–$127,000 higher than the costs of

non-resistant strains [17]. Cost has been particular concern for the healthcare industry because in 2008, the Centers for Medicare and Medicaid Services began denying payments for HAIs [18]. The issue of HAI cost is complex and is influenced by many factors including the type of patient procedure and the type of infectious agent. In a 2011 study by Umscheild et al., they found that the percentage of HAI that were preventable ranged from 69 to 45 %. If prevented, this would correspond to 134,800 fewer infections and 3,100 fewer deaths and a savings to the nation's healthcare system of $160–$630 million annually. Our understanding of which microbes are causing the most significant morbidity, mortality, and cost continues to evolve. *C. difficile* has been assumed to be one of the most significant pathogens because of its environmental resistance and its high prevalence in hospitals. Stewart and Hollenbeak found that *C. difficile*'s contribution to costs and mortality have been overestimated due to reporting bias [19]. They found that previous researchers had not controlled for differences in types of hospitals, differences in patient populations, and the presence of comorbidities such as diabetes when estimating the total costs of *C. difficile* to healthcare systems. This is an important consideration because concern over limiting the spread of *C. difficile* infections has been a major factor in the push to find alternative disinfection modalities.

Until recently, the common assumption has been that the number one risk factor for HAI is direct contact spread between a carrier and the patient or autoinfection of the patient due to colonizing organisms [20, 21]. According to the Centers for Disease Control and Prevention (CDC), there have only been a few reports documenting "cause and effect" between environmental contamination and infection [22]. In the last several years, there have been a number of studies that suggest a more important role of the environment in HAI [3, 6, 21, 23–34]. There are a number of factors that contribute to the contamination of healthcare environments. Patients infected with MRSA and other communicable diseases will shed these microorganisms and may potential serve as a source of HAIs [24, 27, 29, 35–38]. Since *C. difficile* is a spore-former, it would be expected to be viable for months [39]. More importantly, the patient's environment has long been known as an important reservoir for *C. difficile* infections [40]. Even gowns and gloves worn to protect the healthcare worker have been found to be contaminated and to serve as potential vehicles of transmission [41]. A particular concern is that hospital textiles such as linens, surgical drapes, uniforms, patient apparel and many more may play a role in infection transmission [42]. There is evidence that the type of species or strain of microorganism affects survival in the environment. For example, *A. baumannii* strains survive desiccation better than other *Acinetobacter sp.* [35, 43]. In the case of *C. difficile*, there is evidence that the organism has mutated and now has greater toxin production, pathogenicity, and infectivity [4, 44]. Akerlund et al., refer to this mutated organism as "hypervirulent." While there is ample evidence that certain microbes can survive in the environment for long periods of time, the assumption that the environment was the cause of the infection has been based primarily on correlation studies. The actual mechanism of transfer of the infectious agent to the patient is not well understood. A large scale

observational study attempted to identify the kinds of contacts between patients, healthcare personnel, and visitors that present a risk of nosocomial infection [45]. The researchers found that in 21.6 % of interactions between patient and healthcare provider or a visitor, there was no contact with either the patient or the environment. The most frequent interaction was contact with the patient's environment (33.5 %), followed by contact with intact patient skin (27.1 %). The interaction with the highest potential for infection transfer was contact with the patient's blood or body fluids (17.8 %). A particular concern was the proper use of gloves while touching blood or body fluids. The study revealed that healthcare providers complied over 94 % of the time, whereas only 33 % of visitors did. This is an important consideration because failure to comply with hand hygiene and gloving policy by visitors may significantly increase the potential for infection transmission.

While hand hygiene is the cornerstone of modern infection prevention, there are inherent weaknesses in standard infection control practices in U.S. hospitals. The CDC has promoted the use of alcohol-based hand scrubs as the standard of practice for hand hygiene for many years [46]. Unfortunately, alcohol is ineffective in destroying *C. difficile*, and in the case of *A. baumannii*, Edwards et al., found that it enhances the growth and pathogenicity of the organism [47]. These authors believe that for *C. difficile* and *A. baumannii*, there should be a return to basic hand washing as the primary approach to hand hygiene.

The design and maintenance of healthcare building construction or mechanical systems is important in preventing HAIs. For example, poor construction or maintenance can contribute to water infiltration and mold growth or leaking isolation rooms. Even if the isolation rooms were initially constructed properly, renovations and other modifications such as cable installations can result in barrier penetrations. A serious problem with mechanical systems occurs when isolation rooms have substandard air exchange rates. Researchers have found isolation rooms that were not air tight due to penetrations and inadequate exchange rates [48–52]. In one study, the authors found 9 % of negative pressure isolation rooms were actually under positive pressure relative to the corridor [52]. The problem with insufficient pressurization was most pronounced in isolation rooms with suspended ceilings.

Chemical fumigation was used in the 1960s to supplement standard environmental surface disinfection in hospital isolation rooms and other critical areas [53]. There was an assumption that surface disinfection was inadequate and that a chemical fog would destroy microorganisms in hard to reach locations. The CDC, in their *Guidelines for Environmental Infection Control in Health-Care Facilities*, recommended against the practice of using chemical fogging for general infection control in routine patient care areas because there was a lack of evidence of efficacy [22]. In 2001, a fumigation technique using chlorine dioxide effectively destroyed bacteria and their spores in the heavily contaminated Hart Senate Office Building and some U.S. Post Office facilities [54]. Because of the success in destroying anthrax using fumigation, healthcare officials have begun adopting fumigation techniques in hospitals and similar institutional environments as an adjunct to routine cleaning methods.

There have been a number of chemicals tested for use as fumigants. Examples of these include chlorine dioxide, hydrogen peroxide vapor, hydrogen peroxide and silver dry mist, super-oxidized water, or ozone for terminal disinfection of hospitals contaminated with mold and bacteria [37, 55–59]. In the past, paraformaldehyde, has been used to decontaminate biological safety cabinets and in entire buildings [60]. Fumigants such as chlorine dioxide and hydrogen peroxide continue to be the two agents most frequently examined to decontaminate hospitals, animal research facilities or similar environments. One of the selling points of these products is that their composition as a gas or vapor allows them to be deposited on hard to reach areas [37]. Research has consistently demonstrated the ability of these fumigants to kill microorganisms in the laboratory; however, there are still concerns for the safety of patients, workers, or research animals that may be inadvertently exposed to these toxic chemicals when used in the field. Some of the health effects resulting from exposure to certain fumigants may include neurological signs and respiratory damage [61]. Other symptoms from exposure may include severe nausea, vomiting and dizziness. Limiting exposure to these toxic chemicals must be considered when using fumigants in areas where humans or animals may be present.

Another modality that has been used in place of or in addition to chemical fumigation is ultraviolet (UV) irradiation. Research has shown that the mode of action of UV is due to damage to nucleotic acids, enzymes, amino acids, phospholipid membranes [62–64]. UV wavelength in the range of 200–295 nm has been determined to be most effective in damaging cellular DNA (most commercial lamps primarily produce UV at a 254 nm wavelength.).

UV has a long history of use in healthcare settings. For example, UVA has been used for many years to cure plastic resins and as a black light. Black lights have been used to identify the presence of rodent infestations and in hand hygiene demonstrations (Glo Germ™). This same approach has been recently applied by environmental services to assess the quality of surface disinfection [65]. UVB is also being used in phototherapy to treat certain types of skin conditions such as psoriasis [66, 67].

UVC has been used for upper air disinfection in the control of tuberculosis or other respiratory diseases for over 100 years [68]. The most effective design involves upper-room placement of the germicidal lamps that are shielded to reduce human exposure. Another effective approach is to place the UVC lamps in the exhaust system ductwork, or they may be used in the disinfection of ventilation system cooling coils [69–71]. To be most effective, the design of the dilution exhaust ventilation should bring the airborne pathogens into close proximity to the UVC germicidal lamps. Chang and Young found that in situations where the UVC radiance is high, the air turbulence is high, and the air velocities are low, germicidal effectiveness is reduced [72]. They emphasized the importance of conducting tracer turbulence studies before considering the use of germicidal UVC to assure that the airborne microorganisms come into close proximity to the lamps.

While there is a long history of using UVC for upper air disinfection, its use in surface disinfection is a relatively recent development [73, 74]. There are a number of challenges using this germicidal modality because in general, microbes are easier to inactivate in the air than on surfaces.

9.2 Methods

Information for this chapter came primarily from peer reviewed and government publications. Papers were sought by performing word searches on a variety of standard internet tools including Medline and Google scholar®. An extensive library of papers on the associated topics of healthcare infection control, fumigation, ultraviolet irradiation, and environmental health and safety were compiled for review and consideration.

Studies of the use of fumigants in settings other than healthcare were also reviewed. Those studies involving fumigation in healthcare explored their use as an adjunct to conventional environmental disinfection [37, 55–58, 75–77]. Fumigation tests were performed under a variety of conditions. Some were efficacy tests in laboratory facilities with controlled environmental conditions, and others were fumigation effectiveness tests administered in the field under ambient conditions. Two studies investigated human exposures during fumigation use in agriculture or residential settings [78, 79]. Fumigation is being marketed as an effective means of controlling undesirable microorganisms. The challenge faced by fumigation researchers is to develop an approach that is successful in killing harmful microorganisms, while preventing health effects and environmental harm from exposure. Unfortunately, most studies only addressed the degree of microbial disinfection efficacy, not safety. Also, with the exception of reports by the Environmental Protection Agency (EPA), environmental damage from exposure to materials or equipment was only mentioned occasionally in the studies investigated [37, 60, 80].

There were many sources that discussed UVC irradiation as a means of upper air disinfection [68, 81–83]. However, in terms of research on UVC for surface disinfection, the literature was limited, and only laboratory based research was located [84–87].

9.3 Results

9.3.1 Fumigation Benefits: Efficacy, Effectiveness and Efficiency

Reports of the efficacy and efficiency of different types of fumigation approaches were examined. See Table 9.1 for definitions of *Efficacy*, *Effectiveness*, and *Efficiency*. An early method of fumigation was to use a high velocity-fogger to spray quaternary ammonium compounds in hospital rooms [53]. The unit was operated with the room air conditioners off and the doors and windows closed. The apparatus was placed in the middle of the hospital room and the fogging cycle was 10–15 min. Researchers believed that this approach was effective because they observed a reduction from the average of 10–15 detectable bacteria per cubic foot (0.0283 m^3) of air prior to fogging to less than 2 or 3 detectable microorganisms after fogging. Similar reductions were seen on surface samples.

Table 9.1 Definitions of efficacy, effectiveness, and efficiency

Efficacy	Did the agent work under controlled conditions such as in the laboratory?
Effectiveness	Did the agent work as intended in field conditions? This category included safety of the product for humans, laboratory animals, environmental surfaces or equipment
Efficiency	If the agent was determined to be effective, does the benefit exceed the cost? It is important to note that efficacy and effectiveness must be demonstrated before efficiency should be considered

The EPA reviewed the results of four models of fumigation equipment for their ability to destroy three types of spore forming bacteria [80]. The bacteria were: *Bacillus anthracis* Ames strain (*B. anthracis*), *Bacillus subtilis* (*B. subtilis*), and *Geobacillus stearothermophilus* (*G. stearothermophilus*). Test strips of seven types of porous (e.g., carpet) and non-porous (e.g. galvanized metal) materials were treated with a concentration of 10^8 viable biological spores. Two approaches used chlorine dioxide as a fumigant, one used formaldehyde, and one used hydrogen peroxide plasma. The Sabre Technical Services system generated 3,000 ppm of chlorine dioxide in a 3 h treatment. The CDG Research Corporation system was designed to release 2,000 ppm of chlorine dioxide over a 6 h period. The CERTEK, Inc. formaldehyde generator had an average concentration of 1,100 ppm over an 11 h period. The BIOQUELL, Inc. hydrogen peroxide plasma generator achieved a 1,000 ppm concentration in a 1 h cycle. The EPA published tests provided for "worst-case" scenarios for fumigation treatment because it is more difficult to destroy surface contamination than spores dispersed in the air [57]. The EPA tests of sporicidal efficacy found significant differences depending on the type of surface, the type of microbial spore, and the type of fumigant. As expected, all fumigants performed better on non-porous materials, and industrial grade carpet proved most difficult to decontaminate. In general, chlorine dioxide and formaldehyde performed better than hydrogen peroxide plasma in destroying spores. For example, the Sabre Technical Services chlorine dioxide generator achieved a greater than 7.0 log kill of spores in carpeting, whereas the BIOQUELL Inc. hydrogen peroxide generator had only a 0.81 log reduction.

While hydrogen peroxide was found to be less effective in the destruction of bacterial spores, French et al., found it to be more effective than conventional cleaning in destroying MRSA in rooms previously occupied by patients carrying this organism [37]. After treating these rooms for 40 min at a concentration of 500 ppm, they found that MRSA had been destroyed in 84 of 85 locations tested. They also reported the destruction of test samples containing 10^6 *G. stearothermophilus* spores that were applied to some stainless steel disks suspended in the room. Krause et al., had similar success with hydrogen peroxide in decontaminating animal research laboratory areas [76]. They used the VHP1000 system for hydrogen peroxide fumigation of animal rooms, and this unit was designed for direct connection to cages and rooms. Also, due to the design of the rooms, work could be continued in adjacent rooms or areas. The machine operational cycle lasted 3 h and outside monitoring of concentrations of hydrogen

peroxide never exceeded 0.02 ppm. After fumigation no contamination was observed. They also found that this fumigant did not appear to be corrosive or damaging to surface materials.

In a before-after intervention study, Boyce et al. assessed the effectiveness of HPV in the control of *C. difficile* [30]. During the intervention period, rooms that had previously housed *C. difficile* patients were fumigated. Each room took approximately 3–4 h to disinfect. The average incidence rates of *C. difficile* infection dropped from 2.28 per 1,000 patient days during the pre-intervention phase to 1.28 ($p = 0.047$) during the intervention phase.

Burton et al., explored the efficacy of chlorine dioxide in destroying bacteria and mold in a private home [57]. Mold was present on the first, second and third floor of a residence. During the treatment process, concentrations were monitored outside and on each floor. The house was enclosed in a plastic tent, and the treatment process did not start until a minimum concentration inside the house reached 500 ppm. The highest concentration in the house was 902 ppm. A variety of microbial air sampling methods were used, including an Andersen N-6 single stage sample, spore traps, fungal PCR, and endotoxin samples. In addition, sticky tape was used to measure total surface fungi. A laboratory evaluation was also conducted using a challenge test sample of 10^6 fungal spores/ml. The laboratory evaluation was conducted inside a plastic chamber. The test was performed using three time periods (4, 8, and 12 h) at 760 ppm. While the fumigant proved to be effective in destroying viable microorganism in the field tests (kill rates of vegetative organisms and spores ranging from 84.9 to 97.6 %), researchers found an increase in endotoxins or mycotoxin levels. When they repeated their experiments in a laboratory setting, they obtained similar but slightly lower efficacies. One possible reason for the decrease in kill rate was that the temperature and RH in the laboratory was lower than in the field study. Temperature and RH are critical factors in microbial viability.

Clark et al., used a Dyna-Fog model to dispense superoxidized water fog to kill MRSA and *Acinetobacter baumannii* organisms [58]. The superoxide fog solution was marketed under the trade name of Sterilox. In this study, ceramic tiles were treated with 10^9 concentrations of the organisms and allowed to dry. The Sterilox fumigant was released into a laboratory using a 3.8 L fogging machine that created an airborne concentration of 180 ppm of free chlorine at a pH of 5.2. The fog was released for 10 min at a maximum setting of 19 L/min. An hour later, the samples were removed for testing. The MRSA strains showed approximately a 10^4 – fold reduction and the *Acinetobacter* strain showed a greater reduction (approximately 10^6 – fold). No information was provided on the effects of this product on surfaces or equipment.

Other approaches were not as successful. For example, Berrington and Pedler found that ozone killed microorganisms only in the immediate vicinity of the generator. At greater distances, ozone was deemed to be ineffective in its ability to kill MRSA [56]. There are a number of challenges associated with the use of ozone, including the need for high relative humidity (RH) to be effective.

9.3.2 Fumigation Risks: Health and Safety and Costs

While efficacy is important, so is the safety of workers and other building occupants. Nine greenhouse workers were accidently exposed to methyl bromide in a greenhouse [78]. In this incident, one of the sections of the greenhouse was being fumigated at the same time the workers were working in an adjacent section. It was believed that the workers were safe from exposure because their section was separated with a glass partition wall. Unfortunately, the fumigant traveled up a sewage pipe into the occupied section of the greenhouse. It was noted that exposure lasted for up to 6 h and reached a concentration that peaked at 200 ppm. This concentration of methyl bromide was 200 times the accepted exposure limit of 1 ppm [61]. All nine workers experienced nausea, repeated vomiting, and dizziness. Some had symptoms that included twitching of the limbs and generalized seizures, and two of the workers were placed in intensive care for several weeks. Fortunately, the use of methyl bromide as a fumigant has been decreasing due to its toxic effects.

Another study investigating the safety of fumigation involved methyl bromide exposure to a family of three [79]. The accident occurred when a neighboring house was being fumigated, and again the fumigant moved from the target structure through sewer lines to the occupied house. This incident resulted in the death of a newborn and severe illness to the parents. The family was exposed for an estimated 5–6 h. While actual methyl bromide level inside the home of the victims was not measured, the concentration was estimated to be 12,850 ppm. The infant experienced vomiting and severe diarrhea. The symptoms lasted 6–7 h, and upon arrival at the hospital, the infant was declared dead. An autopsy revealed that the infant had received severe lung tissue damage. The cause of death was due to acute pneumonia due to aspiration from inhalation of methyl bromide. The two adults experienced dry cough, sore throat, nausea, vomiting, dizziness and drowsiness.

In both situations, the site of fumigation was unoccupied, but in each case, the fumigant breeched containment, exposing the workers and the family. As stated earlier, the effect of fumigants on environmental surfaces or equipment was not routinely evaluated in studies. The EPA found that formaldehyde did not appear to damage surfaces; whereas, chlorine dioxide caused bleaching of surfaces, and hydrogen peroxide discolored dyes and had unfavorable interactions with nylon [60, 80].

There was little evidence presented in the peer reviewed literature on the occupational and environmental exposure that results from fumigation activities in healthcare. On the other hand, numerous case studies and peer reviewed reports are available on occupational and environmental exposures in non-healthcare incidents. Some of these exposures occurred during routine operations.

9.3.3 UVC Germicidal Irradiation Risks and Benefits

As previously mentioned, the vast majority of information on UVC use as a germicide deals with upper air disinfection. Unfortunately, there are few laboratory-based efficacy studies on UVC for surface disinfection, and no field-based microbiology studies of UVC as of this writing.

One laboratory study of surface disinfection used 40-W UVC lamps at approximately 2.4 m above nutrient agar plates cultured with a variety of bacterial and fungal species [84]. The duration of exposure was 30 min resulting in a four log reduction in all organisms tested except *Candida, Bacillus subtilis* spores, *Aspergillus* spores and *Mycobacterium fortuitum*. *Aspergillus* spores exhibited the highest resistance with only 2.5 log reduction. *Penicillium* and *Stachybotrys* have shown similar resistance in other studies. Prions and viruses were not evaluated. Another study used four 8-W UVC lamps at a distance of 30.5 cm, for durations between 3 s and 6 min at 55 % RH and again at 85 % RH against four different bacteriophages [87]. Tseng and Li found that 90 % reductions were similar to those achieved with non-spore forming bacteria and that viruses are more susceptible in air than on surfaces. They also found that survival was inversely related to dose (as expected). Double stranded DNA viruses were found to be most resistant, and viral inactivation required higher doses of UVC at 85 % RH compared to 55 %. These results are consistent with Riley and Kaufman's earlier work with *Serratia marcescens*. These authors found the organism to have exceedingly low death rates at humidities above 80 % and that photo-reactivation of the organism was likely. RH level is a critical factor influencing the effectiveness of UVC as a germicide [88]. In their research on the effects of UVC irradiation on *Deinococcus sp*., Bauermeister et al. found that it was more detrimental to bacterial cells at a higher RH than at low RH (33 %) and that desiccated cells were highly resistant to the effects of UVC. The above studies suggest that RH levels between 50 and 60 % are essential for UVC irradiation to be effective.

Researchers also explored the potential for using UVC irradiation to disinfect medical supplies and equipment. For example, Fisher and Shaffer believed that UVC could be used to disinfect N95 disposable respirators [85]. The reason to do this is that during an epidemic of influenza or other airborne pathogen, there may be a shortage of single use N95 and that UVC germicidal treatment and respirator reuse may extend the supply. Nerandzic et al. used a handheld, UVC wand to disinfect hospital surfaces such as keyboards and portable medical equipment [89]. There were three settings, and the highest setting is called "deodorize" because it produces small amounts of ozone (<0.05 ppm). This 1.8 kg shielded wand was attached to a wheeled power pack and produced a radiant dose of 100 mJ/cm^2 after a 5 s exposure at the highest setting. The unit produces far-ultraviolet radiation (185–230 nm) and was tested for germicidal effect against *C. difficile*, MRSA, and vancomycin-resistant *Enterococcus* (VRE). The unit manufacturer used the far-ultraviolet spectrum in the belief that the higher photon energy would increase the speed of microbial inactivation. The distance from the wand to the surface was not specified, and no mention was made of ambient conditions such as RH and temperature. The highest reduction was demonstrated against VRE (6.9 $\log_{10}$CFU), and the lowest reduction was against *C. difficile* (4.4 $\log_{10}$CFU). In the presence of organic matter, the unit was less effective with only a 3.4 $\log_{10}$CFU reduction of *C. difficile*. This is not surprising given the limited penetration ability of UV in the 185–230 nm range. The authors concluded that additional research was needed to determine if this technology could serve as a useful adjunct to routine environmental services in healthcare facilities.

One of the major reasons that researchers are exploring the use of additional treatment modalities such as UVC irradiation and chemical fumigation is the belief that spore forming microbes such as *C. difficile* may be difficult to control using conventional environmental disinfection methods [30, 33]. Microbial spores present a particular challenge because their inner and outer coats are highly resistant to inactivation by chemical and physical means. Furthermore, during sporulation, the microbe may be able to repair the damage from the germicidal treatment [90]. Moeller et al. found that certain metabolites such as cysteine that are released during sporulation, do reduce the ability of UVC to inactivate *B. subtilis* spores. In addition, if these spores had been previously desiccated, the lowered RH in the spores further reduced the effectiveness of the irradiation. The authors identified similar problems when using HPV as the germicidal agent.

Recently efforts have been made to increase UV germicidal efficacy in water and on surfaces by combining it with photocatalysts like TiO_2 or Ag [91]. Some researchers have found that even UVA has germicidal effects on vegetative organisms in the presence of TiO_2/Ag. This is an area of new research and as of this writing; the mechanism is not well understood.

It has been long known that exposure to UV can cause deleterious effects to the skin, eyes, and immune system [92]. It is also known that all forms of UV can cause skin cancer [93, 94]. However, it is important to note that UVC is the least likely to cause skin cancer because the depth of penetration is so shallow, and the radiation is absorbed in the outer layer of dead skin cells [68]. The primary health effects of UVC overexposure are erythema of the skin, photoketaoconjunctivitis and photokeratitis. Nevertheless, overexposure should be avoided because the injuries such as sunburn, snow blindness, and "welder's flash" can be painful and debilitating, at least in the short-term. As of this writing, there were no studies that documented worker or patient exposures to UVC during surface disinfection; however, First et al. measured 8-h doses to workers and patients from upper room UV irradiation ranging from 7.9 to 34 mJ/cm^2 [81]. This exposure was well above the Threshold Limit Value^{TM} (TLV) for UVC of 3 mJ/cm^2 as an 8-h time weighted average [61]. The authors concluded that this exposure was not a concern because the position of the dosimeter significantly overestimated the actual dose, and they believed that the TLV could be doubled and still be protective. It is important to remember that the American Conference of Industrial Hygienists (ACGIH), the association that publishes the TLVs, recommends against using their standards as a basis of protecting members of the general public. Their reasons for making this recommendation are that the members of the general public may be exposed for more than an 8 h duration and because members of the general public may be more susceptible to chemical or physical exposures than healthy workers. Patients in a healthcare setting would be expected to be exposed for more than 8 h and to be more susceptible to the deleterious effects of overexposure.

There are other concerns associated with the use of UVC as a germicidal modality. Said et al. found that *Escherichia coli* exposed to UVC could be rendered non cultivable but still viable [95]. Photoreactivation after exposure to UVC has long been known to be a problem [96]. With photoreactivation, what appear to be

dead microbes are revived after exposure to longer wavelength radiation due to DNA repair mechanisms [97]. The longer wavelength light provides the energy for this DNA repair. A final concern is that many healthcare facilities are attempting to minimize the presence of mercury in the environment. Ultraviolet lamps, if broken will spill elemental mercury into the immediate area [62].

9.4 Discussion

This chapter described the purpose for and risks and benefits of the use of chemical fumigation and UVC irradiation for surface disinfection. See Table 9.2 for a summary of pros and cons of these alternative modalities. This study looked at the efficacy and effectiveness of fumigation in institutional environments. There are other areas that frequently use this practice, and by observing consequences of fumigation in other environments, the findings may be analyzed for relevance in healthcare or research institutions. One of the most common uses of fumigation is for the control of pests and mold in homes and other buildings. The standard approach is to evacuate people and pets and then fill the space with a gaseous pesticide to kill the target organism. A similar method is also used in controlling pests in soil, grain and produce. The record of fumigation safety suggests that even industries with many years of experience with chemical fumigation still have over exposure incidents.

A number of chemicals have been used over the years to clean and disinfect critical environments. For example, formaldehyde will kill microorganisms, including their resistant spores, but as Krause points out, it is slow, difficult to generate

Table 9.2 Pros and cons of chemical fumigation and UVC germicidal irradiation for surface disinfection

Modality		Pro's	Con's[a]
Fumigation	Chlorine dioxide	Good biocidal properties on all surfaces, a gas that will easily disperse throughout a space	Highly toxic (TLV = 0.1 ppm, STEL = 0.3 ppm), must be generated on site, may damage surfaces & equipment
	Hydrogen peroxide plasma (HPP)	High kill rate on hard surfaces, easier to generate	Toxic (TLV = 1 ppm), less effective on porous surfaces, no easy method to monitor
	HPP plus silver	Same as HPP	Same as HPP & no method to measure silver exposure
UVC irradiation		Easy to administer	Limited efficacy data, many challenges including variability in organism susceptibility, limited to RH range of 50–60 %, assuring minimum contact distance, photo-reactivation likely

[a]Containment of the gas, vapor, or radiation is essential to prevent human exposure

and potentially carcinogenic to humans [76]. Formaldehyde is also extremely irritating and both a dermal and respiratory sensitizer [98]. These undesirable properties have limited its use as a chemical fumigant. Some other chemicals that have been considered were hydrogen peroxide, hydrogen peroxide and silver dry mist, ozone, superoxidized water (Sterilox) and chlorine dioxide. Ozone appears to be limited in its effectiveness as a fumigant, and Sterilox was only evaluated in one study with modest success [56, 58]. A major advantage of hydrogen peroxide is that it breaks down into oxygen and water, leaving no toxic residues [76]. One vendor claims that the addition of silver to hydrogen peroxide provides residual inhibition of microbial growth; however, no independent confirmation was located.

While fumigants can be successful in killing microorganisms, some chemical agents have been found to be more effective than others in certain test environments [80]. For example, chlorine dioxide achieved a higher kill rate on test samples of industrial carpeting than did hydrogen peroxide. On the other hand, chlorine dioxide is more likely to bleach the color from exposed materials and is more toxic than hydrogen peroxide [61]. While these fumigants have demonstrated high kill rates, in occupied hospital rooms, environmental surfaces will be constantly re-contaminated. CDC does not recommend chemical fogging for general infection control in routine patient-care areas because of the issue of recontamination and the lack of evidence that chemical fogging will reduce nosocomial infection rates [22]. While the CDC has not taken a position on the newer approaches to chemical fumigation, these approaches have yet to provide convincing evidence of effectiveness in lowering infection rates or in their ability to be used safely.

Another issue is that, while chemicals such as chlorine dioxide effectively kill viable microorganisms, they will not affect the toxicity associated with non-viable microorganisms and their endotoxins or mycotoxins [57, 99]. This is an important limitation because the primary health effect from mold exposure is an allergic reaction, not an infection [100]. So unless water infiltration or other sources of the mold are eliminated, fumigation will have little benefit.

Fumigation is being considered because gases and vapors can permeate areas that are not easily reachable. However, this characteristic also means ventilation ducts, plumbing fixtures, doors, windows and any other openings must be sealed with a material that will resist penetration. The problem associated with blocking ventilation supply, return and exhaust ducts is that it will disrupt the air balance in all rooms served by the same blowers. This could change room pressurization in other locations, including isolation rooms. Also, Rice and others have discovered problems with room leakage in many healthcare facilities [49, 50, 52]. Blocking ventilation ducts causing changes in room pressurization or increasing room leakage could contribute to infection spread [101]. The issue of disruption to the building ventilation systems has not been addressed by fumigation equipment vendors.

One of the most important measures of disinfectant effectiveness would be the demonstration of a reduction in HAI rates. As of this writing only one study demonstrated a significant reduction in HAI rates, and by the authors own admission, this study had severe design issues that prevented them from linking the rate

reduction to the fumigation procedure [30]. The authors noted that the infection rate was already below the hospital's action threshold of 1.1 infections per 1,000 patient days the month before the intervention started. It was also observed that during the last 3 months of the HPV intervention, there was a steady increase in incidence. The last month's rate was above the hospital's action threshold. If fumigation was an effective infection control method, then one would expect consistently low incident rates throughout the 10-month intervention period.

The decision to use fumigation in occupied buildings must be carefully considered, since a breach in containment could injure patients, visitors, or personnel and, in the case of chlorine dioxide and hydrogen peroxide, damage surfaces. To avoid operator errors, the safest approach would be to evacuate during fumigation. However, this would be costly, and relocating displaced patients should be carefully considered to assure there is adequate bed-capacity in other facilities.

Generally, a cost-benefit analysis should not be performed until both efficacy and effectiveness have been firmed demonstrated. Unfortunately, in the case of chemical fumigation, safety and other effectiveness concerns are unresolved. Nevertheless, Otter et al., examined the feasibility of routinely using hydrogen peroxide fumigation [102]. They noted that the time to pre-clean, prepare and administer the fumigant, and wait for the vapor to clear took 4–5 h which was more than 3 times longer than disinfecting with dilute bleach solution. They also noted that as hospital occupancy rates went up, the difficulty in scheduling a time consuming fumigation procedure increased, and more rooms targeted for fumigation were missed. At this point, it is hard to see how busy modern medical facilities could afford the loss of room space during fumigation.

Complex environmental factors may limit the effectiveness of ultraviolet irradiation. To be effective, RH must be within a narrow range (50–60 %) [87, 88]. Also, source to target distance will influence beam intensity, duration of exposure must be sufficient to inactivate microbes, and in the case of upper air disinfection, insufficient air movement and turbulence will significantly reduce effectiveness [72]. The type of organism varies significantly in susceptibility to UVC disinfection. For example, double stranded viruses were more resistant than single DNA viruses, bacterial and fungal spores are highly resistant to inactivation, and photoreactivation has been demonstrated [84, 87, 96]. The use of unshielded UVC lamps significantly increases the likelihood of worker, patient or visitor overexposure. While the depth of penetration of UVC reduces its potential to cause cancer, the U.S. National Toxicological Program lists UVC as an agent "*reasonably anticipated to be a human carcinogen*" based on limited human cell data and sufficient evidence in animal studies [94]. The most common effects of UVC exposure are photoketaoconjunctivitis (snow blindness) and photodermatitis (sun burn) [68]. According to Memarzadeh, the use of germicidal UV as a means of infection control must be evaluated under controlled experimental conditions, rather than reliance on observational and retrospective studies [103]. Results from poorly controlled observational studies would be subject to the Hawthorne effect.

In summary, chemical fumigation of a healthcare facility has merit under certain conditions such as in response to a bioterrorism attack [59]. If a building is heavily

contaminated with dangerous pathogens, fumigation may allow the building to be safely re-occupied. In other situations where patients are shedding organisms such as MRSA, this approach must be used with caution. It is unclear how fumigation can be effective when there is a likelihood of continuous recontamination.

There appears to be an assumption that since UVC is effective for upper air disinfection, so it must also be effective for surface disinfection. This assumption is seriously flawed because it is much easier to inactivate organisms in the air than on surfaces. As of this writing, there is limited research on the effectiveness of UVC for surface disinfection under actual use conditions. This lack of scientific support suggests that the use of this modality should limited to such applications as upper air and ventilation cooling coil disinfection until such time that convincing evidence of effectiveness on surfaces is demonstrated.

If ineffective cleaning and conventional surface disinfection is the problem, then strategies that improve housekeeping effectiveness should be considered. Dancer and his colleagues addressed the problem with MRSA contamination in a hospital in the United Kingdom by improving patient screening and isolation of patients infected with MRSA and by implementing an enhanced cleaning protocol [1]. This enhanced protocol simply involved adding an additional housekeeper, more frequent cleaning and careful monitoring of cleaning performance. The enhanced cleaning resulted in a 32.5 % reduction in aerobic colony counts. There was also a reduction in new nosocomial MRSA cases. However, the authors noted that the study lacked sufficient power to determine if the reduction in infections was significant. According to the authors, the increased cost of an additional staff member and additional supplies was more than offset by the reduction in MRSA infections and the costs associated with patient care. Goodman et al. did a similar study in an intensive care unit targeting MRSA and VRE contamination [104]. Their approach was to study "high-touch" surfaces, train housekeeper to focus on these surfaces, and then monitor the effectiveness of cleaning using microbial cultures and a backlight. Their enhanced cleaning procedures also resulted in significant reductions in MRSA and VRE.

The fact that accidental releases of chemical fumigants in healthcare have not yet been reported does not mean that they will not happen or have happened and were not reported. The evidence from non-healthcare applications demonstrates that accidents are possible, and consequences can be severe. Occupational and environmental exposures in non-healthcare operations have been published, and there is little evidence to indicate that similar exposures cannot occur in healthcare.

There appears to be a lack of consensus and guidance on the safe application protocols for the use of fumigants in healthcare. Validated methods for the recognition and control of hazards must be developed and used to protect workers, patients, and the general public. Exposure limits for these chemicals for patients or other non-occupational groups currently do not exist, making it difficult to determine the safe concentration of chlorine dioxide or HPV for someone who may be in a weakened state. The current Occupational Safety and Health Administration (OSHA) Permissible Exposure Limit (PEL) for chlorine dioxide is 0.1 ppm, and OSHA's sampling method (ID-202) has a limit of detection for a

120-L sample of 0.004 ppm. When applying an occupational exposure limit to a member of the general public, it is essential to apply a safety factor. If a 100-fold safety factor were applied to the PEL, the exposure limit of 0.001 ppm could not be detected using OSHA's validated sampling method. Currently there are no fully validated methods for measuring hydrogen peroxide. OSHA's partially validated method (VI-6) can detect as low as 0.043 ppm, and their other partially validated method (ID-126-SG) can detect only 0.1 ppm. Since the PEL is 1.0 ppm, these methods would not be low enough to determine the safety of a non-occupational exposure.

If, at some point in the future, either fumigation or UVC irradiation are determined to be effective, the cost efficiency should be determined. The assessment of cost must consider more than just the vendor fees or purchase of the equipment. The time that rooms are removed to be fumigated or irradiated should be considered. Fumigation techniques using hydrogen peroxide vapor typically reported a 2–4 h per room cycle time. A greater than 2-h delay could affect room turnover rates and potentially create a significant burden on the short supply of beds in hospitals. The downtime using UVC irradiation would be expected to be less, but the medical or legal costs of overexposure to either ultraviolet radiation or chemical fumigation must be considered. An additional cost is that rooms to be fumigated must be checked by a qualified person for potential leakage or for potential deleterious effects on the facility's ventilation system. The cost of exposure monitoring should be factored into the total price of a fumigation or UVC irradiation procedures.

9.5 Conclusion

Fumigation and ultraviolet germicidal irradiation in healthcare facilities and other related institutions should be limited to those instances where the benefits clearly exceed the risks of human exposure or environmental damage. Fumigation of an unoccupied building following a bioterrorism incident would meet this criterion. In situations where the building is occupied, and the potential for recontamination is high, the benefits of fumigation do not appear to exceed the risks. There is limited research on the effectiveness of UVC as a surface germicide. Therefore, its use should be restricted to areas with a proven benefit such as upper air and ventilation system coil disinfection. Before potentially risky procedures such as fumigation or ultraviolet irradiation are considered, simpler and safer approaches such as enhanced cleaning should be considered first.

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