M. Bonten, E. Stobberingh, J. Philips, A. Houben

Antibiotic Resistance of *Escherichia coli* in Fecal Samples of Healthy People in Two Different Areas in an Industrialized Country*

Summary: Fecal samples of 310 healthy persons, from two populations from different areas in the Netherlands, were examined for the presence of *Escherichia coli* resistant to ampicillin, tetracycline, sulfamethoxazole, trimethoprim and nitrofurantoin. High prevalences of resistance were found in both populations, ranging from 28% for trimethoprim to 89% for ampicillin. The percentages of the fecal samples with a dominantly resistant *E. coli* flora (> 50% resistance) were distinctly lower, ranging from 1% for nitrofurantoin to 21% for tetracycline. No significant differences in the level of resistance were observed between these two comparable populations in two different areas. The susceptibilities to 11 antimicrobial agents of 456 at random isolated *E. coli* were determined. The percentages of resistance varied widely: from 80% for chloramphenicol to 9% for nitrofurantoin. Only 19% of the isolates were susceptible to all antibiotics tested and 14% were resistant to more than four of the agents tested. Great differences in resistance rates between the two populations examined were seen for chloramphenicol (80% to 41%) and trimethoprim (16% to 36%). The results of this study underscore the presence of a human reservoir of antibiotic resistant microorganisms.

Zusammenfassung: *Antibiotikaresistenz von Escherichia coli in Stuhlproben gesunder Personen in zwei verschiedenen Regionen eines Industrielandes. Escherichia coli*-Resistenz gegen Ampicillin, Tetracyclin, Sulfamethoxazol, Trimethoprim und Nitrofurantoin wurde in Isolaten aus Stuhlproben von 310 gesunden Personen aus zwei Bevölkerungsgruppen verschiedener Regionen der Niederlande geprüft. In beiden Populationen fanden sich hohe Resistenzraten von 28% gegen Trimethoprim bis 89% gegen Ampicillin. Der Prozentsatz von Stuhlproben mit dominant resistenter *E. coli*-Flora (mehr als 50% resistente Stämme) war eindeutig geringer mit 1% für Nitrofurantoin und 21 für Tetracyclin. Der Grad der Resistenz war zwischen den vergleichbaren Populationen dieser zwei verschiedenen Regionen nicht signifikant verschieden. Die Empfindlichkeit der von 456 Personen isolierten *E. coli*-Stämmen gegen 11 Antibiotika wurde untersucht. Die Resistenzraten zeigten erhebliche Unterschiede von 80% für Chloramphenicol bis 9% für Nitrofurantoin. Nur 19% der Isolate waren gegen alle Testantibiotika empfindlich, 14% waren gegen mehr als vier der Testsubstanzen resistent. Die beiden Populationen wiesen große Unterschiede in den Resistenzraten gegen Chloramphenicol (80% gegenüber 41%) und Trimethoprim (16% gegenüber 36%) auf. Die Ergebnisse dieser Studie bestätigen das Vorliegen eines Reservoirs an resistenten Mikroorganismen beim Menschen.

Introduction

Since the development and introduction of antimicrobial agents about 60 years ago, there is a growing concern about the increasing incidence of antibiotic resistance [1]. As a result, the therapeutic value of originally effective antibiotics became significantly reduced over time, increasing the need for newer, more effective compounds. However, neither the development of new drugs nor the use of combinations of antibiotics could solve the problem of resistance and even to the newest group of agents, the fluoroquinolones, resistance has been described [2–6].

There is no question about the effect of antibiotic susceptibility on the clinical outcome of therapy. However, initial antibiotic therapy is used empirically in many situations, such as in outpatient clinics as well as in hospitals, and is based more on epidemiological data and on resistance trends than on the antibiotic susceptibility of individual isolates [7]. In contrast to data on antibiotic susceptibility of hospital isolates, which are generally available via microbiology laboratories, little is known about susceptibilities of community strains [8,9]. However, a high level of resistance in microorganisms colonizing healthy persons may be an indicator of resistance in the infecting microorganisms, since the latter arise from the former [10]. The largest amount of colonizing microorganisms is found in the intestines of healthy subjects. Knowledge about the susceptibilities of these isolates may improve the efficacy of initial antibiotic therapy and may further elucidate the future potential for resistance in infections [11].

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M. Bonten, M. D., Dept. of Internal Medicine, *E. Stobberingh*, Ph. D., *J. Philips*, *A. Houben*, Dept. of Medical Microbiology, State University of Limburg, P. O. Box 616, NL-6200 MD Maastricht, The Netherlands.

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As no recent data were available on the Dutch population, the prevalence of antibiotic resistant fecal *Escherichia coli* and the antibiotic resistance level of the fecal flora of healthy persons from Maastricht (in the south of the Netherlands) and Zwolle (in the north) were determined. In addition, the susceptibilities of the 456 microorganisms isolated were determined.

Materials and Methods

Sample population: Fecal samples were collected in two medium-sized cities, Maastricht and Zwolle, located about 250 km apart. The samples were obtained from healthy persons living in four neighbourhoods, two in each city, with a comparable socioeconomic standing. Information was supplied to all inhabitants of the four neighbourhoods by letter, followed by personal information by one of the authors. Participants answered a questionnaire that included such items as age, sex, history of antibiotic use and hospitalization in the past three months.

Sample collection and processing: Fresh fecal samples were collected directly into small containers which were transported to the laboratory immediately. One gram of feces was mixed in 9 ml of distilled water (with 20% glycerol and 0.9% NaCl) and stored at –20°C. From this suspension, serial tenfold dilutions were made up to a final dilution of 10^{-4}. To determine the number of *E. coli*, 0.037 ml of the 10^{-2} to 10^{-4} dilutions were applied to Levine-agar plates (Oxoid CM 69 Ltd., Basingstoke, England) using a spiral plater (Lameris Laboratorium B.V., Breukelen, Netherlands). By using this method and the above serial dilutions the number of microorganisms could be quantified; they ranged from 200 to 1 x 10^8 cfu/ml. On Levine agar colonies *E. coli* have a purple pigmentation with a black centre and a green metallic screen. Only colonies that corresponded to this description were included in the analysis. The total number of *E. coli* in 1 g feces was determined by plating the dilutions on agar without antimicrobial agent. To determine the number of antibiotic resistant microorganisms agar containing one of the following selecting agents – ampicillin 25 mg/l, tetracycline 25 mg/l, sulfamethoxazole 100 mg/l, trimethoprim 8 mg/l and nitrofurantoin 50 mg/l – were used. The degree of antibiotic resistance of each fecal sample to one of the selecting agents was expressed as the proportion (in %) of the number of colony forming units (cfu) of resistant *E. coli* to the total number of *E. coli*. As the experimental error for the determination of bacterial counts is half an order of magnitude and most bacterial counts exceed 10^6 cfu/ml, the proportion of resistant bacteria in 1 g feces was divided into two levels: level I, the proportion of resistance was less than 50% and level II, more than 50%. Thus, level II means that the majority of the fecal *E. coli* flora was resistant to the selecting agent.

A total of 456 strains were picked up randomly from agar plates without antibiotics: 279 from the population of Maastricht and 177 from the population of Zwolle. The antibiotic susceptibility was determined using a microbroth dilution method in Iso-Sensitest broth (Oxoid CM 471 Ltd., Basingstoke, England). An inoculum of 10^5 cfu/ml was used, obtained by dilution of an overnight culture in the same broth. The minimum inhibitory concentration (MIC) was defined as the concentration at which no visible growth was apparent after overnight incubation for 18–24 h at 37°C. The antibiotics tested and the breakpoints for resistance were as follows: > 2 mg/l for trimethoprim, > 4 mg/l for gentamicin, > 8 mg/l for nalidixic acid and chloramphenicol, > 16 mg/l for ampicillin, tetracycline, cephalothin, kanamycin and streptomycin, > 32 mg/l for nitrofurantoin and > 128 mg/l for sulfamethoxazole. The critical MIC values were graded according to the guidelines of the Dutch Working Party on Antimicrobial Susceptibility Testing. For other purposes, more than one strain was isolated from the samples derived from the inhabitants of Maastricht. From the samples from the inhabitants of Zwolle only one strain was isolated. Susceptibilities of all strains were determined. The mean MIC for each antimicrobial agent was used in the analysis if the susceptibility of more than one strain per sample was tested. Statistical analysis was done using the Chi-square test, $p < 0.05$ was considered significant.

Results

Sample Population

Fecal samples from 310 healthy subjects were collected: 126 healthy inhabitants from Maastricht and 184 healthy subjects from Zwolle. The male/female distribution was 0.7:1. The mean ages were 29.3 ± 12.4 and 39.0 ± 14.2 years for the respective groups. Antibiotic use in the last three months (8%) was also quite similar in the two groups: ten and 13 participants, respectively, from the populations of Maastricht and Zwolle. Six subjects used tetracyclines, six others used a broad spectrum penicillin derivative and another six did not know which antibiotic they had used. Six of the study participants had been hospitalized in the previous three months.

Susceptibilities of *E. coli* strains to 11 antimicrobial agents were determined using isolates from 96 and 173 samples from the populations of Maastricht and Zwolle, respectively.

Prevalence of Antibiotic-Resistant Fecal Escherichia coli in the Two Populations

The prevalence of fecal *E. coli* resistant to ampicillin ranged from 62% to 89% for the two populations in Maastricht and Zwolle (Table 1). Differences in prevalence between the two populations were also found for tetracycline (68% and 49%), sulfamethoxazole (71% and 49%) and nitrofurantoin (48% and 28%). None of the differences in prevalence rates reached statistical

Table 1: Prevalence of antibiotic-resistant fecal *Escherichia coli* in the populations studied.

Selecting agent (mg/l)		Prevalence of antibiotic resistance (%)	
		Population Maastricht n = 126	Population Zwolle n = 184
Ampicillin	(25)	78 (62)	164 (89)
Tetracycline	(25)	86 (68)	90 (49)
Sulfamethoxazole	(100)	89 (71)	90 (49)
Trimethoprim	(8)	57 (45)	77 (42)
Nitrofurantoin	(50)	61 (48)	51 (28)

Table 2: Degree of antibiotic resistance of *Escherichia coli* in fecal samples.

Antimicrobial agent	Population	Degree of resistance[a]	
		I	II
Ampicillin	Maastricht	111 (88)	15 (12)
	Zwolle	160 (87)	24 (13)
Tetracycline	Maastricht	100 (79)	26 (21)
	Zwolle	158 (86)	26 (14)
Sulfamethoxazole	Maastricht	103 (81)	23 (19)
	Zwolle	156 (85)	28 (15)
Trimethoprim	Maastricht	114 (90)	12 (10)
	Zwolle	171 (93)	13 (7)
Nitrofurantoin	Maastricht	125 (99)	1 (1)
	Zwolle	183 (99)	1 (1)

[a]Number of resistant *Escherichia coli* (percent of total *Escherichia coli*) level I = < 50%, II = ≥ 50%.

Table 3: Antibiotic resistance (%) of isolated *Escherichia coli* from two populations for 11 antimicrobial agents.

Antimicrobial agent	Population		
	Maastricht n = 96 %	Zwolle n = 173 %	p[a]
Ampicillin	21	13	< 0.05
Cephalothin	14	12	ns
Chloramphenicol	80	41	< 0.01
Gentamicin	37	21	< 0.01
Kanamycin	51	44	ns
Nalidixic acid	16	16	ns
Nitrofurantoin	9	10	ns
Tetracycline	20	21	ns
Streptomycin	27	25	ns
Sulfamethoxazole	40	47	ns
Trimethoprim	16	36	< 0.01

[a]The p-value was analysed using the Chi-square test, ns indicates p > 0.05.

significance. The prevalence of trimethoprim resistance was quite similar in both groups (45% and 42%).

Antibiotic Resistance Level of Fecal Samples

In the two populations studied a similar percentage of the samples had a dominant resistant fecal flora (≥ 50%, i.e. level II) with 11–13% and 1–3% of *E. coli* being resistant to either ampicillin or nitrofurantoin (Table 2). For tetracycline and trimethoprim the fecal samples of the population of Maastricht showed a higher percentage at the ≥ 50%-level of resistance than the Zwolle population (21% to 14% and 10% to 7%, respectively). However, these differences were not statistically significant.

Antibiotic Resistance Patterns of Escherichia coli

The percentage of resistance of individual isolates to the antibiotics tested is shown in Table 3. The lowest percentage of resistance was observed for nitrofurantoin (9%). For ampicillin, cephalothin and nalidixic acid these

Table 4: Multiple resistance of isolated *Escherichia coli* (n = 456).

Number of antimicrobial agents	Frequency n	%	Cumulative %
0	88	19.3	19.3
1	85	18.6	37.9
2	88	19.3	57.2
3	70	15.4	72.6
4	37	8.1	80.7
5	25	5.5	86.2
6	22	4.8	91.0
7	25	5.5	96.5
8	9	2.0	98.5
9	4	0.9	99.4
10	3	0.6	100.0

percentages were 13%, 12% and 16%, respectively. Resistance to sulfamethoxazole was found in 40-47% and to kanamycin in 44–51% of the strains.

Great differences were seen in the resistance rates against trimethoprim and gentamicin: 16% and 36% and 37% and 21% for the populations of Maastricht and Zwolle, respectively. The differences in resistance to chloramphenicol between the two groups were even larger: 80% of the strains isolated from the population of Maastricht and only 41% in the strains from the population of Zwolle.

Multiple Resistance

Out of 456 *E. coli* strains, 283 were resistant to two or more antimicrobial agents. Only 19% were susceptible to all antimicrobial agents tested and another 19% were resistant to only one agent. Nine percent of the isolates tested were resistant to six or more antimicrobial agents (Table 4).

Discussion

The prevalence rates of *E. coli* resistant to antimicrobial agents observed in this study were relatively high compared to percentages reported in other studies performed in industrialized areas in other developed countries [10–13]. Moreover, similar high prevalence rates were observed by us in a previous study performed among students in the Netherlands [14]. In both studies we used the spiral plating method to perform a quantitative analysis. By plating serial tenfold dilutions 200 antibiotic resistant *E. coli* in a total flora of 1 x 10^7 *E. coli* per gram feces could be quantified. The sensitivity of this method probably resulted in high prevalence rates. As a matter of fact, this method resulted in a higher percentage of samples with a small proportion of resistant *E. coli*; thus, much lower percentages of fecal samples with a dominant resistant flora were observed. The percentage of fecal samples with a dominant resistant flora was more similar to that found in other studies [12,13,15]. In fecal samples from healthy adults in the Boston area, a dominant

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resistant flora to ampicillin was observed in 16.7% and to tetracycline in 18.7% [15]. Eight years later, in 1987, in the same area, 11.3% and 21.3% of the fecal samples had a dominant resistant flora to ampicillin and tetracycline, respectively [15]. In the Netherlands a dominant tetracycline-resistant *E. coli* flora was observed in 12% of healthy adults in 1980 and in only 6% in 1987. In contrast, the percentages observed for ampicillin were 5% in 1980 and 11% in 1987 [12,13]. Both studies were performed in an area different from that of the present study. Despite differences in methods, populations and breakpoints of resistance between the studies mentioned, the percentages of a dominant resistant flora where quite similar. It is therefore likely that 10 to 20% of the healthy population in industrialized countries carry a fecal *E. coli* flora dominantly resistant to ampicillin or tetracycline. It is also likely that these percentages are lower for trimethoprim and nitrofurantoin, although further studies are needed to confirm this. Ten to 20% of healthy persons carry at least 5 x 10^6 *E. coli* resistant to one of these antibiotics. These results underscore the presence of a large intestinal reservoir of microorganisms resistant to commonly used antimicrobial agents [16]. The relevance of this reservoir as a hazard for infection or a source of resistance plasmids spreading to susceptible microorganisms still has to be confirmed.

In contrast to samples with a large amount of resistant *E. coli*, most fecal samples contained a flora in which less than 5% was resistant to one of the five antimicrobial agents tested. The meaning of the relatively few resistant bacteria in an overwhelming susceptible flora remains unclear. On the one hand, even these few resistant microorganisms could have a selective advantage over the susceptible ones if that particular antibiotic was used. Subsequently, the risk for colonization and infections with resistant bacteria might increase. On the other hand, subjects with susceptible bacteria would remain susceptible after antimicrobial therapy, unless there was a mutation or a transfer of resistant bacteria from an exogenous source. The latter possibility is more likely in places where a majority of the population harbors resistant bacteria [17]. This theory was supported by studies on trimethoprim resistance. Almost no one acquired *E. coli* resistant to trimethoprim while using the drug in the United States, but almost all students who had used trimethoprim in Mexico, where the resistance level against this compound is high, carried resistant *E. coli* after therapy [17]. There is no doubt that the use of any antibiotic will lead to development of resistant bacteria, probably by mutation, even if no resistant strains were present before [18]. However, transfer and selection remain much more fruitful sources of high levels of resistance in bacterial populations, especially in populations with high levels of resistant microorganisms [7].

The differences observed in this study strongly suggest the existence of variations in antibiotic resistance between similar populations in distinct areas in a country as small as the Netherlands. It is difficult to give a definite explanation for these differences. The relationship between antibiotic use and increase of resistance rates has been well established [18]. However, as expected, this relationship was not observed in this study, as only a few participants had used antibiotics in the previous three months. Neither were the results influenced by age and gender. There appear to be other factors influencing antibiotic susceptibility of the fecal flora of healthy persons. Differences in the overall use of antimicrobial agents between the two regions might have led to variations in antibiotic resistance. The choice of antimicrobial agents of medical doctors could influence their overall use in a region, but until now data available about antibiotic prescriptions in different areas in the Netherlands have been too limited to confirm this. Ecological differences between the two areas and subsequent antibiotic use in bioindustry and veterinarian practice might also contribute to the variations in antibiotic susceptibilities [19,20]. Several studies reported more frequent drug resistance in bacteria isolated from humans on farms with livestock given antibiotic-supplemented feed than those on farms without animals [19,21–24]. Also, handling of carcasses, both in abattoirs and at home for cooking, led to the transfer of resistant bacteria from animals to humans [25,26]. But this relationship was only established for persons who had direct contact with the animals, their antibiotic-supplemented feed or the carcasses. The effects of these transfers of resistance to the population without direct contact to these sources need further study.

The high percentage of resistance to chloramphenicol of 41 to 81% is also difficult to explain. It is unlikely that any of the participants was ever treated parenterally with this agent, which is rarely used in the Netherlands. In a previously performed study among students, 73% of the isolated *E. coli* strains were resistant to chloramphenicol [14]. These high percentages were in contrast to those observed in other countries. In England, where this drug is also hardly ever used, 14.5% of hospital isolates and 13% of community isolates of *E. coli* were resistant to chloramphenicol using a breakpoint of 32 mg/l, one step higher than the breakpoint used in the present study [27]. And in the U.S., 4% of fecal samples contained *E. coli* resistant to chloramphenicol, using a breakpoint of 25 mg/l [15]. High frequencies of resistance to chloramphenicol were indeed reported from countries where the drug is frequently prescribed or available without prescription, such as Indonesia, Mexico, Venezuela and China [19,28]. In the present study, resistance to chloramphenicol was always correlated with resistance to other antimicrobial agents. Development of resistance to another antibiotic, due to mutation or transconjugation, could have led to multiple resistance including resistance to chloramphenicol. Cross-resistance of *E. coli* to chloramphenicol and other structurally unrelated antimi-

crobial agents located on plasmids or chromosomes has been described [29,30]. As multiple resistance was observed in many strains, resistance to other antimicrobial agents, such as aminoglycosides, also could have been a result of one of these mechanisms.

High prevalence rates to several antimicrobial agents were observed in fecal *E. coli* derived from healthy adults in the Netherlands. Ten to 20% of the samples contained a dominant resistant flora to ampicillin or tetracycline and multiple resistance was frequently observed. The results of this study underscore the presence of a human reservoir of antibiotic resistant microorganisms. For the reasons discussed, the maintenance of a low level of resistance in the fecal flora of healthy persons is important as a precaution against the future development of infections caused by common pathogens that already are or will become resistant to antibiotics.

References

1. **O'Brien, T. F. and the Members of Task Force 2:** Resistance of bacteria to antibacterial agents: report of Task Force 2. Rev. Infect. Dis. 9 (1987) (Suppl. 3) S244–S260.
2. **Cohen, S. P., McMurray, L. M., Hooper, D. C., Wolfson, J. S., Levy, S. B.:** Cross-resistance to fluoroquinolones in multiple-antibiotic-resistant (Mar) *Escherichia coli* selected by tetracycline or chloramphenicol: decreased drug accumulation associated with membrane changes in addition to OmpF reduction. Antimicrob. Agents Chemother. 33 (1989) 1318–1325.
3. **Fischer, L. M., Lawrence, J. M., Josty, I. C., Hopewell, R., Margerrison, E. E. C., Cullen, M. E.:** Ciprofloxacin and the fluoroquinolones. New concepts on the mechanism of action and resistance. Am. J. Med. 87 (1989) (Suppl. 5A) 2–8.
4. **Parry, M. F., Panzer, K. B., Yukna, M. E.:** Quinolone resistance. Susceptibility data from a 300-bed community-hospital. Am. J. Med. 87 (1989) (Suppl. 5A) 12–16.
5. **Aoyama, H., Sato, K., Kato, T., Hirai, K., Mitsuhashi, S.:** Norfloxacin resistance in a clinical isolate of *Escherichia coli*. Antimicrob. Agents Chemother. 31 (1987) 1640–1641.
6. **Kresken, M., Wiedemann, B.:** Development of resistance to nalidixic acid and the fluoroquinolones after the introduction of norfloxacin and ofloxacin. Antimicrob. Agents Chemother. 32 (1988) 1285–1288.
7. **Greenwood, D.:** Antimicrobial chemotherapy, second edition. Oxford University Press, New York 1989, pp. 129–135.
8. **Kunin, C. M., Lipton, H. L., Tupasi, T., Saks, T., Scheckler, W., Jivani, A., Goic, A., Martin, R., Guerrant, R., Thamlikitkul, V.:** Social behavioral and practical factors affecting antibiotic use worldwide; report of Task Force 4. Rev. Infect. Dis. 9 (1987) (Suppl. 3) 270–285.
9. **Hawkey, P. M.:** Resistant bacteria in the normal human flora. J. Antimicrob. Chemother. 18 (1986) (Suppl. C) 133–139.
10. **Lester, S. C., del Pilar Pla, M., Wang, F., Perez Schael, I., Jiang, H., O'Brien, T.:** The carriage of *Escherichia coli* resistant to antimicrobial agents by healthy children in Boston, in Caracas, Venezuela, and in Qin Pu, China. N. Engl. J. Med. 323 (1990) 285–289.
11. **World Health Organization, Scientific Working Group on Antimicrobial Resistance:** Control of antibiotic-resistant bacteria; memorandum based on report from WHO meeting. Bull. WHO 61 (1984) 423–433.
12. **Degener, J. E., Smit, A., Michel, M., Valkenburg, H., Muller, L.:** Faecal carriage of aerobic gram-negative bacilli and drug resistance of *E. coli* in different age-groups in Dutch urban communities. J. Med. Microb. 16 (1983) 139–145.
13. **Degener, J. E., van Hooft, I., van Stiphout, W., Luchmun, R.:** Veranderende gevoeligheid van *Escherichia coli* voor antibiotica in de bevolking. Ned. Tijdschr. Geneeskd. 134 (1990) 2296–2299.
14. **Bonten, M., Philips, J., Houben, T., Stobberingh, E.:** High prevalence of antibiotic resistant *Escherichia coli* in faecal samples of students in the south-east of the Netherlands. J. Antimicrob. Chemother. 26 (1990) 585– 592.
15. **Levy, S. B., Marshall, B., Schluederberg, S., Rowse, D., Davis, J.:** High frequency of antimicrobial resistance in human fecal flora. Antimicrob. Agents Chemother. 32 (1988) 1801–1806.
16. **Levy, S. B.:** Starting life resistance-free. N. Engl. J. Med. 323 (1990) 335–337.
17. **Murray, B. E., Rensimer, E. R., DuPont, H. L.:** Emergence of high-level trimethoprim resistance in fecal *Escherichia coli* during oral administration of trimethoprim or trimethoprim-sulfamethoxazole. N. Engl. J. Med. 306 (1982) 130–135.
18. **Timmis, K. N., Gonzales-Carrero, M. I., Sekizaki, T., Rojo, F.:** Biological activities specified by antibiotic resistance plasmids. J. Antimicrob. Chemother. 18 (1986) (Suppl. C) 1–12.
19. **Linton, A. H.:** Flow of resistance genes in the environment and from animals to man. J. Antimicrob. Chemother. 18 (1986) (Suppl. C) 189–197.
20. **Corpet, D. E.:** Ecological factors influencing the transfer of plasmids *in vitro* and *in vivo*. J. Antimicrob. Chemother. 18 (1986) (Suppl. C) 127–132.
21. **Wiedemann, B., Knothe, H.:** Epidemiological investigations of R factor-bearing enterobacteria in man and animal in Germany. Ann. NY Acad. Sci. 182 (1971) 380–382.
22. **Guinée, P. A. M.:** Bacterial drug resistance in animals. Ann. NY Acad. Sci. 182 (1971) 40–51.
23. **Moorhouse, E.:** Prevalence of R^+ bacteria in infants in Ireland. Ann. NY Acad. Sci. 182 (1971) 65–71.
24. **Levy, S. B., Fitzgerald, G. B., Macone, A. B.:** Changes in intestinal flora of farm personnel after introduction of a tetracycline-supplemented feed on a farm. N. Engl. J. Med. 295 (1976) 583–588.
25. **Linton, A. H., Howe, K., Hartley, C. L., Clements, H. M., Richmond, M. H., Osborne, A. D.:** Antibiotic resistance among *Escherichia coli* O- serotypes from the gut and carcasses of commercially slaughtered broiler chickens: a potential public health hazard. J. Appl. Bacteriol. 42 (1977) 89– 110.
26. **Ozanne, G., Bédard, P., Ducic, S., Panisset, J.:** Antibiotic multiresistance among coliforms isolated from the gut of swine and abattoir workers: evidence of transfer from animal to man. Can. J. Public Health 78 (1987) 340–344.
27. **Philips, I., King, A., Gransden, W., Eykin, S.:** The antibiotic sensitivity of bacteria isolated from the blood of patients in St. Thomas' Hospital 1969–1988. J. Antimicrob. Chemother. 25 (1990) (Suppl. C) 59– 80.
28. **Levy, S. B., Hedges, R. W., Sullivan, F., Medeiros, A. A., Sosroseputros, H.:** Multiple antibiotic resistance plasmids in *Enterobacteriaceae* isolated from diarrhoeal specimens of hospitalized children in Indonesia. J. Antimicrob. Chemother. 16 (1985) 7–16.
29. **Hachler, H., Cohen, S. P., Levy, S. B.:** MarA, a regulated locus which controls expression of chromosomal multiple antibiotic resistance in *Escherichia coli*. J. Bacteriol. 173 (1991) 5532–5538.
30. **Senerwa, D., Mutanda, L. N., Gathuma, J. M., Olsvik, O.:** Antimicrobial resistance of enteropathogenic *Escherichia coli* strains from a nosocomial outbreak in Kenya. APMIS 99 (1991) 728–734.

