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Antibiotic Resistance Is Selected Primarily in Our Patients

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ABSTRACT

The potential for bacterial resistance probably existed prior to the arrival of humans on earth and bacterial populations isolated before the antibiotic era surely contained antibiotic-resistant organisms. Antibiotic resistance has undergone an explosive development following the introduction of antibiotics in medical practice and in agriculture, and there is no doubt that the higher prevalence of bacterial resistance is closely related to human activities. Strict infection control policies limit the risk of patient-to-patient transmission of resistant as well as susceptible bacteria *(Infect Control Hosp Epidemiol* 1994;15:472-477).

INTRODUCTION

An increasing frequency and variety of antimicrobial resistances is being documented in both the hospital and the community.[1] Recent emergence of multiple-drug resistance in a variety of bacterial species such as *Streptococcus pneumoniae, Staphylococcus aureus, Mycobacterium tuberculosis,* or *Enterococcus* species poses a serious public health challenge and raises the possibility of untreatable infections, as they were encountered before the antimicrobial era. Determining why resistance progresses is a difficult but essential task for those involved in the fight against this modern plague. Numerous factors associated with the emergence of resistance have been identified, including antibiotic use and abuse, microbial abilities to select and to transmit resistance phenotypes and resistance genes, environmental conditions promoting persistence or dissemination of resistance determinants, and presence of bacterial reservoirs.[1] This article focuses on the selection of antibiotic resistance in patients receiving antibiotic therapy.

THE ANIMAL MODEL

A murine model was developed that allowed detection and quantification of the bacterial resistance that emerges during or after antibiotic therapy.[2-4] Peritonitis was established in mice by intraperitoneal bacterial challenge. Treatment started 2 hours later and consisted of one to six subcutaneous antibiotic doses. Therapeutic results were evaluated in comparison with untreated control animals, according to mortality, severity of the peritonitis, colony-forming units (CFUs) in peritoneal fluid, and antibiotic susceptibility patterns. Resistance emerged rapidly when it occurred, often after the first antibiotic dose. Emergence of resistance was not necessarily associated with therapeutic failure. However, after a first antibiotic exposure in the animal, gram-negative rods with post-therapeutic resistance kept the virulence of the parent strain, and were subject to further selection of a higher level of resistance after a subsequent antibiotic challenge in the same model.

In gram-negative bacteria, low-level post-therapeutic resistances to β-lactam and quinolones (four- to eightfold

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increase in the minimum inhibitory concentration [MIC]) was caused by outer membrane impermeability, whereas the highly resistant bacteria combined impermeability and production of chromosomal β-lactamase or altered DNA-gyrase.[4-6] The outer membrane defects often caused cross-resistance to structurally unrelated antibiotics. For example, quinolones regularly selected quinolone-resistant *Pseudomonas aeruginosa* with decreased susceptibility to imipenem associated with decreased expression of OprD, the porin channel that catalyzes facilitated diffusion of imipenem.[4,5] Emergence of resistance depended on several parameters. *Enterobacter cloacae* and *P aeruginosa* were the most prone to manifest resistance, followed by *Serratia marcescens* and *Klebsiella pneumoniae,* as opposed to *Escherichia coli* and S *aureus.*

Aminoglycosides, imipenem, and cefepime were associated less with resistance than were third-generation cephalosporins and quinolones (Table 1). In particular, studies have demonstrated the possibility of selecting methicillin-resistant S *aureus* that also is quinolone-resistant during quinolone treatment of experimental endocarditis.[7] Underdosing, either by lowering antibiotic doses or by enlarging dosing intervals, promoted occurrence of resistance,[2,4] as did the higher inocula, or the presence of talcum used as a foreign body in the peritoneal cavity.[8] By contrast, the combination of β-lactam plus aminoglycoside or quinolone plus aminoglycoside clearly reduced the risk of resistance during therapy.[9] In other *P aeruginosa* models, aminoglycoside-ß-lactam combinations reduced the emergence of resistant organisms in neutropenic rat peritonitis[10] but not in rabbit aortic valve endocarditis.[11]

TABLE 1

EMERGENCE OF RESISTANCE AFTER SHORT-TERM THERAPY WITH DIFFERENT ANTIBIOTICS IN MICE CHALLENGED WITH ***ENTEROBACTERCWACAE***

Antibiotic	Dose (mg/kg)	No. of Treated Mice	% Yielding Resistant Bacteria
Ceftriaxone	50	23	87
Cefotaxime	50	36	76
Ceftazidime	50	24	58
Aztreonam	50	24	42
Carumonam	25x2	24	21
Imipenem	50	24	<5
Cefepime	25x2	19	≤5
Piperacillin	200	12	≤8
SCH 34343	25x2	20	≤5
Pefloxacin	25	27	41
Ciprofloxacin	25	20	40
Amikacin	15	20	≤5

Adapted from Michéa-Hamzehpour, Lucain, and Péchère.[5]

DEVELOPMENT OF RESISTANCE

Monotherapy

Patients receiving antibiotic therapy often experience the increase of resistant bacteria, as is well documented by a number of prospective clinical studies.[12] Resistance emerging during therapy occurs both among colonizing bacteria and in the pathogens targeted by the treatment. In many cases, the MIC increases are substantial, with organisms passing from the "susceptible" to the "resistant" category during antibiotic exposure. One possible problem for evaluating the changes of susceptibility patterns in this context is the actual identity of isolates over the course of time, even when they belong to the same species. DNA probing has confirmed that failures to eradicate *P aeruginosa* in clinical infections can be due to development of resistance within a given clone, as opposed to selection of new populations or re-infection,[13] but further studies are required to evaluate the respective contribution of selection versus transmission of resistance.

Careful analysis of prospective studies that included large numbers of patients[12] showed that the mean resistance rates during monotherapy in strains that seemingly caused infection (excluding colonizing bacteria) ranged from 4.7% to 13.4% (Table 2). In accordance with the animal model, *P aeruginosa* appeared particularly to be able to produce resistance during therapy, followed by the *Serratia* species and, in the case of therapy with broad spectrum penicillins, *Proteus* species. Other problematic organisms include *Enterobacter* species (notably during *Enterobacter* bacteremia[14]), strict anaerobes, *Staphylococcus epidermidis,* etc. In addition to prospective studies, numerous anecdotal reports have documented emergence of resistance in infections caused by *Salmonella* species.[15,16] *Helicobacter pylori* in the context of peptic ulcer,[17] S *pneumoniae*,[18] and, needless to say, *M tuberculosis.* Resistance can emerge with almost any site of infection: skin wound, pneumonia, meningitis, bacteremia, osteomyelitis, etc. Although, to my knowledge, no study has addressed this question specifically, it has been suggested that some host factors might promote emergence of resistance, such as impaired host-defense mechanisms[19] or cystic fibrosis.[20]

Emergence of resistance during therapy also depends on the drug administered to the patient. According to an extensive review dealing with a great number of patients,[12] and in accordance with in vitro stepwise selection of resistance[21], aminoglycosides appeared to be efficient selectors when used alone or when the concomitantly given antibiotic was not effective against the invading pathogen (Table 2). The ability to produce resistance has been observed with all aminoglycoside drugs, particularly in urinary tract infections, where monotherapy with antibiotics is a common practice.

Development of resistance to ciprofloxacin was detected in *8 (36%)* of *22 P aeruginosa* isolates and in 3 (14%) of 22 S *aureus* isolates from patients on ciprofloxacin therapy, according to a recent prospective study.[22] In accordance with in vitro[21] and experimental[4] findings, ciprofloxacin selected OprD deficient mutants, with reduced susceptibility to imipenem. In addition, enoxacin therapy has been shown to select cross-resistance affecting quinolones, some β-

TABLE 2
DEVELOPMENT OF RESISTANCE DURING MONOTHERAPY

Antibiotics	Studies (Patients)	Percent of Patients with: Acquired Resistance	Percent of Patients with: Therapeutic Failure	Most Frequent Pathogens
Broad-spectrum penicillins	6 (170)	9.2	5.2	*Pseudomonas aeruginosa, Proteus* species, *Serratia* species
2nd-3rd-generation cephalosporin	9 (350)	8.6	4.3	*Pseudomonas aeruginosa, Serratia* species
Imipenem	5 (277)	4.7	2.5	*Pseudomonas aeruginosa*
Ciprofloxacin	7 (322)	11.8	4.4	*Pseudomonas aeruginosa, Serratia* species
Aminoglycosides	5 (142)	13.4	11.4	*Pseudomonas aeruginosa, Serratia* species

Adapted from Milatovic and Braveny.[12]

lactams, and chloramphenicol, with OmpF deficiency and altered lipopolysaccharide as possible mechanisms.[25] Development of quinolone resistance seems to be especially rapid in methicillin-resistant *S aureus*.[23-24] Quinolone resistance emerges most frequently in hospitalized and nursing-home patients,[7] especially where large numbers of microorganisms are present.[7] Emergence of *P aeruginosa* infections in the course of ciprofloxacin therapy was associated frequently with underdosing, anatomical abnormalities, or the presence of foreign bodies.[26]

Resistance emerged in 3.6% to 35.7% of patients receiving third-generation cephalosporins, with an especially high risk attached to cefsulodin in *P aeruginosa* infections.[27] These resistances may occur despite combination with aminoglycosides[27] and produced clinical failures in about half the cases,[12] some with serious consequences in patients with impaired host defenses.[19]

Imipenem selected resistance in about 5% of patients,[12] almost solely in *P aeruginosa* infections, where the carbapenem can select OprD-deficient mutants.

Combined Therapy

The actual contribution of antimicrobial combinations in limiting occurrence of resistance still is not clarified entirely, but it seems likely in some areas. From the very earliest studies, it appeared that during the treatment of tuberculosis, resistance to any single antibiotic developed readily, and that combinations of antibiotics were necessary to limit the emergence of resistance.[28] For the same purpose, it is also common practice to use combined antibiotic therapy for serious *P aeruginosa* infections. Two recent prospective studies showed that, with a new β-lactam or ciprofloxacin with concurrent use of an aminoglycoside, the risk of emergence of resistance was reduced in hospital practice,[22,29] but other studies were unconclusive,[30,31] and more investigations aiming at comparing development of resistance in monotherapy and combination therapy still are necessary to get a definitive answer to this important issue.

POSSIBLE ROLE OF INTESTINAL FLORA

The studies quoted above demonstrate that selection of resistance is a common event among the bacteria causing various types of infections, but the normal flora of a host receiving antibiotics also deserve consideration. The bowel represents, by far, the main human reservoir for bacteria. The intestine contains several hundred obligate anaerobic species,[32] for a total of 10^{11} to 10^{12} CFU/g of feces. The concentration of the aerobic flora is one thousand times lower, represented by a small number of species of gram-negative bacilli (*E coli* is predominant) and gram-positive cocci, notably enterococci. The skin, the oropharynx, and the vagina can be considered as additional reservoirs of lesser importance in terms of absolute numbers of bacteria and bacterial concentrations they contain. Selection of resistant bacteria can occur in any of these reservoirs during antibiotic therapy, but the intestine is probably the dominant niche of this selection due to the abundant and rich intestinal flora where resistant genes can persist and circulate.

A concept called "colonization resistance" has been developed, which posits that indigenous anaerobic flora suppress the overgrowth of potentially pathogenic, mostly aerobic flora.[33] The impact of antimicrobials on intestinal microflora can be described according to four categories[33]: very limited effect; selective decontamination of potential pathogens preserving colonization resistance; inhibition of colonization resistance with possible overgrowth of resistant pathogens; and unselective decontamination. A number of studies have demonstrated that administration of antibiotics may result in the overgrowth of intestinal microorganisms resistant to the administered drug.[33,34]

Table 3 indicates that overgrowth of resistant strains in the intestine lumen can accompany most antibiotic therapies, with a few exceptions. Some drugs, like cephradine, enjoy complete digestive absorption and rapid renal excretion, providing probably very low levels in feces and consequently, limited effects on fecal flora.[36] Imipenem interferes little with colonization resistance and produces no signifi-

cant resistance selection.[37] Oral administration of nitroimidazoles causes no significant changes in the intestinal flora despite their potent in vitro antibacterial effect against a majority of intestinal obligate anaerobes.[34] Oral quinolones cause major effects on enterobacteriaceae, but do not seem to predispose to development of resistant bacteria,[34] and consequently can be used as selective decontaminating agents. The fact that these antibiotic agents apparently are unable to select bacterial resistance in the bowel suggests the possibility that resistance to these agents is produced elsewhere, perhaps in the infectious foci or in the environment. It is also worthwhile to mention that of 11 studies that provide data about emergence of resistance during selective decontamination, 10 reported no increase in resistant microorganisms.[38]

RELATION TO ANTIBIOTIC USE

If antibiotic resistance is selected in our patients, extensive use of antibiotics in human medicine should fuel the resistance crisis.[39] A causal relationship between antibiotic usage and bacterial resistance is supported by concurrent variations in hospital practice.[40] Some observations also may suggest that the same trait is observed in the community. The extremely high incidence of antibiotic resistance in *S pneumoniae* in Hungary may have resulted from uncontrolled use and easy access to antibiotics in this country.[41] It also has been suggested that erythromycin resistance in pneumococci may increase in countries where the drug is used extensively.[42] Recent European data provide evidence that ciprofloxacin-resistant strains of various species are more frequent in southern Europe, where the consumption of fluoroquinolones per capita also is higher.[43] The correlation between antibiotic use and bacterial resistance is not straightforward, however, and many other parameters have to be considered besides antibiotic policies, such as infection control practices, presence or absence of resistance genes in a given niche, selectivity and transmissibility of these resistance genes, and antibiotic dosages. Examples of noncorrelation between antibiotic consumption and antibiotic resistance exist. The introduction of a number of antibiotics in U.S. hospitals was not followed by substantial changes in the incidence of resistant strains 3 to 5 years later.[44] In one hospital, high-level use of gentamicin was associated with rapid increase in aminoglycoside resistance, whereas high-level use of amikacin produced the opposite results.[45] Decreased incidence of antibiotic resistance among S *aureus* despite increased antibiotic usage has been reported at least once.[46]

TABLE 3
EFFECT OF ANTIBIOTICS ON INTESTINAL MICROFLORA

	Overgrowth of Resistant Strains in the Dowel During Therapy	
Antibiotic Class	Yes	No
Penicillins	Ampicillin, amoxicillin, clavulanate,* pivampicillin, talampicillin, pivmecillinam, azlocillin	Phenoxymethypenicillin, bacampicillin, temocillin, ticarcillin/clavulanate,
Cephalosporins	Cefuroxime, cefazolin, cefaclor,* cefoxitin, cefotiam, cefixime, cefotaxime, cefoperazone, ceftriaxone, cefmenoxime	Cephradine
Other β-lactams	Aztreonam*	Imipenem
Macrolides	Erythromycin	
Tetracyclines	Tetracycline, doxycycline	
Imidazoles		Metronidazole, tinidazole
Lincosamines	Clindamycin	
Quinolones		Ciprofloxacin,* enoxacin, norfloxacin,* ofloxacin,* pefloxacine, lomefloxacin

* Some studies have shown a different impact.
Adapted from Nord and Edland.[34]

CONCLUSION

The potential for bacterial resistance probably existed prior to the arrival of humans on earth and bacterial populations isolated before the antibiotic era surely contained antibiotic-resistant organisms: penicillinase was first described in 1940 by Abraham and Chain, when no penicillin was available for treating patients. However, antibiotic resistance has undergone an explosive development following the introduction of antibiotics in medical practice and in agriculture, and there is no doubt that the higher prevalence of bacterial resistance is closely related to human activities. Resistance can be selected in the environment, notably through farming activities,[47] but an array of evidence indicates the important role of antibiotic therapy in our patients.

How could we limit the selection of antibiotic resistance? Despite the introduction of potent new drugs (third- and now fourth-generation cephalosporins, carbapenems, and quinolones), more bacterial resistance is observed today than 20 years ago, both in the community and in the hospital,[48] and it is unlikely that discovery and development of even more potent antibiotics will solve the problem.

Development of antibiotic policies often is presented as essential,[39] but action in this field is not obvious. At the world level, certain incorrect practices should be eliminated: the availability of antibiotics without prescription, over-the-counter sales, poorly regulated manufacturing leading to underdosed antibiotic products, antibiotic misuses (such as aid treatment of immunocompetent patients with common

upper respiratory tract infections of viral origin), abusive antibiotic promotions by unscrupulous drug companies, or the "just-in-case" philosophy adopted by anxious physicians willing to cover "everything."

Education is a key word here, but the task is immense. Beyond obvious measures, many questions concerning an appropriate antibiotic policy remain unanswered. In many institutions, some recent and expensive antibiotics are kept for very restricted indications and require a formal approval before use; is this "reserve" policy correct in terms of selection of bacterial resistance? Apart from some situations in which the evidence is clear, such as tuberculosis, what are the infections requiring a combined therapy for limiting the risk of emerging resistance? In addition to imipenem in *non-Pseudomonas* infections, what antibiotics are less prone to select resistance and should be preferred on this basis? For example, oral cephalosporins increasingly are used instead of penicillin for treating bacterial pharyngitis. What is the actual influence of this trend on bacterial resistance? Should we avoid standardized therapy in which the same drug is used any time and everywhere for a given disease (for example, penicillin for gonorrhea), and instead, introduce the concept of cycling the use of antibiotics? If so, what cycle rhythm? In many developing countries, the resistance crisis is present, and only a few cheap and old antibiotics are available. What newer antibiotics should be introduced?

There certainly is a need for molecular epidemiology to delineate more precisely the respective contribution of the selection of resistance in patients receiving antibiotics and the diffusion of resistance from person to person, but European prevalence studies in intensive care units (Euronis, EPIC, and personal unpublished data) indicate that countries with the lower rates of nosocomial infections (ie, northern Europe) also enjoy lower rates of methicillin resistance. Although this association may have several explanations, there is at least one point on which a majority of authorities will agree: strict infection control policies limit the risk of patient-to-patient transmission of resistant as well as susceptible bacteria.

REFERENCES

1. Cohen MT Epidemiology of drug resistance: implications for a post-antimicrobial era. Science 1992;257:1050-1055.
2. Marchou B, Michéa-Hamzehpour M, Lucain C, Péchère JC. Resistance after p-lactam therapy in a murine model of *E cloacae* infection. *J Infect Dis* 1987;156:369-373.
3. Péchère JC, Marchou B, Michéa-Hamzehpour M, Auckenthaler R Emergence of resistance after therapy with antibiotics used alone or combined in murine model. *J Antimicrob Chemother* 1986;17(suppl A):11-18.
4. Michéa-Hamzehpour M, Auckenthaler R, Regamey P, Péchère JC. Resistance occurring after fluoroquinolone therapy of experimental *Pseudomonas aeruginosa* peritonitis. *J Antimicrob* Chemother 1987;31:1803-1808.
5. Michéa-Hamzehpour M, Lucain C, Péchère JC. Resistance to pefloxacin in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 1991;35:512-518.
6. Lucain C, Regamey P, Bellido F, Péchère JC. Resistance emerging after pefloxacin therapy of experimental *Enterobacter cloacae* peritonitis. *Antimicrob Agents Chemother* 1989;33:937-943.
7. Dalhoff A. Quinolone resistance in *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Development during therapy and clinical significance. *Infection* 1994;(suppl 2):51-69.
8. Péchère JC. Emergence of antibiotic resistance during therapy in an animal model. *Current Topics Infect Dis Microbiol* 1990;3:27-30.
9. Michéa-Hamzehpour M, Péchère JC. Marchou B, Auckenthaler R. Combination therapy: a way to limit emergence of resistance? *Am J Med* 1986;80(suppl 6B):138-142.
10. Johnson DE, Thompson B, Calia FM. Comparative activities of piperacillin, ceftazidime, and amikacin alone and in all possible combinations, against experimental Pseudomonas *aeruginosa* infections in neutropenic rats. *Antimicrob Agents Chemother* 1985;27:735-739.
11. Bayer AS, Norman D, Kim KS. Efficacy of amikacin and ceftazidime in experimental aortic valve endocarditis due to *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* 1985;28:781-785.
12. Milatovic D. Braveny I. Development of resistance during antibiotic therapy. *Eur J Clin Microbiol* 1987;6:234-244.
13. Ogle JW, Reller LB, Vasil ML. Development of resistance in *Pseudomonas aeruginosa* to imipenem, norfloxacin and ciprofloxacin during therapy: proof provided by typing with a DNA probe. *J Infect Dis* 1988;157:743-748.
14. Chow JW, Fine MJ, Shlaes DM, et al. *Enterobacter* bacteremia: clinical features and emergence of antibiotic resistance during therapy. *Ann Intern Med* 1991;115:585-590.
15. Ling JM, Hui YM. Cheng AF, French GL. Development of trimethoprim resistance in Salmonella *typhi* during therapy. *Pathology* 1992;24:190-193.
16. Bellido F, Vladoianu IR, Auckenthaler R. et al. Permeability and penicillin-binding protein alterations in Salmonella *muenchen:* stepwise resistance acquired during p-lactam therapy. *Antimicrob Agents Chemother* 1989;33:113-115.
17. Malfertheiner P. Compliance, adverse events and antibiotic resistance in *Helicobacter pylori* treatment. *Scand J Gastroenterol* 1993;196:34-37.
18. Dixon JMS. Pneumococcus resistant to erythromycin and lincomycin. *Lancet* 1967;1:573.
19. Follath F, Costa E, Thommen A, Frei R. Burdeska A, Meyer J. Clinical consequences of development of resistance to third-generation cephalosporins. *Eur J Clin Microbiol* 1987;6:446-450.
20. Mouton JW, Den Hollander JG, Horrevorts AM. Emergence of antibiotic resistance amongst *Pseudomonas aeruginosa* isolates from patients with cystic fibrosis. *J Antimicrob Chemother* 1993;31:919-926.
21. Michéa-Hamzehpour M, Kahr A, Péchère JC. In vitro stepwise selection of resistance to quinolones β-lactams and amikacin in nosocomial gram-negative bacilli. *Infection* 1994;21(suppl 2):S1-S6.
22. Wallrauch-Schwarz C. Voss A. Milatovic D. Braveny I. Development of resistance to new β-lactams and ciprofloxacin during therapy: a prospective study. Presented at the 33rd International Conference on Antimicrobial Agents and Chemotherapy; October 17-20, 1993; New Orleans, Louisiana. Abstract 627.
23. Shalit I, Berger SA, Gorea A, Frimerman H. Widespread quinolone resistance among methicillin-resistant *Staphylococcus aureus* isolated in a general hospital. *Antimicrob Agents Chemother* 1989;33:593-594.
24. Hori S, Ohshita Y, Hiramatsu K. Sequential acquisition of norfloxacin and ofloxacin resistance by methicilllin-resistant and -susceptible *Staphylococcus aureus*. *Antimicrob Agents Chemother* 1993;37:2278-2284.
25. Piddock JJ, Wijnards WJA, Rise R. Quinolone/ureidopenicillin cross resistance. *Lancet* 1987;1:907-908.
26. Ball P. Emergent resistance to ciprofloxacin amongst Pseudomonas *aeruginosa* and *Staphylococcus aureus:* clinical significance and therapeutic approaches. *J Antimicrob Chemother* 1990;26(suppl F):165-170.
27. Nichols L, Gudmundsson S, Maki DG. Experience with cefsulodin therapy for lower respiratory tract infections caused by *Pseudomonas aeruginosa* in adults without cystic fibrosis or granulocytopenia. *Rev Infect Dis* 1984;6(suppl 3):711-720.
28. Bloom BR, Murray CJL. Tuberculosis: commentary on a reemergent killer. *Science* 1992;257:1055-1062.
29. Jacobson KL, Cohen SH, King JH, Lippert WE, Inciardi JF, Iglesisas T. The relationship between resistance to extended spectrum cephalosporins and antecedent antibiotic use in type I β-lactamase producing organisms. Presented at the 33rd Interscience Conference on Antimicrobial Agents and Chemotherapy; October 17-20, 1993; New Orleans, Louisiana. Abstract 625.
30. Nichols L, Maki D. The emergence of resistance to β-lactam antibiotics during treatment of Pseudomonas aeruginosa lower respiratory tract infections: is combination therapy the solution? *Chemotherapia* 1985;4:102-108.
31. Collins T, Gerding DN. Aminoglycosides versus β-lactams in gram-negative pneumonia. *Sem Respir Infect* 1991;6:136-146.
32. Moore WEC, Holdeman LV. Human fecal flora: the normal flora of 20 Japanese-Hawaiians. *Applied Microbiology* 1974;27:961-979.
33. Vollaard EJ, Clasener HAL. Colonisation resistance. *Antimicrob Agents Chemother* 1994;38:409-414.

34. Nord CE, Edland C. Ecological effects of antimicrobial agents on the human intestinal microflora. *Microbiol Ecol Health Dis* 1991;4:193-207.
35. Clasener HAL, Vollaard EJ, Van Saene HKF. Selective decontamination and long-term systemic prophylaxis in leukopenia and in mechanical ventilation. *Rev Infect Dis* 1987;9:295-338.
36. Vollaard ES, Clasener HAL, Janssen AJHM. Influence of cephradin on microbial colonisation resistance in healthy volunteers. *Microb Ecol Health Dis* 1992;5:147-153.
37. Nord CE, Kager L, Philipson A, Stiernstedt G. Impact of imipenem/cilastin therapy on faecal flora. *Eur J Clin Microbiol* 1984;3:2475-477.
38. Van Saene HK, Stoutenbeek CC, Stoller JK. Selective decontamination of the digestive tract in the intensive care unit: current status and future prospects. *Crit Care Med* 1992;20:691-703.
39. Neu HC. The crisis in antibiotic resistance. Science 1992;257:1064-1073.
40. McGowan JE Jr. Antimicrobial resistance in hospital organisms and its relation to antibiotic use. *Rev Infect Dis* 1983;5:1033-1048.
41. Marton A, Gulyas M. Munoz R, Tomasz A. Extremely high incidence of antibiotic resistance in clinical isolates of *Streptococcus pneumoniae* in Hungary. *J Infect Dis* 1991;163:542-548.
42. Ward J. Antibiotic-resistant *Streptococcus pneumoniae:* clinical and epidemiological aspects. *Rev Infect Dis* 1981;3:254-266.
43. Kresken M, Hafner D, Mittermayer H, et al. Prevalence of fluoroquinolone resistance in Europe. *Infection.* In press.
44. Barry AL, Jones R. Bacterial antibiotic resistance before and after clinical application in the United States. *Bull NY Acad Med* 1994;63:217-230.
45. Gerding DN, Larson TA, Hugues RA, Weiler M. Shanholtzer C, Peterson LR. Aminoglycoside resistance and aminoglycoside usage: ten years of experience in one hospital. *Antimicrob Agents Chemother* 1991;35:1284-1290.
46. Bulger RJ, Sherris JC. Decreased incidence of antibiotic resistance among *Staphylococcus aureus:* a study in a university hospital over a 9-year period. *Ann Intern Med* 1968;69:1099-1108.