RELAZIONI A INVITO

Chairman:

C. GRASSI – Clinica Tisiologica e delle Malattie dell'Apparato Respiratorio, Università degli Studi, Pavia, Italia

D. VAN DER WAAIJ and H.G. DE VRIES-HOSPERS – Laboratory for Medical Microbiology, Gröningen, The Netherlands

COLONIZATION RESISTANCE OF THE DIGESTIVE TRACT: MECHANISM AND CLINICAL CONSEQUENCES
RESISTENZA ALLA COLONIZZAZIONE DEL TRATTO INTESTINALE: MECCANISMI E IMPLICAZIONI CLINICHE

C.E. NORD, A. HEIMDAHL and L. KAGER – National Bacteriological Laboratory, Stockolm, Sweden

ANTIMICROBIAL AGENTS AND THE HUMAN OROPHARYNGEAL AND INTESTINAL MICROFLORA
ANTIMICROBICI E MICROFLORA INTESTINALE UMANA

Ann. Ist. Super. Sanità
Vol. 22, N. 3 (1986), pp. 875-882

COLONIZATION RESISTANCE OF THE DIGESTIVE TRACT; MECHANISM AND CLINICAL CONSEQUENCES

D. Van der Waaij & de Vries-Hospers, H.G.

Laboratory for Medical Microbiology, University of Gröningen, The Netherlands.

Summary. - Colonization by (resistant) potentially pathogenic microorganisms inside the digestive tract appears to be controlled by a complex mechanism which is partially of host origin. Individual differences in the degree in which this mechanism - called Colonization Resistance (CR) of the digestive tract - operates may explain the variability in the occurrence of infections of otherwise comparable individuals. Treatment with some antibiotics was found to dramatically decrease the CR by eradicating many anaerobic bacterial species which play a role in the CR of the gi-tract; other antibiotics appeared to have no adverse effect on the CR when used in therapeutical dosages. On the contrary, the latter were found to be effective in selectively eliminating sensitive potentially pathogenic organisms from the digestive tract without decreasing the CR: selective decontamination of the digestive tract.

Riassunto (Resistenza alla colonizzazione del tratto intestinale: meccanismi e implicazioni cliniche). - La colonizzazione del tratto intestinale da parte di batteri potenzialmente patogeni appare sotto il controllo di un meccanismo complesso parzialmente legato all'ospite. Differenze individuali nel grado con il quale questo meccanismo, detto Resistenza alla Colonizzazione (CR), opera, possono spiegare la variabilità del verificarsi di infezioni in individui per altri versi comparabili. Il trattamento con alcuni antibiotici è risultato abbassare drammaticamente la CR con l'eliminazione di molte specie anaerobie. Altri antibiotici non sono risultati avere effetto negativo sulla CR se usati in dosaggi terapeutici. Al contrario si sono mostrati efficaci nell'eliminazione selettiva di batteri potenzialmente patogeni senza diminuire la CR: decontaminazione selettiva del tratto intestinale.

Introduction

The Colonization Resistance (CR) has been defined as the resistance which a potentially pathogenic microorganisms encounters when it tries to colonize a "landing site" on the mucosa in one of the three tracts that have an open communication with the outside world. In the respiratory and in the urinary tract the CR determining forces - mucus, IgA-secretion, cell desquamation and mechanical cleansing mechanisms (ciliar movement and bladder emptying) - are entirely of host origin. In the digestive tract, however, the resident microflora plays a major role in maintaining colonization resistance. The indigenous (resident) flora consists predominantly of anaerobes. CR-determining host factors comparable to those in both other tracts are present in the digestive tract and effectively contribute to the CR.

The various anaerobes involved in the intestinal CR appear to adhere

particularly to the mucosal lining of the ileum and the colon, forming "living wall paper". The indigenous flora - viridans streptococci in the oral cavity and anaerobic bacteria in the intestines - are susceptible to a number of antibiotics. Colonization by potentially pathogenic bacteria is perhaps only possible when they have found open patches in the otherwise confluent "anaerobic wall paper" of the colon. In the oropharynx and in the mouth other indigenous bacteria such as α-haemolytic streptococci, may interfere with mucosal adherence of potentially pathogenic microorganisms (1).

Antibiotic treatment may increase the size and number of the open patches or even may peal the "anaerobic wall paper" off the gut wall. Adherence of large numbers of potentially pathogenic bacteria (colonization) appears associated with detectable penetration of the epithelial lining and bacterial migration into the lymphatic organs (trans-location of bacteria). Anaerobes shedded from the "wall paper" and those multiplying in the intestinal contents appear to compete successfully with potentially pathogenic bacteria for nutrients which is another effective way of controlling the number of potentially pathogenic microorganisms.

Since antimicrobial drugs have been available for the treatment of infections, resistance has emerged (2-13). The increase in the number of antibiotics and in the diversity of their spectrum of activity in the past decades has not solved this problem. So far, the use of new antibiotics has invariably led to the development of resistance after a shorter or longer treatment period. Bacteria of any different species have shown to have an almost endless capacity to develop sublines of strains which are resistant; often to more than one antibiotic.

Development of resistance

The development of resistance to antimicrobial drugs appears to occur relatively rarely at the site of the infection. Adequate dosing of drugs which have the pharmacokinetic properties to reach the focus of the infection in a sufficient concentration can in general prevent development of resistance. In the throat or in the intestinal lumen, however, resistance may develop during therapy among strains which are marginally susceptible to the average (constantly varying) concentrations of the antibiotic. Many if not all anti-bacterial drugs are to a greater of lesser extent excreted with the saliva into the throat and/or with the bile into the intestinal lumen.

Effect of antibiotics on indigenous flora

The dose, the frequency of administration, and the type of the antibiotic selected for treatment are determined by the bacteria isolated from the infection. If no culturing results are available, an antibiotic choice is made on the basis of the bacteria which are expected to be involved in the infection. In general little attention is paid to what occurs in the alimentary canal during treatment. The only concern of the physician regarding the patient's gi-tract is the occurrence of side effects which cause discomfort to the patient, such as nausea and/or diarrhoea. However, the marginally biologically effective, often discontinuous, concentration of an antibiotic during therapy which establishes in the oropharynx and/or in the intestines, favours the selection of resistant strains.

Mutation and selection

During CR-decreasing antibiotic therapy, resistant potentially pathogenic strains may increase to concentrations of 10^9 bacteria per gram of faeces. This is about a 1000-fold higher than the average concentration of these bacteria when the CR is unaffected. Since (depending on the species) bacteria may mutate each 10^6-10^8 divisions, the presence of (1000-fold) more bacteria implies a

proportionally higher change for the development of new mutants. Mutation and more often selection may not only concern resistance to an antibiotic used. It may also concern adaptation of resistant bacteria with improved ability to colonize the oropharynx or the gut. Mutation may occasionally result in the development or modification of adherence pili of bacteria. The strain involved may thereafter adhere better to the mucosal lining. However, this event occurs rarely, which can be explained by the fact that the majority of the mutations which result are not compatible with survival in the alimentary canal. It is of importance, however, to realize that the length of a CR-decreasing treatment plays an important role. The longer a patient is treated with a CR-decreasing antibiotic which establishes only marginally effective (suppressive) concentrations to (potentially) pathogenic bacteria in the intestinal lumen, the higher the risk that a (better colonizing) mutant may develop.

Development of resistance and of pili for mucosal adherence occurs less frequently when combinations of antibiotics are used for therapy. However, multi-resistant strains do occur. Therefore, mutation may even be possible under circumstances of multi-antibiotic therapy. consequently, the use of combinations of antibiotics implies an improvement over mono-treatment in this respect. However, it is still not yet the final solution of the problem of selection of resistance or mutation to improved adherence or other pathogenic factors.

Transfer of resistance

If the antibiotic is not only marginally suppressive to potentially pathogenic strains but also suppressive to those anaerobic bacteria which play a role in the colonization resistance (CR) (14, 15), then transfer of plasmids coding for antibiotic resistance may occur (16). At the same time, genetic information for adherence factors (17) or enterotoxin production may be exchanged in vivo (18) although this is questioned by some others (19). This leads to the conclusion that there is transfer of genetic information about resistance and/or pathogenicity factors appears possible in vivo when the CR is decreased. A decreased CR allows a strong increase in concentration of resistant strains (20). This is more generally referred to as "bacterial overgrowth". As outlined above, decrease of the CR can be due to severe disease (decreased host CR factors) as well as to CR-decreasing antimicrobial treatment. In the case of severe underlying illness genetic information concerning resistance could be transferred from resistant bacteria to sensitive ones, which has, however, not yet been reported. During therapy with one CR-decreasing antibiotic, plasmids coding for multi-resistance can be exchanged between related species.

Clinical consequences of mutation and selection during antibiotic treatment

The predominantly anaerobic component of the intestinal microflora contributes considerably to the CR of the digestive tract of the patient and is therefore highly beneficial to the patient. Maintenance of the CR is particularly important during antibiotic therapy. In an environment with a complex nosocomial flora, often consisting of resistant strains, the CR of individuals in that environment will control the number of colonizing resistant potentially pathogenic microorganisms and thereby minimize the chance of a "superinfection". When one applies antibiotics clinically, one should realize that the dramatic events outlined above may occur as they have apparently taken place since 1945 (2-13). In practice this means that for the selection of an antibiotic for therapy, one should realise that not only the infectious process is treated, but also the indigenous microflora of the patient.

Antimicrobial drugs and the colonisation resistance

Screening tests in both man and mice as well as general experience with patients (6-10, 21-30) have indicated that antimicrobial drugs can be classi-

fied according to their effect on the CR. Drugs such as ampicillin and several other penicillins are strongly CR-suppressive, except in individuals carrying beta-lactamase which produces bacteria in their intestinal flora. In those patients the antibiotic is inactivated. Other antimicrobial drugs exert no effect on the CR. A third group has only a negative influence on the CR during treatment with high daily doses.

Antimicrobial drugs which are not CR-suppressive, are either almost completely absorbed in the small bowel following oral administration or biliary excretion (oral cephalosporins), or they have a small spectrum of activity which does not involve the CR-associated anaerobic species (polymyxin, polyene antibiotics, co-trimoxazole, malidixic acid, doxycyclin etc. (31). The intermediate group consists largely of aminoglycosides and a number of parenteral cephalosporins (29, 31, 32).

On the basis of the considerations mentioned above, preference should be given to CR-indifferent drugs to avoid the development or transfer of resistance in vivo and to minimize the acquisition of (multi-) resistant strains during therapy. Resistance however, may still develop when (absorbable) CR-indifferent drugs are used and no attention is paid to the amount of the drug that remains (following oral administration) in the intestinal lumen or the amount excreted into the digestive tract. If such CR-indifferent antimicrobial drugs are dosed with the only intent to treat an infection, the fraction of the dose that remains in the intestines or is excreted into the intestines may be too low to suppress the potentially pathogenic flora in the intestinal canal (33). Experience to date has indicated that resistant strains may then emerge (2-13). To avoid development of resistance or transfer of genetic information in vivo, the dose of an antibiotic required for the treatment of an infection should either be so high enough to virtually suppress all susceptible strains of potentially pathogenic bacteria which colonize the alimentary tract, or else so low enough to develop concentrations which are well below the minimal inhibitory concentration of the colonizing potentially pathogenic bacteria which colonize the alimentary tract.

In case CR-decreasing antibiotics are used for the treatment of an infection particularly in immunocompromised patients, a combined treatment systemic CR-decreasing antibiotics with non-absorbable CR-indifferent drugs such as polymyxin should be considered, the latter to suppress the Gram-negative bacilli which otherwise might develop resistance. The latter kind of prophylactic treatment is called selective decontamination (see later).

"Overgrowth" of the digestive tract by yeasts during CR-decreasing therapy can in general be limited effectively or even prevented when the patient is concomitantly treated orally with a polyene antibiotic (amphotericin B or nystatin) to control yeast proliferation. Obviously, these CR-indifferent oral drugs must be dosed sufficiently high and frequently per day to constitute a cidal concentration inside the alimentary tract to Candida and Torulopsis species. For example, to control overgrowth in adult patients, amphotericin B should be given in a dose of 0.5 g q.i.d. and polymyxin in amounts of 200 mg q.i.d. (34). If patients who are systemically treated with antibiotics which (at the dose level applied) decrease the CR, are colonized by polymyxin resistant Gram-negative bacilli, low oral doses of 300 mg aztreonam per day (35) will in the majority of the cases adequately control the Gram-negative "overgrowth". Polymyxin resistant Gram-negative bacilli include Proteus and some Serratia species which are naturally resistant and rarely some nosocomial strains which have become resistant and may be present in the ward. Oral treatment with neomycin of tobramycin is not recommended since it is easily overdosed and then may decrease the CR (29).

Gram-positive bacteria such as St.aureus - resistant to the CR-decreasing drug selected for therapy - may soon disappear from the oropharynx following treatment with cephradine in daily doses of 6.0 g (36).

Selective decontamination (SD) of the digestive tract

As mentioned above some antimicrobial drugs have been found to have no suppressive effect on the CR following "high therapeutic" and even higher doses. In animals, oral treatment with such drugs, in sufficient daily dosages, suppresses several endogenous potentially pathogenic aerobic or facultative anaerobic Gram-negative bacilli completely. Soon after the start of such treatment they can no longer be isolated from the digestive tract (oral swabs and faeces). Nalidixic acid as well as other quinolone derivates, co-trimoxazole and polymyxin and aztreonam have for example been found capable to eliminate the susceptible Gram-negative bacilli (28, 29, 35, 37). Because (anaerobic) bacteria are naturally resistant to the polyene antibiotics nystatin and amphotericin B, it is plausible that these drugs can be applied in high oral doses without an adverse effect on the CR. These observations have also seemed to apply to humans (35, 38, 39).

Because the CR-associated, predominantly Gram-positive anaerobic flora remains intact during treatment with CR-indifferent antibiotics, resistant strains if acquired, do not "overgrow" any gi-tract area. They can mostly be eliminated by additionally supplying one of the above mentioned anti-microbial drugs to which they were susceptible. This means that potentially pathogenic Gram-negative bacilli (Enterobacteriaceae and Pseudomonadaceae species) can more or less be selectively eliminated from the digestive tract both in man and in animals. Therefore, this treatment aimed at clearing the digestive tract of Gram-negative (potentially) pathogenic bacilli, is called selective decontamination (SD). When the drugs involved in SD are properly dosed and the combination applied is adjusted to the patient's admission flora as well as to the nosocomial flora in the ward, SD has been proven to be an important method for infection prophylaxis in severely neutropenic patients (38, 40-44). Insufficient dosing of CR-indifferent drugs for SD (45) and combination of CR-indifferent with CR-decreasing drugs for infection propylaxis, have appeared to be less successful (46).

Selective Decontamination (SD) of the digestive tract in immune compromised patients

Prophylactic treatment of patients with antimicrobial drugs which do not decrease the CR but selectively eliminate the endogenous potentially pathogenic organisms (Gram-negatives, Candida species and ST.aureus) following adeguate oral dosing has been studied in prospectively randomized trials. Several groups (Sleijfer and coworkers) (38) have randomized 103 neutropenic patients - who had, or would soon have, less than 1000 granulocytes/mm^3 blood -into either a SD-group or into a non-prophylactically treated control group. SD was performed until sufficient recovery of the bone marrow was achieved.

The control group patients were, like the SD-group, bacteriologically monitored three times a week. Throat swabs and faeces were cultured to isolate potentially pathogenic microorganisms and to determine their sensitivity to current antibiotics to establish the condition for optimal antibiotic therapy in case of infection.

In SD patients, bacteriological monitoring was also done, however, with a different aim namely of investigating the effectiveness of oral SD-treatment. SD patients received nalidixic acid, co-trimoxazole and polymyxin E either alone or in combination to eliminate Gram-negative bacteria from the digestive tract. Amphotericin B was given orally to these patients to suppress yeasts. SD treatment revealed a highly significant decrease of the incidence of infections. Nine patients died in the control group and no patient in the SD-group. This result could only be acquired by "Tailor made" prophylactic SD treatment: the continuous bacteriological monitoring during the SD-treatment period made adaptation of SD-treatment possible when the patients - who were not isolated as they were treated in the general ward and received normal hospital food - were found to have a Gram-negative bacterium that persisted in their throat or

faecal cultures (40). Because of the practically normal (only slightly reduced: see above) CR also in this kind of patient, previous positive samples were in general followed spontaneously by negative samples without readjustment of treatment.

In conclusion: particularly in immunocompromised patients, maintaining of CR is of great importance both for: prevention of acquisition and colonization by potentially pathogenic microorganisms as well as for infection prevention by antimicrobial treatment (SD).

REFERENCES

1. SPRUNT, K. & REDMAN, W. 1968. Evidence suggesting importance of role of interbacterial inhibition in maintaining balance of normal flora. Ann. Int. Med. 68: 579-590.
2. JULIANELLE, L.A. & SIEGEL, M. 1945. The epidemiology of acute respiratory infections conditioned by sulfanomides. II Gross alterations in the naso-pharyngeal flora associated with treatment. Ann. Int. Med. 22: 10-20.
3. WEINSTEIN, L. 1947. The spontaneous occurrence of new bacterial infections during the course of treatment with streptomycin or penicillin. Am. J. Med. Sci. 214: 56-63.
4. SMITH, D.T. 1952. The disturbance of the normal bacterial ecology by the administration of antibiotics with the development of new clinical syndromes. Ann. Int. Med. 37: 1135-1143.
5. LOURIA, D.B. & KAMINSKI, Th. 1962. The effects of four antimicrobial drug regimens on sputum superinfection in hospitalized patients. Am. Rev. Resp. Dis. 85: 649-665.
6. FINEGOLD, S.M., HARADA, N.E. & MILLER, L.G. 1965. Lyncomycin: Activity against anaerobes and effect on normal human fecal flora. Antimicrob. Agents Chemother. 5: 659-667.
7. SOMPOLINSKI, D., YARON, V. & ALKAN, W.J. 1967. Microbiological changes in the human fecal flora following the administration of tetracyclines and chloramphenicol. Am. J. Protocology 18: 471-478.
8. ROSE, H.D. & SCHREIER, J. 1968. The effect of hospitalization and antibiotic therapy on the Gram-negative fecal flora. Am. J. Med. Sci. 225: 228-236.
9. SHAW, E.J., DATTA, N., JONES, G., MARR, F.M. & FROUD, W.J.B. 1973. Effect of stay in hospital and oral chemotherapy on the antibiotic sensitivity of bowel coliforms. J. Hyg. 71: 529-534.
10. HIRSH, D.C., BURTON, G.C. & BLENDEN, D.C. 1974. The effect of tetracycline upon establishment of Escherichia coli of bovine origin in the enteric tract of man. J. Appl. Bact. 37: 327-333.
11. GAMAN, W., CATES, C., SNELLING, C.I.F., LANK, B. & RONALD, A.R. 1976. Emergence of gentamicin- and carbicillin-resistant Pseudomonas aeruginosa in a hospital environment. Antimicrob. Agents Chemoter. 9.
12. ROBERTS, N. & DOUGLAS, R.G. 1978. Gentamicin use and Pseudomonas and Serratia resistance: effect of a surgical prophylaxis regimen. Antimicrob. Agents Chemoter. 13: 214-220.
13. CHOW, A.W., TAYLOR, Ph.R., YOSHIKAWA, T.T. & GUZE, L.B. 1979. A nosocomial outbreak of infections due to multiply resistant Proteus mirabilis: role of intestinal colonization as a major reservoir. J. Infect. Dis 139: 621-627.
14. VAN DER WAAIJ, D., DE VRIES, J.M. & LEKKERKERK, J.E.C. 1971. Colonization resistance of the digestive tract in conventional and antibiotic-treated mice. J. Hyg. 69: 405-610.
15. VAN DER WAAIJ, D. 1982. Colonization resistance of the digestive tract: clinical consequences and implications. J. Antimicrob. Chemother. 10: 263-270.

16. LAUFS, R., FOCK, R., VON HULST, F.B. & RITTER, R. 1979. Transfer of R-factors during antibiotic treatment in the hospital. In: New criteria for antimicrobial therapy: maintenance of digestive tract colonization resistance, D. Van der Waaij & J. Verhoef, (Eds). Excerpta Medica (ICS477) Amsterdam-Oxford. pp. 74-88.
17. EVANS, D.G., SILVER, R.P., EVANS, D.J., CHASE, D.G., GORBACH, S.L. 1975. Plasmid-controlled colonization factor associated with virulence in Escherichia coli enterotoxin for humans. Infect. Immunol. 12: 656-667.
18. NANDADASA, H.G., SARGENT, G.F., BROWN, M.G.M., McNEISH, A.S. & WILLIAMS, P.H. 1981. The role of plasmids in adherence of adhesive Escherichia coli to mammalian cells. J. Infect. Dis. 143: 286-290.
19. ECHEVERRIA, P. & MURPHY, J.R. 1980. Enterotoxigenic Escherichia coli carrying plasmids coding for antibiotic resistance and enterotoxin production. J. Infect. Dis. 142: 273-278.
20. VAN DER WAAIJ, D. 1979. The colonization resistance of the digestive tract in experimental animals and its consequences for infection prevention, acquisition of new bacteria and the prevention of spread of bacteria between cage mates. In: New criteria for antimicrobial therapy: maintenance of digestive tract colonization resistance. D. Van der Waaij & J. Verhoef, (Eds). Excerpta Medica (ICS477) Amsterdam-Oxford, pp. 43-52.
21. LOURIA, D.B. & BRAYTON, R.G. 1963. The efficacy of penicillin regimens: with observations on the frequency of superinfections. J.A.M.A. 186: 937-990.
22. GORBACH, S.L., SPANKNEBEL, G., WEINSTEIN, L., PLANT, A.G., NAHAS, L. & LEVITAN, R. 1969. Studies of intestinal microflora. VIII Effect of Lincomycin on the microbial population of the human intestine. J. Infect. Dis. 120: 298-304.
23. HINTON, N.A. 1970. The effect of oral tetracyclin-HCL and doxycycline on the intestinal flora. Curr. Ther. Res. 12: 341-352.
24. HARTLEY, Ch.L., CLEMENTS, H.M. & LINTON, K.B. 1977. Effects of cephalexin erythromycin and clindamycin on the aerobic Gram-negative faecal flora in man. J. Med. Microbiol. 11: 125-135.
25. HEIMDAHL, A. & NORD, C.E. 1982. Effect of erythromycin and clindamycin on the indigenous human anaerobic flora and new colonization of the gastrointestinal tract. Eur. J. Clin. Microbiol. 1: 38-48.
26. HEIMDAHL, A., KAGER, L., MALMBORG, A.S. & NORD, C.E. 1982. Impact of different betalactam antibiotics on the normal human flora, and colonization of the oral cavity, throat and colon. Infection 10: 120-124.
27. THIJM, H.A. & VAN DER WAAIJ, D. 1979. The effect of three frequently applied antibiotics on the colonization resistance of the digestive tract of mice. J. Hyg. 82: 397-405.
28. EMMELOT, C.H. & VAN DER WAAIJ, D. 1980. The dose at which neomycin and polymyxin B can be applied for selective decontamination of the digestive tract in mice. J. Hyg. 84: 331-340.
29. VAN DER WAAIJ, D., ABERSON, J., THIJM, A.H. & WELLING, G.W. 1982. The screening of four aminoglycosides in the selective decontamination of the digestive tract in mice. Infection 10: 35-40.
30. WIEGERSMA, N., JANSEN, G. & VAN DER WAAIJ, D. 1982. Effect of twelve antimicrobial drugs on the colonization resistance of the digestive tract of mice and on endogenous potentially pathogenic bacteria. J. Hyg. 88: 221-230.
31. VAN DER WAAIJ, D. 1979. Colonization resistance of the digestive tract as a major lead in the selection of antibiotics for therapy. In: New criteria for antimicrobial therapy: maintenance of digestive tract colonization resistance. D. Van der Waaij & J. Verhoef, (Eds). Excerpta Medica (ICS477) Amsterdam-Oxford. pp. 271-280.
32. VAN DER WAAIJ, D., HOFSTRA, W. & WIEGERSMA, N. 1982. The effect of two cephalosporins and two broad-spectrum penicillins on the colonization resistance of the digestive tract. J. Infect. Dis. (in press).

33. MURRAY, B.E., RENSIMER, E.D. & DUPONT, H.L. 1982. Emergence of high-level trimethoprim resistance in fecal Escherichia coli during oral administration of trimethoprim or trimethoprimsulfamethoxazole. N. Engl. J. Med. 306: 130-135.
34. HOFSTRA, W., DE VRIES-HOSPERS, H.G. & VAN DER WAAIJ, D. 1982. Concentration of amphotericin B in faeces and blood of healthy volunteers after the oral administration of various doses. Infection 10: 223-227.
35. DE VRIES-HOSPERS, H.G., WELLIN, G.W., SWABB, E.A. & VAN DER WAAIJ, D. 1984. Selective decontamination of the digestive tract with aztreonam: a study of 10 healthy volunteers. J. Infect. Dis. 150: 636-642.
36. VAN SAENE, H.K.F. & DRIESSEN, L.H.M.N. 1979. Importance of the treatment of Staphylococcus aureus carriership in the prevention and therapy of Staphylococcus aureus infections. In: New criteria for antimicrobial therapy: maintenance of digestive tract colonization resistance. D. Van der Waaij & J. Verhoef, (Eds). Excerpta Medica (ICS477) Amsterdam-Oxford. pp. 197-205.
37. VAN DER WAAIJ, D. & BERGHUIS-DE VRIES, J.M. 1974. Selective elimination of Enterobacteriaceae species from the digestive tract in mice and monkeys. J. Hyg. Camb. 72: 205-211.
38. SLEIJFER, D.I., MULDER, N.H., DE VRIES-HOSPERS, H.G., FIDLER, V., NIEWEG, H.O., VAN DER WAAIJ, D. & VAN SAENE, H.K.F. 1980. Infection prevention in granulocytopenic patients by selective decontamination of the digestive tract. Eur. J. Cancer 16: 859-869.
39. SCHIMPFF, S.C., GREENE, W.H., YOUNG, V.M., FORTNER, C.L., JEPSEN, L., CUSACK, N., BLOCK, J.B. & WIERNIK, P.H. 1975. Infection prevention in acute nonlymphocytic leukemia. Laminar air flow rooms reverse isolation with oral, nonabsorbable antibiotic prophylaxis. Ann. Intern. Med. 82: 351-358.
40. DE VRIES-HOSPERS, H.G., SLEIJFER, D.I., MULDER, N.H., VAN DER WAAIJ, D., NIEWEG, H.O. & VAN SAENE, H.K.F. 1981. Bacteriological aspects of selective decontamination of the digestive tract as a method of infection prevention in granulocytopenic patients. Antimicrob. Agents Chemother. 19: 813-830.
41. GUIOT, H.F.L., VAN DER MEER, J.W.M. & VAN FURTH, R. 1981. Selective antimicrobial modulation of human microbial flora: infection prevention in patients with decreased host defense mechanisms by selective elimination of potentially pathogenic bacteria. J. Infect. Dis. 413: 644-654.
42. KURRLE, E. 1981. Infektprophylaxe bei der Induktion Therapie akuter Leukämien. Klin. Wochenschr. 59: 1079-1085.
43. KURRLE, E., BHADURI, S., KRIEGER, D., PFLIEGER, H. & HEIMPEL, H. 1983. Antimicrobial prohylaxis in acute leukaemia: prospective randomized study comparing two methods of selective decontamination. Klin. Wochenschr. 61: 691- 698.
44. DEKKER, A.W., ROZENBERG-ARSKA, M., SIXMA, J.J. & VERHOEF, J. 1982. Prevention of infection by co-trimoxazole in patients with acute non-lymphocytic leukemia. Ann. Int. Med. 95: 555-559.
45. WILSON, J.M. & GUINEY, D.G. 1982. Failure of oral trimethoprim-sulfamethoxazole prophylaxis in acute leukaemia. N. Engl. J. Med. 306: 16-20.
46. WATSON, J.G., POWELS, R.L., LAWSON, D.N., MORGENSTERN, G.R., JAMESON, B., McELWAIN, T.J., JUDSON, I., LUMLEY, H. & KAY, H.E.M. 1982. Co-trimoxazole versus non-absorbable antibiotics in acute leukaemia. Lancet i: 6-9.

Ann. Ist. Super. Sanità
Vol. 22, N. 3 (1986), pp. 883-892

ANTIMICROBIAL AGENTS AND THE HUMAN OROPHARYNGEAL AND INTESTINAL MICROFLORA

C. E. Nord, A. Heimdahl & L. Kager

Departments of General Surgery, Oral Surgery and Microbiology, Huddinge University Hospital and National Bacteriological Laboratory, Karolinska Institute, Stockholm, Sweden

Summary. - The most common and significant cause of disturbances in the normal gastrointestinal microflora is the administration of antimicrobial agents. The microflora can be influenced by antimicrobial agents because of incomplete absorption of any orally administered antimicrobial agent, secretion of an antimicrobial agent by the salivary glands and in the bile, or secretion from the intestinal mucosa. In most cases the influence is not beneficial to the patient because suppression of the indigenous microorganisms often permits potential pathogens to overgrow and cause septic conditions, diarrhea, or colitis. Antimicrobial agents that influence the normal microflora also promote the emergence of antimicrobial-resistant strains.

During the last years the impact of different antimicrobial agents on the human intestinal microflora has been investigated by our research group. Thus the effects on the colonic flora of peroral administration of penicillin, bacampicillin, cefaclor, erythromycin, clindamycin, doxycycline, metronidazole, norfloxacin and ciprofloxacin have been studied. The impact on the intestinal microflora of parenteral administration of ampicillin + sulbactam, azlocillin, aztreonam, piperacillin, cefbuperazone, cefoxitin, cefoperazone, ceftriaxone, moxalactam, imipenem and clindamycin has also been investigated. Pronounced changes were observed in the colonic microflora in patients receiving clindamycin, erythromycin, cefoperazone, ceftriaxone and moxalactam, whereas moderate changes were seen in those patients receiving doxycycline, cefoxitin, ampicillin + sulbactam, azlocillin, piperacillin and ciprofloxacin. Penicillin, bacampicillin, cefaclor, metronidazole and imipenem produced only minor changes. In most patients with an altered microflora, colonization with new microorganisms was found.

Riassunto (Antimicrobici e microflora intestinale e orofaringea umana). - La somministrazione di antimicrobici è la più comune e significativa causa di variazioni nella microflora intestinale normale. Nella maggior parte dei casi questa influenza non è benefica nei confronti del paziente in quanto la soppressione dei microrganismi endogeni spesso permette la crescita di patogeni, causa di sepsi, diarrea o coliti. Gli antimicrobici che agiscono sulla microflora endogena inoltre promuovono l'emergenza di ceppi resistenti. Negli anni passati è stato studiato dal nostro gruppo l'effetto sulla flora del colon della somministrazione orale di penicillina, bacampicillina, cefaclorina, eritromicina, clindamicina, doxiciclina, metronidazolo, morfloxacina e ciprofloxacina. Inoltre è stato studiato anche l'effetto sulla microflora intestinale di somministrazioni parenterali di ampicillina + sulbactam, azlocillina, aztreonam, piperacillina, cefbuperazone, cefoxitin, cefoperazone, ceftriaxone, moxalactam, imipenem e clindamicina. Marcate modificazioni nella microflora sono state osservate in pazienti che avevano ricevuto clindamicina, eritromi-

cina, cefoperazone, ceftriaxone e moxalactam, mentre modificazioni più modeste sono state evidenziate in pazienti trattati con doxiciclina, cefoxitin, ampicillina + sulbactam, azlocillina, piperacillina e ciprofloxacina. Modificazioni ancora minori sono state indotte da penicillina, bacampicillina, cefaclorina, metronidazolo e imipenem. Nella maggior parte dei pazienti con alterazioni della microflora, è stata riscontrata una colonizzazione da parte di nuovi microrganismi.

Normal gastrointestinal microflora in man is a remarkably stable ecosystem. Interpersonal variations exist in the microflora composition and in the number of different microorganisms, but within a given person the flora remains relatively constant over time. However, certain factors are capable of disrupting this ecosystem.

Total extraction of the teeth causes major changes in the oropharyngeal microflora and subsequently in the gastrointestinal microflora. Pathologic conditions that affect normal peristalsis can cause a shift in the intestinal microflora. Gastric surgery is associated with changes in the bacteria of the small intestine. Minor ecologic disturbances are also associated with hospitalization, which may promote colonization of the digestive tract by new microorganisms. The ingestion of a large bacterial inoculum can overwhelm host defenses.

The most common and often the most significant cause of disturbances in the normal gastrointestinal flora is the administration of antimicrobial agents. The importance of antimicrobial agents in the treatment of infectious diseases and in the prophylaxis of infections cannot be minimized. However, some of these agents are not without their detrimental effects on the normal flora, leaving the host susceptible to infection, to superinfection by commensal microorganisms, and to a range of other untoward effects.

In this review article our experience with the impact of different antimicrobial agents on the gastrointestinal microflora is reported.

Prevention of colonization by noncommensal microorganisms

The combination of effects exerted by the physiologic aspects of the host, microbial interaction, and environmental pressures provides a complex ecosystem that is highly resistant to change and allows the microflora to resume its original composition shortly after various environmental insults. The normal microflora prevents colonization by noncommensal microorganisms by the following mechanisms: (1). Competition for nutrients. This is the principal force by which colonization is prevented. Because the available nutrients are used by endogenous microorganisms in the gastrointestinal tract, exogenous species cannot establish themselves. (2). Competition for attachment sites. The mucosal surfaces of the gastrointestinal tract are sites of attachment for infection producing microorganisms. By occupation of attachment sites, the commensal microorganisms are able to prevent colonization by pathogenic microorganisms. The major factor capable of creating changes is the administration of antimicrobial agents having the potential to suppress the indigenous microorganisms, and the disturbed microflora allows potential pathogens to adhere and in some cases cause septicemia and severe infections of the gastrointestinal tract. (3). Production of volatile fatty acids. The anaerobic flora produces volatile fatty acids such as acetic and propionic acids that are toxic for enterobacteria and inhibit their growth, which inhibit the growth of invading enterobacteria. These products are especially effective in an anaerobic environment with low pH such as exists in the large intestine. The amounts of free volatile fatty acids are inversely correlated with the amounts of anaerobic bacteria found in the gastrointestinal tract. The fatty acids are changed during therapy with antimicrobial agents that effect the anaerobic flora. Volatile short-chain fatty acids also serve to prevent overpopulation, which could harm not only the

host but also the microflora. (4). Bacteriocins. These substances are high-molecular-weight antimicrobial compounds produced by commensal microorganisms. These substances produced by Escherichia coli and P.aeruginosa limit the overgrowth of commensal microorganisms in the lower intestinal tract, whereas in the oropharynx Streptococcus salivarius acts as an important producer of bacteriocins active against group A streptococci. Alpha-hemolytic streptococci may also inhibit Gram-negative aerobic rods in the oropharynx. The administration of antibiotics can suppress the number of inhibitory streptococci and thus increase the risk for colonization and overgrowth of exogenous bacteria.

By competing for nutrients and attachment sites and by producing substances such as volatile fatty acids and bacteriocins that limit microbial growth, the normal microflora discourages colonization by exogenous microorganisms. However, this ecologic balance can be disturbed by the administration of antimicrobial agents.

The microorganisms of the gastrointestinal tract are sensitive to many antimicrobial agents. When their number is reduced during therapy, the resistance to colonization is decreased considerably with the following results: (1). Microorganisms resistant to an administered antimicrobial agent are permitted to grow in large concentrations in the gastrointestinal tract. (2). Overgrowth of other bacteria or yeast, or both, takes place. (3). Resistant pathogenic bacteria established in the gastrointestinal tract may colonize other areas of the host. (4). Bacterial overgrowth encourages the transfer of resistance factors among bacteria. (5). The decrease in resistance to colonization lowers the contamination threshold dose.

Pharmacokinetic properties of antimicrobial agents and the resistance to colonization of the gastrointestinal microflora

The potential of an antimicrobial agent to change the colonization is related to its dose and pharmacokinetic properties. Oral agents that are poorly absorbed from the gastrointestinal tract or absorbed but also excreted in active form in the bile or saliva have a significant effect on microbial colonization. Parenterally administered antimicrobial agents excreted in high concentrations in the intestinal tract also cause significant changes in the human microflora. Table 1 summarizes in vitro activities of different antimicrobial agents, pharmacokinetic properties, emergence of resistant microorganisms, and risk of overgrowth or superinfections. As can be seen, some antimicrobial agents induce changes in oropharyngeal and large intestine flora, whereas some others do not affect colonization.

Impact of antimicrobial agents on the oropharyngeal and upper intestinal microflora

Antimicrobial agents administered perorally of parenterally, depending on their pharmacologic properties as mentioned previously, may be secreted in saliva or from the mucous membranes in the oropharynx. The main determinant for such secretion is the lipophilocity of the agent. Antimicrobial agents with low lipophilocity tend to give low concentrations in saliva and pharyngeal secretions, whereas antimicrobial agents with a high lipophilocity usually are found in microbiologically active concentrations in the oropharynx. Oropharyngeal microorganisms susceptible to the antimicrobial agent used can be affected, and disturbances of the oropharyngeal and upper intestinal flora can take place. Rapid selection or emergence of antimicrobial-resistant microorganisms indigenous to the oropharynx and upper intestinal tract may protect from suppression and subsequent colonization and overgrowth of the normal microflora.

Benzylpenicillin, phenoxymethylpenicillin, and ampicillin are found in very low concentrations in saliva and therefore only slightly change the oropharyngeal microflora. However, selection of penicillin resistant viridans streptococci subsequent to prophylaxis with penicillin is well known. The

occurrence of penicillin resistant beta-lactamase-producing anaerobic strains subsequent therapy is now reported in increasing frequency. Selection of such strains during penicillin therapy of oropharyngeal infection may aggravate the infection and eventually cause clinical failure of antimicrobial therapy.

Clindamycin and erythromycin are present in saliva and exert pressure on the normal oropharyngeal and upper intestinal microflora. Susceptible microorganisms are eradicated, and subsequent colonization and overgrowth with resistant aerobic and anaerobic microorganisms frequently are noticed. These colonizing microorganisms are isolated in blood cultures from severely debilitated patients, such as those treated for acute leukemia and severe aplastic anemia.

Tetracyclines are present in the saliva and the gingival crevices. Emergence of resistant aerobic and anaerobic strains causes rapidly overgrowth and therefore maintains the resistance to colonization. The risk of infection with tetracycline resistant strains subsequent to surgery of the oropharynx and esophagus is obvious.

Nitroimidazoles are distributed in virtually all body fluids in concentrations equal to the serum concentration. Salivary concentrations follow concentrations of serum, and therefore high levels of nitroimidazoles are achieved in the oropharynx during therapy. The in vivo susceptibility of anaerobic microorganisms to nitroimidazoles is considerably less than the in vitro susceptibility and relatively large doses of nitroimidazoles have to be administered before suppression of the anaerobic oropharyngeal microflora takes place. The risk of colonization and overgrowth seems therefore to be moderate. Resistance among anaerobic strains isolated from the oropharynx has still not been reported and the agents can safely be administered both therapeutically and prophylactically without risk of infection by resistant anaerobic strains.

Impact of antimicrobial agents on the large intestinal microflora

Many antimicrobial agents cause changes in the intestinal microflora, the severity of which depends largely upon the agent's spectrum and concentration in the luminal contents. Oral antimicrobial agents that are well absorbed in the upper part of the small intestine have minor impact on the microflora in the large intestine. Agents that are poorly or incompletely absorbed can cause significant changes. Parenteral antimicrobial agents secreted in the bile or from the intestinal mucosa can also cause significant disturbances in the large intestinal microflora.

Suppression of the intestinal flora by antimicrobial agents therefore creates a microbiologic vacuum filled by exogenous pathogenic microorganisms or by overgrowth of commensal microorganisms. On the other hand, in certain situations, such as antimicrobial prophylaxis before elective colon surgery, and in the treatment of blind loop syndrome, elimination of microorganisms from the intestinal tract is indicated.

Many surgical infections that appear during antimicrobial therapy are caused by Gram-negative aerobic and anaerobic rods that normally inhabit the intestinal tract. Infectious complications due to Gram-negative bacteria are the major cause of morbidity and mortality in surgical patients. The responsible microorganisms in surgical infections have changed from invading exogenous pathogens to potentially pathogenic indigenous microorganisms. In recent years, the normal gastrointestinal flora has been demonstrated to be of much greater importance in the induction, maintenance, and spread of multiresistant microorganisms in surgical intraabdominal infections than had previously been realized. Figures 4 to 7 show the impact of different antimicrobial agents given perorally and parenterally on the colonic microflora.

Phenoxymethylpenicillin causes no change in the intestinal flora because penicillin is readily absorbed from the intestinal tract and no concentration of penicillin is detected in the large intestine. As might be predicted, no new bacterial colonization or increase in resistance is observed. However, phenoxy-

methylpenicillin has been incriminated in antibiotic associated diarrhea and colitis caused by Clostridium difficile.

Ampicillin is one of the most widely used semisynthetic penicillins because of its broad antibacterial spectrum. Disavantages of the agent are its incomplete absorption and rather high incidence of diarrhea during treatment. Certain esters of ampicillin such as bacampicillin, pivampicillin, and talampicillin are well absorbed when given orally and undergo hydrolysis in the body to yield serum peaks of ampicillin higher than those obtained by ampicillin itself and produce no concentrations in the large intestine. Therefore, compared with ampicillin, these esters are ecologically more favorable.

Azlocillin and piperacillin belong to the fourth generation of penicillins and have an antibacterial spectrum covering many intestinal bacteria. Azlocillin and piperacillin are excreted in high concentrations in the bile, and therefore in most patients the impact on the large intestinal microflora is pronounced. Overgrowth of azlocillin- and piperacillin-resistant enterobacteria and Bacteroides fragilis has been reported.

Third generation cephalosporins have good activity against Gram-negative aerobic and anaerobic rods and have been used in the treatment and prophylaxis of intraabdominal infections. The potent antibacterial activity of some of these cephalosporins in combination with high biliary excretion has resulted in considerable changes in the normal intestinal microflora. The alteration of the microflora has led to undesirable consequences such as superinfection, colitis, and diarrhea. Hypoprothrombinemia and hemorrhage due to impaired vitamin K production have been observed in patients treated with cefoperazone and moxalactam.

Cefoxitin and cefbuperazone are active against a wide variety of aerobic Gram-positive and Gram-negative bacteria and most anaerobic bacteria including B.fragilis. The colon microflora is affected by cefoxitin or cefbuperazone administration, because the agents are present in the large intestine in concentrations above the minimum inhibitory concentrations of many aerobic and anaerobic bacteria. Therefore, colonization with resistant enterococci, clostridia, Pseudomonas, and Enterobacter strains can take place in the intestine.

Cefoxitin and cefbuperazone, when used as prophylaxis in colorectal surgery, should be administered over the shortest period of time, that is, 12 hours to obtain the maximal effect on the microflora without risk of adverse microbial side effects.

Imipenem has a broad antimicrobial spectrum and covers both the aerobic and anaerobic intestinal microflora. Imipenem has been useful as a single agent in the treatment of intraabdominal infections. Fecal elimination is less than 1 percent and only minor changes have been observed in the intestinal microflora of patients receiving the drug.

The concept of combining a beta-lactamase inhibitor with a beta-lactam antibiotic considerably expands the spectrum of safe and well-tried beta-lactam antibiotics. Sulbactam is a beta-lactamase inhibitor that has been combined with ampicillin, and the combination is active against both aerobic and anaerobic microorganisms in the large intestine. When the combination is given to patients, significant changes in the aerobic and anaerobic colon flora are observed. The concentrations of ampicillin and sublactam in feces correlated with the changes in the flora. Overgrowth of yeasts and Pseudomonas resistant to the combination was noticed in some patients.

Erythromycin is often used together with neomycin or kanamycin as prophylaxis in colorectal surgery and is reported to be effective. However, when erythromycin is given as treatment for a longer period, undesirable microbial effects due to high fecal concentrations occur. Aerobic and anaerobic colon flora is changed and new colonization by erythromycin resistant microorganisms occurs.

Clindamycin exhibits high concentrations in the large intestine when administered either perorally or parenterally, which leads to pronounced changes in the aerobic and anaerobic intestinal microflora. Significant decrea-

ses in the number of anaerobic cocci and rods occur and clindamycin resistant enterococci and enterobacteria proliferate. The risk of developing C.difficile diarrheal disease associated with the use of clindamycin is well established.

Tetracyclines have extensively been used in the treatment of many different infections. Diarrhea and superinfection are side effects that limit the use of tetracyclines. One of the tetracyclines, doxycycline, produces minor changes in the number of anaerobic bacteria in the colon microflora, and new colonization of enterobacteria and yeasts is not a common finding because of the rapid emergence of doxycycline resistant anaerobic strains. This finding may explain the good results obtained with doxycycline as prophylaxis in elective colorectal surgery. The modest changes of microorganisms in the intestinal microflora may also explain the few cases of pseudomembraneous colitis.

Nitroimidazoles are selectively active against anaerobic microorganisms. They have been used in both the treatment and prophylaxis of anaerobic infections. When tinidazole was administered perorally in small doses, no significant changes in the aerobic and anaerobic colon flora could be noticed. No microbiologically active concentration of tinidazole could be recovered in feces specimens. When tinidazole was given intravenously in large doses, marked changes were observed in the colon microflora. Enterococci and streptococci increased, whereas anaerobic cocci, Gram-positive rods, fusobacteria, and bacteroides significantly decreased. Concentration of tinidazole higher than the minimum inhibitory concentrations of anaerobic bacteria were noticed in the intestinal tract.

Table 1. - Impact of different antimicrobial agents on the human oropharyngeal and intestinal microflora. In vitro activities, pharmacologic properties, emergence of resistant indigenous microorganisms and risk for overgrowth or superinfection.

	Activity in vitro			
Antimicrobial Agent (PO)	Oropharyngeal flora		Lower intestinal flora	
	Aerobes	Anaerobes	Aerobes	Anaerobes
Penicillin	+++	+++	-	++
Bacampicillin	+++	+++	++	++
Clindamycin	+	+++	-	+++
Erythromycin	+	++	++	++
Metronidazole	-	+++	-	+++
Doxycycline	++	++	++	++
Norfloxacin	+	-	+++	-

Activity in vitro

Antimicrobial Agent (IV)	Oropharyngeal flora		Lower intestinal flora	
	Aerobes	Anaerobes	Aerobes	Anaerobes
Piperacillin	+++	+++	+++	+++
Cefoxitin	+++	+++	+++	+++
Cefoperazone	+++	+++	+++	+++
Imipenem	+++	+++	+++	+++
Aztreonam	++	-	+++	-
Clindamycin	+	+++	-	+++
Metronidazole	-	+++	-	+++

Pharmacologic properties

Antimicrobial Agent (PO)	Salivary Concentration	Intestinal Concentration
Penicillin	Low	Low
Bacampicillin	Low	Low
Clindamycin	Moderate	High
Erythromycin	Moderate	High
Metronidazole	High	Low
Doxycycline	High	Moderate
Norfloxacin	Low	High

Pharmacologic properties

Antimicrobial Agent (IV)	Salivary Concentration	Intestinal Concentration
Piperacillin	Low	High
Cefoxitin	Low	Moderate
Cefoperazone	Low	High
Imipenem	Low	Low
Aztreonam	Low	Moderate
Clindamycin	Moderate	High
Metronidazole	High	Moderate

Emergence of resistance

Antimicrobial Agent (IV)	Oropharyngeal flora		Lower intestinal flora	
	Aerobes	Anaerobes	Aerobes	Anaerobes
Piperacillin	-	-	+	+
Cefoxitin	-	-	+	+
Cefoperazone	-	-	+	+
Imipenem	-	-	-	-
Aztreonam	-	-	(+)	-
Clindamycin	-	-	-	+
Metronidazole	-	-	-	-

Emergence of resistance

Antimicrobial Agent (IV)	Oropharyngeal flora		Lower intestinal flora	
	Aerobes	Anaerobes	Aerobes	Anaerobes
Penicillin	+	+	-	-
Bacampicillin	+	+	-	-
Clindamycin	-	-	-	+
Erythromycin	+	+	++	++
Metronidazole	-	-	-	-
Doxycycline	+++	+++	+++	+++
Norfloxacin	-	-	(+)	-

Overgrowth or superinfection

Antimicrobial Agent (PO)	Oropharyngeal flora	Lower intestinal flora
Penicillin	Low	Low
Bacampicillin	Low	Low
Clindamycin	High	High
Erythromycin	High	High
Metronidazole	Low	Low
Doxycycline	Moderate	Moderate
Norfloxacin	Low	Low

Overgrowth or superinfection

Antimicrobial Agent (IV)	Oropharyngeal flora	Lower intestinal flora
Piperacillin	Low	High
Cefoxitin	Low	Moderate
Cefoperazone	Low	High
Imipenem	Low	Low
Aztreonam	Moderate	Moderate
Clindamycin	High	High
Metronidazole	Moderate	Moderate

REFERENCES

ALESTIG, K., CARLBERG, H., NORD, C.E. & TROLLFORS, B. 1983. Effect of cefoperazone on faecal flora. J. Antimicrob. Chemother. 12: 163-167.

ARONSSON, B., MOLLBY, R. & NORD, C.E. 1982. Clostridium difficile and antibiotic associated diarrhoea in Sweden. Scand. J. Infect. Dis. 35: 53-58.

ARVIDSSON, A., ALVAN, G., ANGELIN, B., BORGÅ, O. & NORD, C.E. 1983. Ceftriaxone: renal and biliary excretion and effect on the colon microflora. J. Antimicrob. Chemother. 10: 207-217.

BARTLETT, J.G., BUSTETTER, L.A., GORBACH, S.L. & ONDERDONK, A. 1975. Comparative effect of tetracycline and doxycycline on the occurrence of resistant Escherichia coli in the faecal flora. Antimicrob. Agents Chemother. 7: 55-57.

BONHOFF, M., MILLERI, C.P. & MARTIN, W.R. 1964. Resistance of the mouse's intestinal tract of experimental Salmonella infection. J. Exptl. Med. 120: 805-816.

BORTHEN, L., HEIMDAHL, A., LINDQVIST, L. & NORD, C.E. 1983. Determination of volatile fatty acids in saliva and faeces by gas-liquid chromatography - a rapid method for detection of antibiotic-induced changes in the oropharyngeal and gastrointestinal microflora. Int. J. Microbiol. 1: 35-45.

BYSTEDT, H., HEIMDAHL, A. & NORD, C.E. 1984. Prophylaxis with tinidazole in oral surgery - tissue penetration and effect on the oral microflora. Int. J. Oral Surg. 13: 200-205.

GORBACH, S.L. 1982. Prophylactic antibiotics. Indications in surgical patients. Scand. J. Infect. Dis. 36: 134-140.

GORDON, J.M., WALKER, C.B., GROODSON, J.M. & SOCRANSKY, S.S. 1980. Sensitive assay for measuring tetracycline levels in gingival crevice fluid. Antimicrob. Agents Chemother. 17: 193-198.

HEIMDAHL, A., NORD, C.E. & OKUDA, K. 1980. Effect of tinidazole on the oral, throat and colon microflora of man. Med. Microbiol. Immunol. 1: 1-10.

HEIMDAHL, A., VON KONOW, L. & NORD, C.E. 1982. Effect of tinidazole on the human oral microflora: a comparison between high single and low repeated doses. J. Antimicrob. Chemother. 10: 157-164.

HEIMDAHL, A. & NORD, C.E. 1982. Effect of erythromycin and clindamycin on the indigenous human anaerobic flora and new colonization of the gastrointestinal tract. Eur. J. Clin. Microbiol. 1: 38-48.

HEIMDAHL, A., VON KONOW, L. & NORD, C.E. 1983. Clinical significance of penicillin-resistant beta-lactamase producing Bacteroides species in human orofacial infections. Proceedings of the Beta-Lactam-Symposium. Kiel: Springer. pp. 49-56.
HEIMDAHL, A. & NORD, C.E. 1983. Influence of doxycycline on the normal human flora and colonization of the oral cavity and colon. Scand. J. Infect. Dis. 15: 293-302.
HEIMDAHL, A., NORD, C.E. & BORTHEN, L. 1984. Impact of phenoxymethylpenicillin, erythromycin, clindamycin and doxycycline on Streptococcus salivarius in the oropharynx. J. Antimicrob. Chemother. 13: 505-509.
HÖJER, H. & WETTERFORS, J. 1978. Systemic prophylaxis with doxycycline in surgery of the colon and rectum. Ann. Surg. 187: 362-368.
KAGER, L., MALMBORG, A.S. & NORD, C.E. 1983. Impact of short-term as compared with long-term prophylaxis with cefoxitin on the colonic microflora in patients undergoing colorectal surgery. Drugs Exptl. Clin. Res. 5: 387-392.
KAGER, L., MALMBORG, A.S., NORD, C.E. & SJÖSTEDT, S. 1983. The effect of piperacillin prophylaxis on the colonic microflora in patients undergoing colorectal surgery. Infection 11: 251-254.
KAGER, L., MALMBORG, A.S., SJÖSTEDT, S. & NORD, C.E. 1983. Concentrations of ampicillin plus sulbactam in serum and intestinal mucosa and effects on the colonic microflora in patients undergoing colorectal surgery. Eur. J. Clin. Microbiol. 2: 559-563.
LEVISON, M.E. 1973. Effect of colon flora and short-chain fatty acids on growth in vitro of Pseudomonas aeruginosa and Enterobacteriaceae. Infect. Immun. 8: 30-35.
NORD, C.E. & KAGER, L. 1983. Tinidazole. Microbiology, pharmacology and efficacy in anaerobic infections. Infection 11: 54-60.
NORD, C.E., HEIMDAHL, A., KAGER, L. & MALMBORG, A.S. 1984. Impact of different antimicrobials on the normal gastrointestinal human microflora. Rev. Infect. Dis. 6: S270-S275.
NORD, C.E., KAGER, L., PHILIPSSON, A. & STIERNSTEDT, G. 1984. Impact of imipenem/cilastatin therapy on faecal flora. Eur. J. Clin. Microbiol. 3: 475-477.
SANDERS, C.C. & SANDERS, W.E. 1982. Enosin: an antibiotic produced by Streptococcus salivarius that may contribute to protection against infections due to group A streptococci. J. Infect. Dis. 146: 683-690.
SIMMON, G.L. & GORBACH, S.L. 1982. Intestinal microflora. Med. Clin. North Am. 66: 557-574.
SPRUNT, K., LEIDY, G.A. & REDMAN, W. 1971. Prevention of bacterial overgrowth. J. Infect. Dis. 123: 1-10.
SUKCHOTIRATANA, M. & LINTON, M. 1975. Antibiotics and oral streptococci of man. J. Appl. Bact. 38: 277-294.
TALLY, F.P., CUCHURAL, G.J., JACOBUS, N.V., GORBACH, S.L., ALDRIDGE, K.E., CLEARY, T.J., FINEGOLD, S.M., HILL, G.B., IANNINI, P.B., McCLOSKEY, R.V., O'KEEFE, J.P. & PIERSON, C.L. 1983. Susceptibility of the Bacteroides fragilis group in the United States in 1981. Antimicrob. Agents Chemother. 23: 536-540.
VAN DER WAAJ, D. 1982. Colonization resistance of the digestive tract. Clinical consequences and complications. J. Antimicrob. Chemother. 10: 263-270.