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EFFECTS OF ANTIMICROBIAL THERAPY UPON DIGESTIVE TRACT MICROFLORA

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INTRODUCTION

Antibiotics may affect the digestive tract microflora in the oropharynx and/or in the intestines, either following incomplete absorption during oral treatment or else by excretion with the bile. They may reach the gut contents and mix with it to establish in the course of several days a steady state concentration. In the oropharyngeal area, the antibiotic concentrations are largely determined by salivary concentrations which may fluctuate with serum levels. Depending on the spectrum of activity of the antibiotic(s) that have reached the digestive tract, sensitive Gram-positive and/or Gram-negative bacteria may suffer of the concentration established. Resistant bacteria on the contrary may flourish under these conditions much better than beforehand and grow out to high numbers. In this process of selective suppression of sensitive bacteria in the oropharynx and/or the gut, either the colonization resistance (CR) associated (*van der Waaij* et al., 1971; *van der Waaij*, 1982) predominantly anaerobic microflora is largely sensitive and suppressed, or not sensitive and largely unchanged. In this last case the aerobic Gram-negative bacteria, which are potentially pathogenic, are generally sensitive and killed (*van der Waaij,* 1982). In the first situation of CR-flora suppression, resistant aerobic Gram-negative bacteria and yeasts may grow out to abnormally high concentrations, a condition commonly described as "overgrowth" (*van der Waaij,* 1979; *Nord* et al., 1984). Such high concentrations of potentially pathogenic bacteria are associated with invasion of the mucous membrane. From there the submucosal tissues and even the mesenteric lymph nodes and spleen are invaded (*van der Waaij* et al., 1972). This event is called "translocation" (*Berg,* 1980).

SELECTIVE SUPPRESSION OF BOWEL FLORA

In case of selective suppression of the aerobic Gram-negatives, the anaerobic fraction of the bowel flora remains largely unaffected (*van der Waaij* et al., 1974). This treatment has been found to reduce Gram-negative infections in the granulocytopenic patient and is named "selective decontamination" (*Sleijfer* et al., 1980). If bacteria replace suppressed flora components during selective decontamination, it normally concerns resident anaerobic species which are resistant to the antibiotic used.

Resident anaerobes do generally not translocate (*Berg* and *Garlington*, 1979). In immunocompromised patients this condition is beneficial as it is associated with a reduction of translocation

in stead of an increase by an overgrowth of Gram-negatives as may occur when the CR-associated flora is largely suppressed by antibiotic therapy (*van der Waaij*, 1982). These phenomena will be discussed in great detail elsewhere in this monograph. Therefore, this overview will be confined to implications of antibiotic therapy for the emergence of resistance in the digestive tract microflora during antibiotic therapy.

FAECAL CARRIAGE OF RESISTANT BACTERIA

Studies which will be quoted hereafter provide further evidence that antibiotic resistance in Gram-negatives may predominantly develop in the intestinal tract and not at the site of infection.

The main anatomical sites at which the occurrence of antibiotic resistant bacteria in the commensal flora have been studied are indeed the intestines, but also the skin and upper respiratory tract. Naturally, attention has focused on the gut because of the large range and number of bacteria present and the association of those bacteria with opportunistic infection such as respiratory tract (*Eickhoff*, 1979) and urinary tract infections (*Ball*, 1986).

Considering the intestines first, there have been a great number of studies but protocols and data analysis vary widely so that direct comparison is difficult. Early classic studies on R-plasmids originate from Japan and mainly concerned the plasmid transfer in the presence of antibiotic treatment (*Watanabe*, 1963).

Although there are a number of studies from the sixties and early seventies of the faecal carriage of antibiotic resistant bacteria in normal individuals, similar comprehensive surveys are not available for the eighties. A study from the early eighties of sensitive enteropathogenic *Escherichia coli* showed that 37% of the isolates obtained, were resistant to ampicillin and 29% to tetracycline (*Gross* et al., 1982). In view of the shift in general practice prescribing habits in the United Kingdom reported, from low ampicillin prescription in 1967 to three fold higher in 1984 while prescription of tetracyclines decreased with about 50% over the same period, such studies would be valuable to confirm changes in resistance rates in relation to prescribing.

Although it is no longer possible to examine urban and rural populations that have not been exposed at some time to therapeutic antibiotics, some studies have attempted to assess the natural occurrence of resistance genes in remote communities that have had little or no contact with antibiotics. The Xhosa communities in South Africa, who avoid Western medicine, were studied in 1973 and 1976. During this time, antibiotic usage increased from zero to moderate usage. During this two year interval, resistance in faecal coliforms increased from 19% to 48% (*Burt* and *Woods*, 1976). A study of an extremely remote island in the Solomon Islands at which modern medicine is shun, revealed only two R-plasmid containing bacteria from 40 samples of faeces and soil, both resistant to tetracycline and streptomycin (*Gardner* et al., 1969). These studies indicate that antibiotic treatment is associated with the emergence of resistant strains in the faecal flora.

ANTIBIOTIC RESISTANCE IN ENTEROBACTERIACEAE FROM HEALTHY VOLUNTEERS

The first survey of the occurrence of antibiotic resistant faecal *E. coli* in normal healthy subjects and animals was reported in 1966 (*Smith* and *Halls*, 1966). The resistance markers studied were relevant. Although only 24 healthy subjects were examined, 62% carried resistant *E. coli*. Approximate analysis of resistance patterns suggested that about 17% were resistant to ampicillin and 70% to tetracycline. The source of the human volunteers was unfortunately not provided.

Again a long time ago in 1966, *Smith* and *Halls* found in 19 of 20 representative *E. coli* from healthy subjects having transferable patterns of drug resistance indicating a high incidence of plasmid-mediated resistance. Concern was expressed in their report and in subsequent studies at the high level of carriage of antibiotic resistant bacteria in apparently normal healthy individuals (*Williams Smith*, 1975; *Leading article*, 1969). Another study in the United Kingdom of urban, rural farming and rural non-farming families by *Linton* et al. (1972) published a few years later confirmed the high incidence of carriage of resistant Enterobacteriaceae. Urban adults had an overall carriage rate of 42% which was considerably lower than the prevalence in children (64%). In contrast rural farming adults carried also in a high percentage (63%) resistant enterobacteria; in children this figure was yet higher (79%). Statistically, the difference in resistance rates between adults and children was significant in both farming and non-farming associated individuals as well as between both groups as a whole. The hypothesis by the author and co-workers was that the difference between children and adults was due to differences in antibiotic use in the two groups. They surveyed the antibiotic prescribing habits of 15 local general practitioners for one week. The highest rate was in school age children, the intermediate in adults and the lowest in children of less than four years of age which led them to the conclusion that antibiotic use could not be the most important factor. *Linton* et al. (1972) however, did not mention the fact that children up to the age of six received a quarter of the prescriptions although they only represent 10% of the total population (*Leading article*, 1974).

It could be argued that the high carriage rates of resistant strains in children are related to their lower standard of personal hygiene and thus their greater chance of acquiring resistant strains. Cross-colonizing of subjects with antibiotic resistant bacteria has been reported both in the absence (*Petrocheilon* et al., 1977) as well as in the presence of antibiotic treatment (*van der Waaij* et al., 1986). Resistant bacteria are not necessarily by themselves good colonizers of the human gut but may persist for a very long time (*Hartley* and *Richmond*, 1975). The explanation for the high carriage rate of resistant Gram-negative enterobacteria in children in comparison to adults may be a combination of: 1. heavy prescribing of oral antibiotics with larger doses for weight in children and 2. a greater chance of cross-contamination in children from siblings and playmates.

The first point may have implicated higher average steady state concentrations in the intestines and, therefore, more often have resulted in suppression of the colonization resistance associated flora in children than in adults. Whether this still holds nowadays in the late eighties is questionable. As reported several years ago about a study in 1971 in the Netherlands (*van der Waaij* et al.,

1986), and as will be reported elsewhere in this monograph, it is likely that in the last two decades inactivation by intestinal contents may have increased particularly as far as ß-lactam antibiotics are concerned. Inactivation of these antibiotics has appeared to he due to ß-lactamases (*Welling* et al., 1987) in the intestinal contents (faeces) and may vary in degree between individuals. Also other antibiotics such as aminoglycosides, polymyxin and quinolones are to a interindividually varying degree inactivated by faecal substances (*Veringa* and *van der Waaij*, 1984).

ANTIBIOTIC RESISTANCE IN ENTEROBACTERIACEAE FROM HOSPITAL PATIENTS

In the late sixties a study of 100 patients admitted to hospital for elective surgery (*Datta*, 1969) was performed. Gram-negative bacilli as well as *E. coli* were studied during hospitalization. A predominance of resistant strains was found amongst *E. coli*, resistant strains being found in 52% of patient's specimens.

Sixty percent of these strains had transferable markers, 17% of patients excreted ampicillin resistant *E. coli* and 34% tetracycline resistant *E. coli*. A comprehensive study by *Moorhouse* (1969) in Dublin in the same period confirmed these figures. A few years later *Shaw* and co-workers (1973) published their findings in comparable groups of 20 to 25 patients who were either not treated or treated with tetracycline or an ampicillin. In this study also an alarming increase of Gram-negative bacteria during hospitalization associated with antibiotic treatment was reported.

R-PLASMID TRANSFER *IN VIVO*

The emergence of resistance among enteric Gram-negative bacilli may exclusively be due to selection of resistant mutants. However, theoretically resistance may spread in hospitals by transfer of resistance plasmids. Whilst all the prevalence studies have usually shown a high incidence of transferability of antibiotic resistance markers to laboratory recipient strains, the relevance of this transfer of R-plasmids amongst members of the resident microflora of the human gut is, however, uncertain (*Lacey*, 1975). A rather recent study by *Platt* and co-workers (1986) , reveals that *in vivo* transfer may occur in hospitalized patients, particularly in *E. coli* during antibiotic treatment. Why other Enterobacteriaceae are not equally able to receive R-plasmids *in vivo* is explained by the authors by giving special emphasis to the fact that the principle habitat of *E. coli* is the human (and animal) gut. In the intestines *E. coli* is known to be predominant to other aerobic Gram-negatives. The Enterobacteriaceae genera other than *E. coli* tend to be minority residents and are also adapted to free-living existence. Plasmid carriage constitutes a biosynthetic burden to Enterobacteriaceae (*Zund* and *Lebek*, 1981). It seems likely that a well-adapted organism like *E. coli* is more readily able to accumulate plasmids than the other enteric Gram-negatives. In the absence of selective pressure, this may confer an energetic disadvantage on the bacteria which carry

R-plasmids. Furthermore, the high spontaneous mutation rate in genera such as *Serratia* (*Platt* and *Sommerville*, 1981) and *Klebsiella* (*Smith*, 1976) may confer sufficient genetic flexibility to reduce the benefits of plasmid carriage.
Finally, anaerobic gut bacteria such as *Bacteroides* spp. are themselves capable of R-plasmid transfer although it seems to be a rare event (*Bawdon* et al., 1982). Although R-plasmid transfer was not seen in the non-antibiotic treated group in a study by *Anderson* and co-workers (1973), there is a reliable report of R-plasmid transfer amongst the bacteria of the faecal flora of a subject who had not received antibiotics for the past 202 days (*Petrocheilon* et al., 1976). Most other reports have involved the administration of antibiotics at or near the time of R-plasmid transfer (*Bawdon* et al., 1982; Lowburry et al., 1969). However, there is one important exception. In patients who have been treated with selective decontamination for Gram-negative infection prophylaxis, the emergence of resistance was not found (*de Vies-Hospers* et al., 1981), despite the fact that acquisition of resistant Gram-negative rods from the environment is apparently not prevented (*Dekker* et al., 1981). Prevention of acquisition of resistant Gram-negatives requires frequent bacteriological monitoring of faeces or the use of a combination of antimicrobials such as colistin (polymyxin) with co-trimoxazole for selective decontamination (*Rozenberg-Arska* et al., 1983).

In summary, if R-plasmid transfer may occur amongst bacteria of the intestinal (faecal) flora of humans not taking antibiotics, transfer is much more likely during antibiotic treatment with a substantial disruption of normal bowel flora and thus of the CR as unavoidable consequence.

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