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# Selective elimination of Enterobacteriaceae species from the digestive tract in mice and monkeys

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## SUMMARY

In mice and in monkeys, 'selective' elimination of Enterobacteriaceae species from the digestive tract of animals with a sensitive flora was accomplished by oral treatment with nalidixic acid (1 mg./g. body weight in mice and 0·4 mg./g. body weight in monkeys). During treatment, the concentration of enterococci (and also of *Candida albicans* in the monkey) remained unaltered. This indicates that the fraction of the anaerobic microflora which is responsible for the colonization resistance of the digestive tract is not affected by the treatment. An important consequence seems to be, that elimination of yeast and fungi with fungistatic drugs can be started at the same time as elimination of Enterobacteriaceae is attempted.

## INTRODUCTION

In patients and animals with impaired immune competence, the digestive tract is often the portal of entry for infections caused by potentially pathogenic (p.p.) micro-organisms such as *Pseudomonas aeruginosa*, *Escherichia coli* and other Enterobacteriaceae species as well as streptococci, staphylococci, yeasts and fungi. Decontamination of the digestive tract by treatment with oral antibiotics is being used increasingly to prevent such infections. However, this approach is of questionable value because decontaminated individuals are easily colonized by bacteria from the environment which are resistant to the antibiotics used for decontamination. Very small numbers of bacteria can colonize a decontaminated individual, because of the previous elimination of certain anaerobic species that are responsible for the colonization resistance (CR) of the digestive tract (van der Waaij, Berghuis-de Vries & Lekkerkerk, 1971). For this reason a different method of decontamination is needed that eliminates only the p.p. micro-organisms while leaving the anaerobes unaffected. The present experiments were conducted to determine whether the use of nalidixic acid is an effective alternative method to antibiotic decontamination for selective elimination of Enterobacteriaceae species from the digestive tract.

Table 1. *Results of* in vitro *test of sensitivity of Entobacteriaceae species present in ten faecal samples from monkeys*

| | mg. drug/ml. | | | |
|---|---|---|---|---|
| Drug tested | 0·027 | 0·29 | 2·1 | 23 |
| Furoxone | 6* | 2 | 0 | 0 |
| Nalidixic acid | 2 | 0 | 0 | 0 |

* Number of positive cultures per 10 samples.

## MATERIALS AND METHODS

### *Antimicrobial agents*

Initially two chemotherapeutic drugs, nalidixic acid and furoxone, gave promising results in *in vitro* sensitivity tests (Table 1). The use of furoxone was discontinued because, when supplied in effective doses, it induced vomiting in several monkeys and caused anorexia in mice. Accordingly, only results with nalidixic acid (N.A.) are reported in this study.

### *Sensitivity testing*

The sensitivity of the Enterobacteriaceae species in the faeces of animals to be treated was determined by suspending 1 part of fresh faeces from each animal in 9 parts of saline. The suspensions were subsequently streaked on Endo agar; disks consisting of 10, 25, 50 and 100 $\mu$g. nalidixic acid were then placed on the plates. After 24 hr. of incubation at 37° C. the plates were read.

### *Animals*

Thirty female ND2 mice, 12 weeks old (30–35 g.) and eight young rhesus monkeys (*M. mulatta*, 2·2–3·5 kg.) were used in this study.

Eight monkeys were found to have a sensitive Enterobacteriaceae flora. Thus any organisms isolated from the faeces of these eight monkeys during treatment would have had to be due to a colonization from an exogenous source.

### *Housing*

All animals were caged individually in clean disinfected cages with wire mesh grids. The mice were given a standard pelleted ration (Hope Farms). The monkeys were maintained on fresh fruit, vegetables, and a standard pelleted primate ration (Hope Farms). Drinking water was supplied *ad libitum*. Food was purposely not sterilized in order to prevent a reduced intake due to a change in palatability. A reduction in food intake during treatment could thus be ascribed to nausea due to nalidixic acid.

### *Oral treatment*

N.A. was supplied to the mice in the drinking water. Commercially available 500 mg. tablets were dissolved in sterilized water (pH = 9) to achieve a concentration of 10 mg./ml. The average dose of 1 mg. N.A./g. body weight was effective

in eliminating Enterobacteriaceae species. To each monkey, 500 mg. N.A. was administered orally twice daily in 5 ml. of water (pH = 9) to obtain an average dose of 0·3–0·5 g./kg. body weight per day. The mice were treated in this way for 3 weeks, and monkeys for 16 days.

*Determination of effect of treatment on CR and disappearance of Enterobacteriaceae species*

CR and effect of the N.A. treatment on the Enterobacteriaceae population were determined by daily quantitative and qualitative studies in the faeces. A sample of 0·1 g. faeces was suspended in 0·4 ml. of Brain Heart Infusion broth (BHI broth, DIFCO); 0·05 ml. of this suspension was transferred to cup 1 of a plastic tray containing 0·5 ml. of broth per cup. The suspension was then serially diluted through eight dilution steps of 1/11 by transferring 0·05 ml. with diluting loops (Flow laboratories). After incubation of the tray, subcultures from each cup were made on Endo agar for the Enterobacteriaceae species and on Aesculin azide agar (Sneath, 1956) for enterococci. The presence of *Candida* species in the cultures from the monkey samples was determined by observing growth on Endo plates after 3 days incubation. In the mouse experiments, subcultures of each dilution were also made. However, the mouse cultures were subcultured on Sabauroud agar, since the yeast species that had been isolated from the mice rarely grew on Endo agar. The concentration both of the enterococci and the yeasts in the faeces was determined because they are not sensitive to nalidixic acid and an increase in number of either would be one indication of a decrease in the CR of the digestive tract.

*Oral sampling*

In the mice the oral cavity was swabbed daily with cotton swabs moistened with broth. The swabs were cultured in BHI broth after which subcultures were made on selective plates as described above. The oral cavity of the monkeys was not swabbed.

## RESULTS

In mice there was a transitory reduction in the water intake during the first 2 days of treatment after which normal intake was restored. Food consumption was normal in both animal species and no weight loss occurred during treatment. The consistency of the faeces remained normal both in mice and monkeys, while all Enterobacteriaceae species were eliminated within 9 days (Figs. 1, 2). The oral swabs in the mice also were negative after 9 days. Thereafter, a positive swab was found occasionally. This is attributed to the fact that the food and water were not sterilized. In no case, however, was a yeast or a fungus species isolated from the oral cavity during treatment. The concentration of enterococci remained constant in the faeces of both mice and monkeys, as did that of *Candida albicans* (Fig. 3), which was present in the faeces of three monkeys at the start of the study. Five other monkeys were negative for candida when they entered the study and

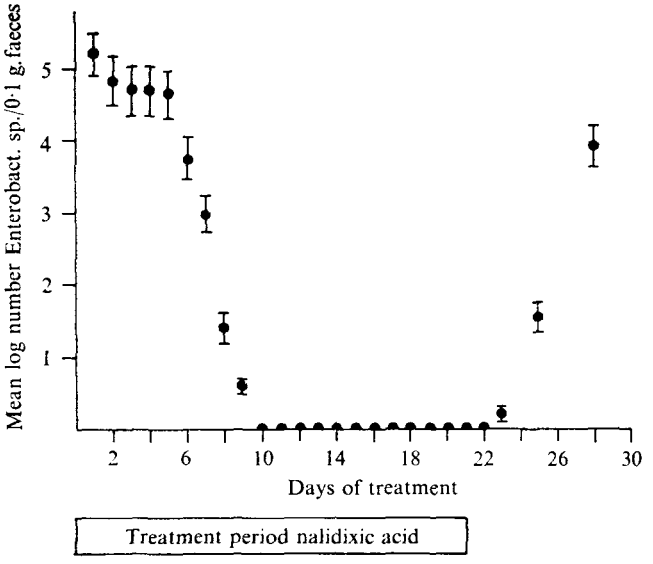


Fig. 1. Mean and standard deviation of the Enterobacteriaceae concentration in the faeces of mice during and after N.A. treatment.

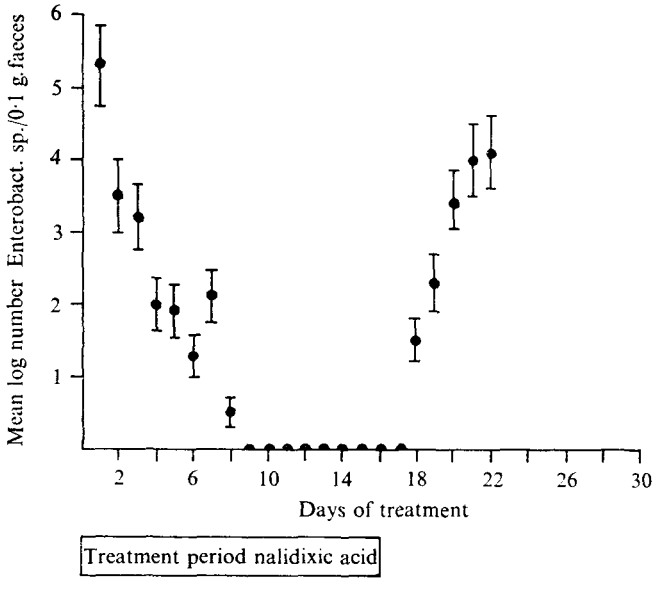


Fig. 2. Mean and standard deviation of the Enterobacteriaceae concentration in the faeces of monkeys during and after N.A. treatment.

remained negative during treatment. No yeasts or fungi were isolated from oral swabs and faeces of the mice.

## DISCUSSION

This study has shown that Enterobacteriaceae can be successfully eliminated from the faecal flora both of mice and monkeys in about 1 week by oral adminis-

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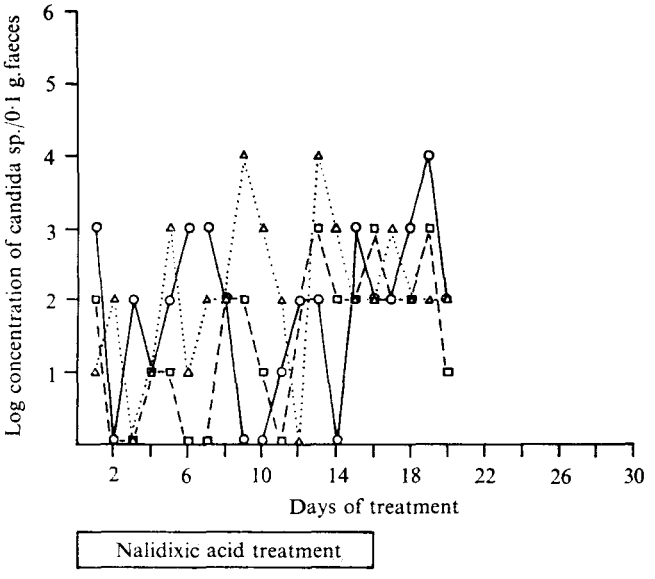


Fig. 3. The concentration of *Candida albicans* in the faeces of three monkeys during and after N.A. treatment.

tration of N.A. Inasmuch as the concentration of enterococci remained unchanged during the treatment, it appears that the CR was unaffected. The fact that the *C. albicans* concentration remained low in the monkeys during treatment further supports the interpretation that N.A. does not affect CR.

The CR of the digestive tract is the resistance to growth and multiplication encountered by micro-organisms after ingestion. The CR for several p.p. bacterial species has been determined both in mice and monkeys, and has been expressed as the log of the oral dose of bacteria of a certain species resulting in a colonization for 2 weeks or longer in 50% of a group of animals (van der Waaij *et al.* 1971). The CR apparently is caused by anaerobic bacterial species (Wensinck & Ruseler-van Embden, 1971) which act largely through the host by stimulating g.i.-peristalsis (Abrams & Bishop, 1967).

In previous experiments it was determined that Enterobacteriaceae species can be eliminated effectively from the digestive tract of mice by systemic treatment with kanamycin (van der Waaij, 1968). It has also been shown that systemic antibiotic treatment reduces the CR to some extent (van der Waaij, Berghuis & Lekkerkerk, 1972). N.A. is only available for oral administration. However, it was used in this study because about 95% is readily absorbed from the g.i. tract, and because it has a limited range which affects Enterobacteriaceae species while leaving enterococci and other bacterial species unaffected. Thus, the enterococci could be used as an indicator of the CR because, if CR decreased as a result of N.A. treatment, the concentration of enterococci in the faeces would increase (van der Waaij *et al.* 1972).

The mechanism by which N.A. eliminates Enterobacteriaceae from the intestinal lumen is not known. However, it is unlikely that it acts directly on the

intestinal contents. A rough estimate of the concentration of N.A. in the faeces of the monkeys was made with a 'sensitive' *Esch. coli* strain. The results indicated that if free N.A. were present in the faeces of these animals, its concentration would not have been more than 7 $\mu$g./g. This concentration is not sufficiently high to eliminate all Enterobacteriaceae species. On the other hand, the absorbed part of the drug may well have reached a sufficiently high concentration in the intestinal mucosa to prevent adherence of Enterobacteriaceae species to the mucosa. Recent observations (Williams & Gibbons, 1972) indicate that immunoglobin A (IgA) may act in about the same way and prevent bacterial colonization by preventing adherence to the mucosa. If prevention of adherence is a factor in the mechanism of N.A. action, this could explain why a week elapsed before the animals were free while, in animals decontaminated with antibiotics, Enterobacteriaceae species disappear in 2 days (van der Waaij, de Vries & Lekkerkerk, 1970).

Clinical observations in patients by Dankert (1973) also indicate that selective decontamination may be feasible. Kidney transplantation patients undergoing moderate immuno-suppression were treated with the combination trimethoprim and sulphamethoxazole. These two drugs were also administered orally. They are also readily absorbed; however, they have a much wider range than N.A. During treatment the concentration of Enterobacteriaceae species in the faeces decreased to zero in about 1 week. As in our experiments, the concentration of enterococci, yeasts and fungi was determined at constant intervals. During treatment no change in *C. albicans* concentration was reported. After treatment, however, a slight increase occurred. The enterococci behaved differently. They rose in concentration from $10^6$/g to $10^8$/g of faeces. This may indicate that the drugs used decrease the CR for enterococci to some extent.

The elimination of yeasts and fungi from the digestive tract by nystatin or amphotericin B is more often successful in the presence of a microflora that causes a good CR than in its absence, such as during antibiotic decontamination. Thus, it is technically preferable to eliminate yeasts before antibiotic decontamination is started. A 4-day treatment period with an adequate antimycotic drug is usually sufficient provided that oral administration of antimycotics is then continued during the antibiotic decontamination period. When N.A. or a similar drug is used, yeasts, fungi and Enterobacteriaceae species can be eliminated simultaneously because the anaerobes responsible for CR remain unaffected.

The results of this study suggest that patients with a N.A. sensitive Enterobacteriaceae flora can now be considered for selective decontamination. It should be stressed, however, in this context, that *Pseudomonas aeruginosa* is not sensitive to N.A. Although N.A. apparently does not affect the CR, so that low dose contaminations with Enterobacteriaceae species will not take, pasteurization of food and oral fluids seems advisable. As in mice, low dose contamination may occur in the oral cavity and thus create a potential hazard.

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