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The effect of three frequently applied antibiotics on the colonization resistance of the digestive tract of mice

By H. A. THIJM and D. van der WAAIJ
Laboratory for Medical Microbiology, University Hospital, Groningen, The Netherlands

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SUMMARY

The influence of treatment with increasing oral doses of three absorbable antibiotics on the colonization resistance of the digestive tract was investigated in mice. Mice treated with ampicillin or epicillin in any of the applied doses had a strongly decreased colonization resistance as demonstrated by 'bacterial overgrowth' after contamination with resistant strains of *Escherichia coli*. After a treatment period of 2 weeks, *Streptococcus faecalis* became resistant in a number of animals. Oral treatment with cephradine on the other hand had no obvious influence on the endogenous flora of the mice, nor was the colonization resistance decreased.

INTRODUCTION

Systemic antibiotic treatment leads often to 'bacterial overgrowth' in the digestive tract by resistant species and strains (Louria & Kaminski, 1962; Selden *et al.* 1971; Pollack *et al.* 1972; Hirsh, Burton & Blenden, 1974; Holzman *et al.* 1974; Westwood, Lagacé & Mitschell, 1974). This unfortunate shift in the bacterial population results from a decrease in the 'colonization resistance' (CR) of the digestive tract (van der Waaij *et al.* 1971). The CR of the digestive tract is a rather complex mechanism in which host and microflora cooperate in preventing colonization of the digestive tract by potentially pathogenic exogenous microorganisms. The CR of the digestive tract is caused by the anaerobic fraction of the intestinal microflora; its strength can be measured and is expressed as $\log_{10}$ of the oral dose of potentially pathogenic bacteria which results in colonization ('take') for a minimum of 2 weeks in 50 % of a group of individuals (men or animals). Since anaerobic bacteria are sensitive to many antibiotics which are daily used in the treatment of infections, the CR of patients with infections often decreases considerably during antibiotic treatment. Because a low CR permits the resistant bacteria to grow out in the intestines to high concentrations of well over 10^8 bacteria/gram (of faeces), transfer of R-factors between related species is to be expected (Kasuya, 1964; Reed, Sieckmann & Georgi, 1969; Jones & Curtiss, 1970). Secondly, a decrease of the CR lowers the 'threshold contamination dose' for colonization of the digestive tract. This means that in antibiotic-treated individuals with a low CR small numbers of resistant bacteria can colonize the digestive tract following oral contamination. This favours the transmission of

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Table 1. *Mean daily doses of the three antibiotics with which animals were treated in the weeks* 3, 4, 5 *and* 6 (7 *in cephradine group*) *of the experiment*

Antibiotic	Group	Mean body weight in g (S.D.)	Antibiotic conc. in the drinking water (μg/ml)	Mean daily dose in mg (S.D.)	Corresponding dose for man 70 kg in g (S.D.)
Epicillin†	1*	31·4 (3·2)	0	0	0
	2	30·4 (2·1)	143	0·6 (0·1)	1·4 (0·4)
	3	29·2 (2·7)	286	1·2 (0·2)	2·9 (0·8)
	4	29·2 (1·1)	715	3·1 (0·4)	7 (2)
	5	30·7 (1·5)	1430	6·2 (0·9)	14 (3)
Cephradine†	6*	25·8 (2·7)	0	0	0
	7	25·8 (1·9)	114	0·3 (0·1)	0·8 (0·2)
	8	25·1 (2·6)	228	0·7 (0·1)	2·0 (0·6)
	9	23·7 (1·9)	570	1·7 (0·3)	5 (1)
	10	24·5 (1·7)	1140	3·4 (0·6)	10 (2)
Ampicillin	11*	31·2 (1·5)	0	0	0
	12	28·4 (2·5)	114	0·5 (0·1)	1·2 (0·3)
	13	30·2 (1·6)	570	2·3 (0·4)	5 (1)
	14	29·4 (1·8)	1140	4·7 (0·7)	11 (3)
	15	31·4 (1·3)	2280	9 (2)	20 (4)

* Groups 1, 6 and 11 served as control groups and were not treated.
† Epicillin and cephradine were kindly provided by SQUIBB-Nederland B.V.

resistant bacteria, which are already prevalent in the environment of most hospitals. (Dempster, Reid & Cody, 1973; Taplin & Mertz, 1973; Berkowitz *et al.* 1974; Cooke, 1974; Hanson & Shelley, 1974; Horwitz, Finlayson & Brede, 1974; Pash, 1974; Shaffer, 1974; Casewell & Phillips, 1977). Finally induction of resistance and selection of less sensitive mutants may occur particularly in patients who are treated with lower doses of antibiotics.

For these reasons two penicillin derivatives (ampicillin and epicillin) which are frequently used in our hospital as well as an oral cephalosporin (cephradine) were used to investigate the effect of different doses of these antibiotics on the CR of mice. Previous experiments (van der Waaij *et al.* 1971, 1972) have indicated that the mouse provides an ideal experimental model for the study of antimicrobial drugs on the CR. Different doses, increasing from 'low clinical dosage' to extremely high dosages, were used to study at which dose the first signs of a decrease of the CR occurred, or in other words, up to which dose the respective drugs were safe – that is do not cause 'bacterial overgrowth'.

MATERIALS AND METHODS

Mice

Conventional female Swiss mice of 8–12 weeks and a mean body weight of 28 g were used. The animals were housed four per cage. Food pellets and water were supplied ad libitum, and during the antibiotic treatment period antibiotics were added to the drinking water.

Table 2. *Strains of* Enterobacteriaceae *species used for oral contamination*

Enterobacteriaceae species and biotype (API 20E)		Resistant to (m.i.c.)
E. coli	5044552	Epicillin (4 mg/ml) and neomycin (2 mg/ml)
E. coli	5144572	Cephradine (1·6 mg/ml) and neomycin (2 mg/ml)
E. coli	5144552	Cephradine (1·4 mg/ml) and kanamycin (2 mg/ml)
Prot. morganii	0174000	Cephradine (1·8 mg/ml) and kanamycin (2 mg/ml)
E. coli	5144572	Ampicillin (2·5 mg/ml) and neomycin (2 mg/ml)

Table 3. ***Biotypes of endogenous*** Enterobacteriaceae *species isolated before the onset of treatment*

	Groups 1 to 5 (epicillin treated)		Groups 6 to 10 (cephradine treated)		Groups 11 to 15 (ampicillin treated)	
	Species	Biotype	Species	Biotype	Species	Biotype
Endogenous Enterobacteriaceae species	*E. coli*	4144572	*E. coli*	4144572	*E. coli*	4144572
	E. coli	5144552	*E. coli*	5144552	*E. coli*	5144552
	E. coli	5144572	*E. coli*	5144572	*E. coli*	5144572
	Prot. mir.	0736000				
	Prot. mir.	0732000				

Antibiotic treatment

A group of 60 mice was subdivided into fifteen subgroups of four animals. During the first 2 weeks the animals were not treated, but only observed bacteriologically. In the third, fourth, fifth and sixth weeks antibiotics were added to their drinking water in concentrations shown in Table 1. Only the cephradine-treated mice were maintained on their antibiotic regimen for an additional (seventh) week to permit experimental contamination on a third occasion.

Bacteriological procedures

Fresh faeces were collected three times a week from each individual mouse. Within 2 h after collection the faeces were suspended in Brain Heart Infusion broth (DIFCO) in a ratio of 1 weight unit to 10 volumes suspension. These suspensions were serially diluted (1/10) for eight steps also in BHI-broth. After overnight incubation each dilution was subcultured on MacConkey agar for the isolation and enumeration of the *Enterobacteriaceae* species, while subculture on aesculin-azide agar (Sneath, 1956) provided information concerning the concentration of *Streptococcus faecalis*. This procedure provided mean values of the concentration of the *Enterobacteriaceae* species and *Strept. faecalis* in the faeces per antibiotic and per group of four animals each week. In the initial observation period of 2 weeks, the *Enterobacteriaceae* species isolated were biotyped. The procedure for isolation and subsequent biotyping to allow a complete inventory to be obtained has been described previously (van der Waaij, Tielemans-Speltie & de Roeck-Houben , 1977).

On the first day of the fifth week of the experiment (third week of antibiotic

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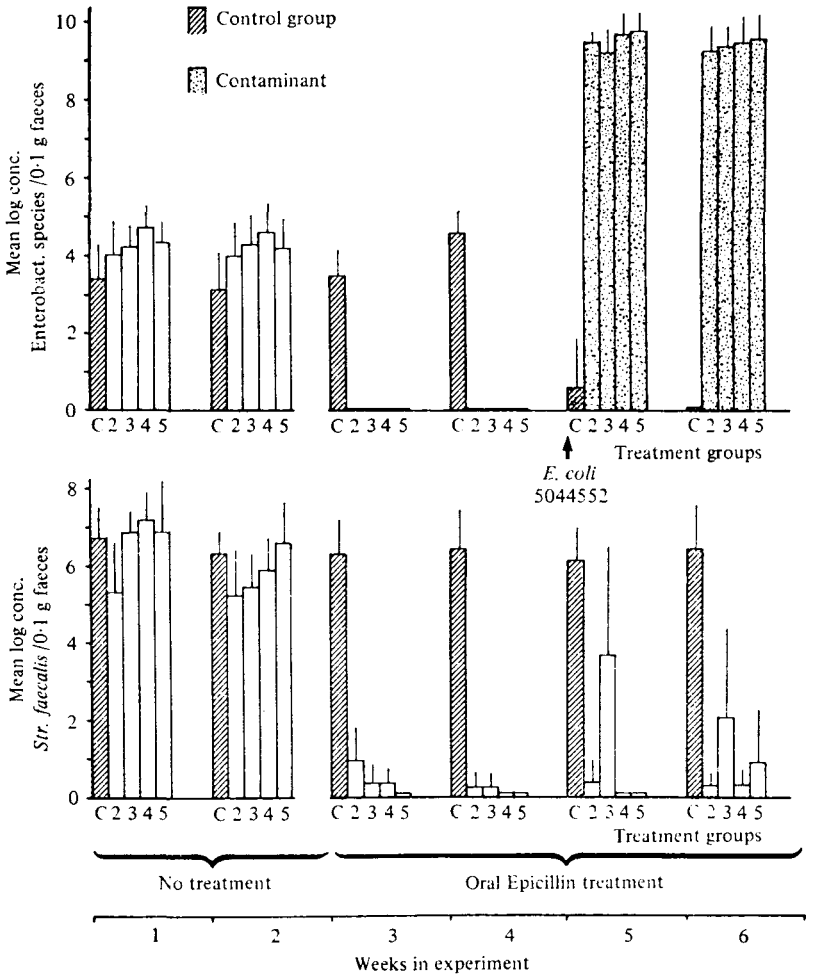

Fig. 1. Concentration of *Enterobacteriaceae* species and *Strept. faecalis* in the faeces of mice (before and during treatment with different doses of Epicillin).

treatment) the animals were contaminated with an *Escherichia coli* strain which was resistant to the antibiotic used and to neomycin or kanamycin. The mice which were treated with cephradine as well as their control group, were also contaminated in the sixth and seventh week. (Table 2).

Selective isolation of the contaminant was obtained by adding neomycin or kanamycin (Table 2) in a concentration of 250 μg/ml to the MacConkey agar. The resistant strains were all resistant to concentrations of more than 1 mg/ml of these aminoglycoside antibiotics.

Oral contamination

The mice were contaminated orally with 0·2 ml of an overnight broth culture of the respective strains (Table 2) containing approximately 10^9 bacteria/ml.

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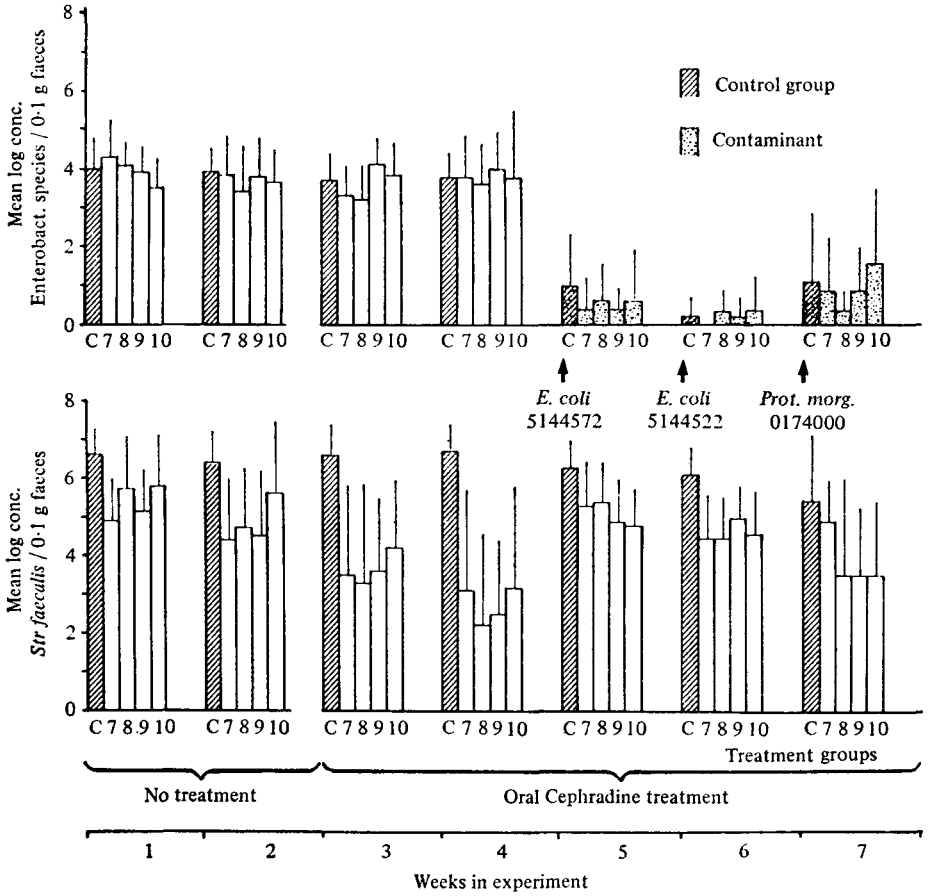

Fig. 2. Concentration of *Enterobacteriaceae* species and *Strept. faecalis* in the faeces of mice (before and during treatment with different doses of Cephradine).

Caecal weight

At the end of the experiment the body weight as well as the wet weight of the filled caecum was determined.

RESULTS

The biotypes which were present in the animals at outset are summarized in Table 3. Only in the cephradine treated animals did these biotypes persist. The concentration of the *Enterobacteriaceae* species and *Strept. faecalis* which are determined as measures of the Colonization Resistance (CR) of the intestinal tract are shown on figures 1, 2 and 3. It appears that both ampicillin and epicillin had a strong effect on the endogenous microflora after even the lowest doses. The influence was the strongest on the *Enterobacteriaceae* species; the *Strept. faecalis* responded only with a reduction in concentration following the lowest doses. It is remarkable that even by the third week of treatment resistant strains were found

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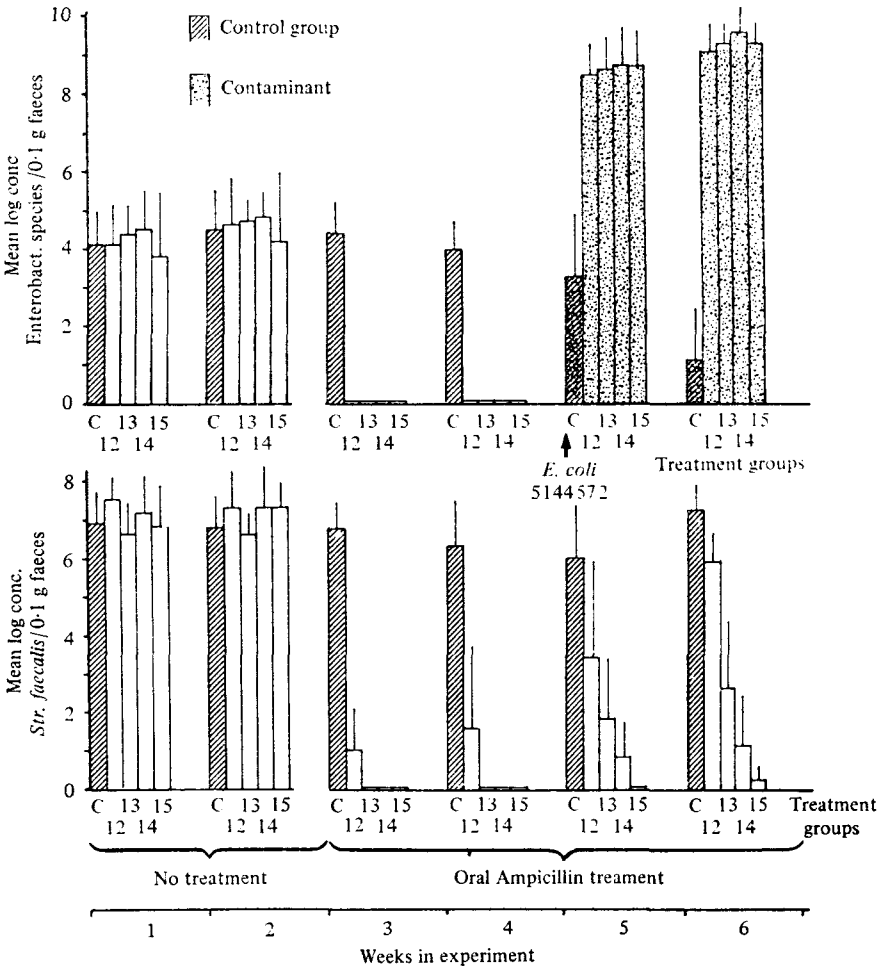

Fig. 3. Concentration of *Enterobacteriaceae* species and *Strept. faecalis* in the faeces of mice (before and during treatment with different doses of Ampicillin).

following treatment with both antibiotics. This occurrence found its expression in the gradual increase in the mean concentration of these enterococci (Figs. 1 and 3) regardless of the fact that the oral daily dose remained constant. The results in the cephradine treated group of mice differed strongly from the other two. Regardless of excessive dosing up to 1140 μg/ml of drinking water (3·4 mg per mouse per day) the concentration of both the *Enterobacteriaceae* species and *Strept. faecalis* remained unchanged (Fig. 2) although both were sensitive to this antibiotic.

Experimental contamination of the mice during treatment differed also in the ampicillin and epicillin treated mice from what was seen in the cephradine group. In the ampicillin and the epicillin treated mice a rapid 'take' of the contaminant was obtained in all groups. A significant difference in concentration was found between the control group and even the group of mice treated with the lowest dose.

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Table 4. *Effect of oral treatment* with epicillin, ampicillin or cephradine on the mean and the relative caecal weight*

Conc. in drinking water (μg/ml)	Mean and (S.D.) of Caecal weight	Body weight	Relative caecal weight
Epicillin			
0	0·4	33	0·012
	(0·1)	(4)	(0·001)
143	0·7	30	0·023
	(0·2)	(3)	(0·007)
286	0·8	30	0·027
	(0·3)	(3)	(0·008)
715	0·8	29	0·027
	(0·2)	(1)	(0·006)
1430	1·0	31	0·031
	(0·1)	(1)	(0·005)
Cephradine			
0	0·3	26	0·012
	(0)	(3)	(0·001)
114	0·3	27	0·012
	(0·1)	(1)	(0·001)
228	0·3	27	0·012
	(0·1)	(1)	(0·002)
570	0·3	25	0·012
	(0·1)	(2)	(0·003)
1140	0·4	25	0·015
	(0·1)	(2)	(0·004)
Ampicillin			
0	0·5	32	0·014
	(0·1)	(2)	(0·001)
114	0·7	29	0·020
	(0·2)	(2)	(0·005)
570	1·1	30	0·04
	(0·3)	(2)	(0·01)
1140	1·1	29	0·04
	(0·2)	(1)	(0·01)
2280	1·1	31	0·03
	(0·2)	(2)	(0·01)

In contrast, no difference in concentration of the contaminant in the faeces was found between the control group and all cephradine treated mice – not even in the mice treated with the highest dose of cephradine. The possibility that an exceptional poorly colonizing strain of *E. coli* was used for contamination was excluded by the two subsequent contaminations with other cephradine-resistant strains. It is also noteworthy that the contaminants were no longer detectable in the faeces a week after oral contamination in the animals treated with cephradine while the contaminant colonized the ampicillin and epicillin treated mice persistently. These results indicate that the CR was strongly decreased during ampicillin and epicillin treatment and not affected by cephradine. The mean and relative caecal weights (Table 4) confirm this conclusion, since these values appear

to be a good indicator of the CR in mice (Koopman, Janssen & van Druten, 1977). These workers found a linear reverse correlation between the CR and the relative caecal weight.

DISCUSSION

The results of the study indicate that oral treatment of mice with ampicillin or epicillin strongly decreases their Colonization Resistance even when extremely low doses of these antibiotics are applied. Cephradine, on the other hand, which is rapidly absorbed to the extent of approximately 98% after oral administration, has no effect at all on the CR. This conclusion concerning the CR was based on two different indices of the CR; (1) the log of the concentration of the contaminant in the faeces as well as the duration of its colonization (Van der Waaij & Berghuis, 1974*c*) and (2) the relative weight of the caecum (Koopman *et al.* 1977).

For comparison with the dosage of these drugs in man, Table 1 includes comparable doses in man calculated on a weight basis. Although direct transfer of the results to man is obviously not possible, the literature nevertheless provides many cases of bacterial overgrowth after systemic antibiotic treatment (Louria *et al.* 1962; Selden *et al.* 1971; Pollack *et al.* 1972; Hirsh *et al.* 1974; Holzman *et al.* 1974; Westwood *et al.* 1974) which justify a clinical study of this kind. The fact that both synthetic penicillin-derivatives ampicillin and epicillin are excreted in the bile may explain their influence on the CR. The anaerobic fraction of the microflora of mice is apparently very sensitive to both antibiotics. This largely excludes the *Bacteroides* species that possess little susceptibility to the ampicillin group from a significant role in the CR, and makes a contribution by certain *Clostridium* species more likely.

In previous experiments with nalidixic acid (Van der Waaij & Berghuis-de Vries, 1974*a*) and with the combination of trimethoprim and sulfamethoxazole (Van der Waaij, Cohen & Anver, 1974*b*) the results were of the 'cephradine type'. However, to provide a better comparison with the present experimental design, these experiments are being repeated. Further experiments in man as well as in animals with these and other antimicrobial agents are also in progress and will be reported soon.

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