

## Colonization resistance of the digestive tract of mice during systemic antibiotic treatment

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### SUMMARY

During systemic treatment of mice with ampicillin or streptomycin, oral contaminations with exogenous bacterial species resulted in an abnormal colonization pattern. The contaminants persisted much longer and in much higher concentrations in the caecum of systemically treated mice than in control animals. Spread of the contaminant into the mesenteric lymph nodes and the spleen was found much more often in the antibiotic treated group. This, however, was only seen when the contaminant was 'resistant' to the antibiotic injected. The experiments suggest that the 'CR-inducing species' of the microflora live in close contact with the mucosa and therefore could be identical with the anaerobic tapered rods described by Savage & Dubos (1968).

### INTRODUCTION

Previously, the occurrence of colonization after oral contamination with various doses of three different Gram-negative bacterial species in mice was investigated (Van der Waaij, De Vries & Lekkerkerk, 1971). Much higher oral contamination doses were required to achieve colonization for 2 weeks or longer in animals with a conventional microflora than in those whose microflora had been altered by prolonged oral antibiotic treatment. This phenomenon, called colonization resistance (CR), was found to depend largely on an anaerobic part of the intestinal microflora. The CR of the digestive tract was expressed as the log of that oral dose which resulted in a 'take' persisting for 2 weeks in 50% of the animals.

An increased concentration of the contaminant in the intestines was found during the first days after contamination (Van der Waaij, De Vries & Lekkerkerk, 1972). This occurred only when the contamination dose had been at or above the CR-determining dose. During this initial colonization phase (ICP) spread of the contaminant into the mesenteric lymph nodes was found in a certain percentage of the mice. In orally antibiotic-treated animals, the effects found during the ICP in conventional animals after high oral contamination doses, persisted throughout the observation period of 2 weeks.

In the present report, the influence of systemic antibiotic treatment on the CR and therefore on the pattern of the ICP following oral contamination, was investigated in mice. This effect was studied with an *Escherichia coli* strain and with a strain of enterococcus. Two broad spectrum antibiotics belonging to different

groups (penicillins and oligosaccharides) were selected for this study: Ampicillin was to some extent excreted with the bile into the intestines and streptomycin was largely excreted by the kidneys.

#### MATERIALS AND METHODS

##### *Mice*

Conventional random bred ND2 female mice of 9–12 weeks of age and 25–35 g. weight were used.

##### *Housing conditions*

The animals were caged separately in polycarbonate autoclavable cages. They were maintained on wire mesh above filter paper to minimize coprophagy. Acidified water (pH = 3) and sterilized food pellets were supplied *ad libitum*.

##### *Oral contamination*

Doses of  $10^8$  and  $10^{10}$  bacteria of the same streptomycin resistant (S.R.)-*E. coli* strain (CR = 7) or with the same levels of a SR-enterococcus strain (CR = 8), were administered in the manner described in a previous paper (Van der Waaij *et al.* 1971). The SR-*E. coli* strain was not only resistant to streptomycin but also to ampicillin. The SR-enterococci were sensitive to 10  $\mu\text{g./ml.}$  ampicillin.

##### *Collection of samples*

During the first 4 days after contamination in mice which received  $10^8$  organisms and at days 6, 8 and 10 in those which received  $10^{10}$  organisms, five mice were killed in each of the three experimental groups (see later). The mesenteric lymph nodes, the spleen and the caecum contents were collected under aseptic conditions and processed in the same way, as previously described (Van der Waaij *et al.* 1972).

##### *Antibiotic treatment*

Before antibiotic treatment, 90 mice were randomly distributed over three groups. The first group was treated twice a day with 5 mg of streptomycin intraperitoneally (i.p.), the second was injected twice daily with 10 mg of ampicillin i.p. and the third group was injected with saline. Antibiotic treatment was started 3 days before contamination. The experiment was performed 4 times.

#### RESULTS

In the present study, a strong negative influence of systemic treatment with streptomycin and to some extent also of systemic treatment with ampicillin was observed on the CR (Fig. 1). After marginal effective oral doses of  $10^8$  SR-*E. coli* cells and  $10^8$  SR-enterococci (just above the CR-determining dose), the control animals showed the same type of colonization in the ICP as was seen in previous experiments (Van der Waaij *et al.* 1972) in conventional mice. In the antibiotic-treated mice, however, the contaminant was recovered from the caecal contents in increased concentrations. This effect was stronger in the streptomycin-treated animals than in the ampicillin-treated mice. The effect was seen after contamination

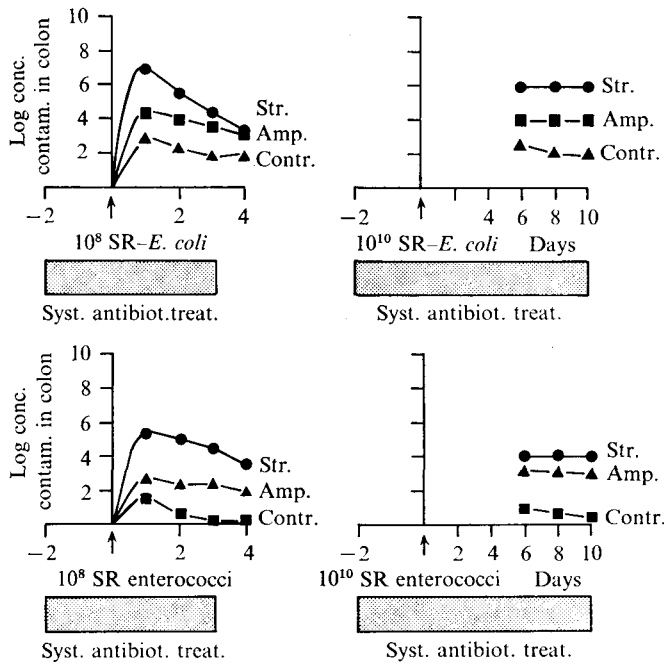


Fig. 1. Colonization pattern during systemic treatment with ampicillin and streptomycin.

Table 1. Spread of S.R.-E. coli into mesenteric lymph nodes\* during systemic antibiotic treatment

Treatment	Days after contamination				Mean	Days after contamination			Mean
	1	2	3	4		6	8	10	
Streptomycin	3	5	1	2	2.7	4	0	2	2
Ampicillin	6	4	4	3	4.2	6	4	5	5
Controls	0	0	0	0	0	1	0	0	0.3

\* Figures in the body indicate the number of positive cultures per 20 mice (total of four experiments with five animals per group).

with both *E. coli* and enterococci. Increased concentrations of the contaminants were not confined to the ICP, but appeared to persist thereafter since they were also observed at days 6, 8 and 10, after the higher dose of  $10^{10}$  bacteria (Fig. 1).

The spread of the contaminant into the mesenteric lymph nodes and spleen was also clearly influenced by systemic antibiotic treatment. The two contaminating organisms showed different effects (Tables 1 and 2). Although the CR was found to be decreased for both bacterial strains used for contamination in the ampicillin-treated animals as well as in the streptomycin-treated mice, all cultures of the mesenteric lymph nodes and the spleen remained sterile in the ampicillin-treated enterococci-contaminated animals (Table 2). In the *E. coli*-contaminated animals spread of ampicillin-resistant SR-*E. coli* was obviously not inhibited by ampicillin; on the contrary positive cultures were seen in more animals than in the strepto-

Table 2. *Spread of S.R.-enterococci into mesenteric lymph nodes\* during systemic antibiotic treatment*

Treatment	Days after contamination				Mean	Days after contamination			Mean
	1	2	3	4		6	8	10	
Streptomycin	2	6	2	2	3.0	1	1	1	1
Ampicillin	0	0	0	0	0	0	0	0	0
Controls	1	1	3	0	1.2	1	0	0	0.3

\* Figures in the body indicate the number of positive cultures per 20 mice (total of four experiments with five animals per group).

mycin-treated group following contamination with *E. coli* (Table 1). This is presumably due to the fact that the enterococci were sensitive to ampicillin and the *E. coli* strain was not. Spread into the lymphatic organs is usually confined to the first 4–6 days after contamination (Van der Waaij *et al.* 1972). This was also seen in the controls. In the antibiotic-treated mice, it evidently persisted longer since it was also found on days 8 and 10 after contamination.

#### DISCUSSION

The dubious effect that systemic antibiotic treatment can exert on the intestinal microflora has been described by various authors – predominantly clinicians. Changes have been reported in the microflora of the nose (Aly, Maibach, Strauss & Shinefield, 1970), the oropharynx (Louria & Kaminski, 1962) and the faecal flora (Altemeier, Culbertson & Hummel, 1970; Gaya, Admitt & Turner, 1970). In most cases the changes imply colonization with antibiotic-resistant bacterial strains for longer periods and, most often, in usually high concentrations (Louria & Kaminski, 1962). Colonization with *Salmonella typhimurium* persisted longer in patients treated with antibiotics than in an untreated group (Dixon, 1965; Aserkoff & Bennett, 1969).

Studies in human volunteers contaminated with *Pseudomonas aeruginosa* in various doses indicated that long-lasting colonization occurred only when ampicillin had been administered (Buck & Cooke, 1969; Stoodley & Thom, 1970).

Since we found a similar colonization pattern after experimental oral contamination with SR-*E. coli*, SR-*P. aeruginosa*, and SR-enterococci in conventional monkeys (Van der Waaij, De Vries & Lekkerkerk, 1970) and in conventional mice (Van der Waaij *et al.* 1971) it was decided to use the mouse as a model subject to study the influence of parenteral antibiotic treatment on the CR.

In the present study an obviously negative influence of high dose systemic antibiotic treatment on the CR was found. The concentration of both bacterial strains used for contamination recovered from the caecum was clearly increased during the ICP. Thereafter, a significant difference between antibiotic-treated and control animals was only found after the high dose of  $10^{10}$  cells.

Evidence of spread into the mesenteric lymph nodes was seen much more often in the antibiotic-treated animals than in the controls. Only the SR-enterococci

(which were sensitive to ampicillin) were not recovered from the mesenteric lymph nodes during ampicillin treatment. During treatment with streptomycin (to which the enterococci were resistant), positive mesenteric lymph nodes were seen more often than in the control groups. The *E. coli* strain used in this experiment, being resistant to both the antibiotics used, also invaded more often in the antibiotic-treated groups than in the controls.

The decrease of the CR, as reflected in intestinal concentration, was greater during streptomycin treatment than during ampicillin treatment.

Our observations indicate that, under the conditions described, systemic treatment with antibiotics such as ampicillin and streptomycin enhances the possibility of colonization by exogenous bacterial species. This seems not to be confined to strains resistant to the antibiotics injected such as were found during oral antibiotic treatment (Van der Waaij *et al.* 1972). Spread from the intestines into the lymphatic tissues, on the other hand, appears to be confined to strains which are insensitive to the antibiotic supplied systemically as was to be expected. Although ampicillin is known to be excreted with the bile, in our experiments it evidently has not reached high enough concentrations in the colon to influence growth of the 'sensitive' enterococci used for contamination. Nevertheless, the CR-inducing part of the intestinal microflora appeared to be negatively influenced by the antibiotics supplied. Streptomycin, which is presumably not excreted with the bile, had a stronger 'negative' effect on the CR than ampicillin. This may indicate that these antibiotics influence the 'CR-responsible micro-organisms' via another route; possibly directly through the intestinal mucosa. If this is the case, it could be concluded that the 'CR-inducing' anaerobes are among those species that are described as living in close contact with the mucosa (Savage & Dubos, 1968).

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