J. Hyg., Camb. (1984), **93**, 27–34
Printed in Great Britain

Binding to and antibacterial effect of ampicillin, neomycin and polymyxin B on human faeces

By M. P. HAZENBERG, A. M. PENNOCK-SCHRÖDER, M. VAN DEN BOOM and J. P. VAN DE MERWE

Department of Medical Microbiology, Erasmus University Rotterdam, P.O. Box 1738, 3000 *DR Rotterdam, The Netherlands*

(*Received* 4 *November* 1983; *accepted* 7 *March* 1984)

SUMMARY

Neomycin and polymyxin B, used during selective decontamination of the gastrointestinal tract, were studied for their effect on the human faecal flora *in vitro*. The selective effect was found to be associated with a relative insusceptibility of the obligate anaerobic flora as compared with the facultatively anaerobic Gram-negative rods (*Escherichia coli*). Both neomycin and polymyxin B were bound by human faeces, in contrast to ampicillin. The results may explain the selective effect of neomycin and polymyxin B on the human flora *in vivo*.

INTRODUCTION

Neomycin and polymyxin B are antibiotics used for selective decontamination (SD) of the gastrointestinal tract of patients with impaired host defences (Guiot, Van der Meer & Van Furth, 1981; De Vries-Hospers *et al.* 1981; Guiot *et al.* 1983). Obligate anaerobes, the major part of the intestinal flora, remain resident, whilst the Gram-negative facultatively anaerobic rods, endogenous or acquired during treatment, disappear during SD (Guiot *et al.* 1981; De Vries-Hospers *et al.* 1981; Guiot *et al.* 1983; Young, 1983). The aim of such treatment is to free the patient of life-threatening bacteria while the remaining flora contributes to colonization resistance (Van der Waaij, Berghuis-De Vries & Lekkerkerk-Van der Wees, 1971).

SD depends on the different susceptibilities of obligately anaerobes and facultatively anaerobes to antibiotics. Previous studies showed that the obligate anaerobic bacteria of the faecal flora of a healthy subject were moderately susceptible to antimicrobial agents used for SD, as compared with the Gram-negative facultatively anaerobic rods (Hazenberg *et al.* 1983*a*). Polymyxin B and neomycin were 90% bound to the solid fraction of human faeces. This finding extends the results of Gotoff & Lepper (1965) and of Wagman, Bailey & Weinstein (1974) on the binding of polymyxin E and aminoglycosides to faeces.

In the present study the susceptibility to ampicillin, neomycin and polymyxin B of obligately anaerobic bacteria and facultatively anaerobic Gram-negative rods from the faecal flora of eight subjects was studied. Binding of the antibiotics to faeces was also determined.

MATERIALS AND METHODS

Human faeces

Faecal samples from eight healthy laboratory workers were used.

Intestinal flora

Anaerobic culture. Faeces were cultured within 1 h after passage as described previously (Hazenberg, Bakker & Verschoor-Burggraaf, 1981) on non-selective medium (Schaedler broth, Oxoid, Basingstoke, U.K.) solidified with 2% agar.

Aerobic culture. Dilutions of faecal samples were plated on blood agar in triplicate and incubated for 24 h at 37 °C. Gram stains of all colony types were made; Gram-negative rods were subcultured and identified with the API-20 system for Enterobacteriaceae (API Benelux B.V., The Netherlands).

Antibiotics

The following antibiotics were used in the present study: ampicillin (Beecham, Heppignies, Belgium), neomycin (Pharmachemie B.V., Haarlem, The Netherlands) and polymyxin B (Pfizer N.V., Brussels, Belgium). Antibiotic solutions were sterilized by membrane filtration (disposable filters, pore size 0·2 μm, Schleicher and Schűll, Dassel, Federal Republic of Germany).

Minimal inhibitory concentration

Cultures of *Escherichia coli* were diluted and aliquotes containing 10^5 bacteria were incubated in Schaedler broth with antibiotic concentrations ranging from 0·125 to 128 mg/l in twofold dilution steps (in triplicate). The minimal inhibitory concentration (MIC) was defined as the lowest concentration of the antibiotic that inhibited growth during 24 h at 37 °C.

Inhibition of the human faecal flora in vitro *by antibiotics*

Inhibition of the obligate anaerobic flora by antibiotics was tested by comparing numbers of colonies on anaerobic media without antibiotics with those on media with increasing concentrations of antibiotics. The inhibitory effect was tested with ampicillin in concentrations ranging from 0·5 to 64 mg/l and with neomycin and polymyxin B in concentrations ranging from 8 to 1024 mg/l in twofold dilution steps. Colonies were counted after incubation of the samples for 48 h at 37 °C. Inhibition was expressed as a percentage according to the formula $100\% - (A/W \times 100\%)$ where A = the number of colonies on medium with antibiotic and W = the mean number of colonies on two media without antibiotic. Inhibition was only calculated if a minimum of 100 colonies of anaerobes was counted on each of the media without antibiotic.

Antibiotic assay

Concentrations of antibiotic in faecal supernatant (see below) were determined with the agar-plate diffusion technique (Bennet *et al.* 1966). Indicator organisms were *Staphylococcus aureus* (MIC value of ampicillin and neomycin respectively 0·25 and 0·5 mg/l) and *E. coli* (MIC value of polymyxin B 0·5 mg/l). Petri dishes (diameter 14 cm) with 25 or 50 ml DST agar (Oxoid) were covered with 10 ml of

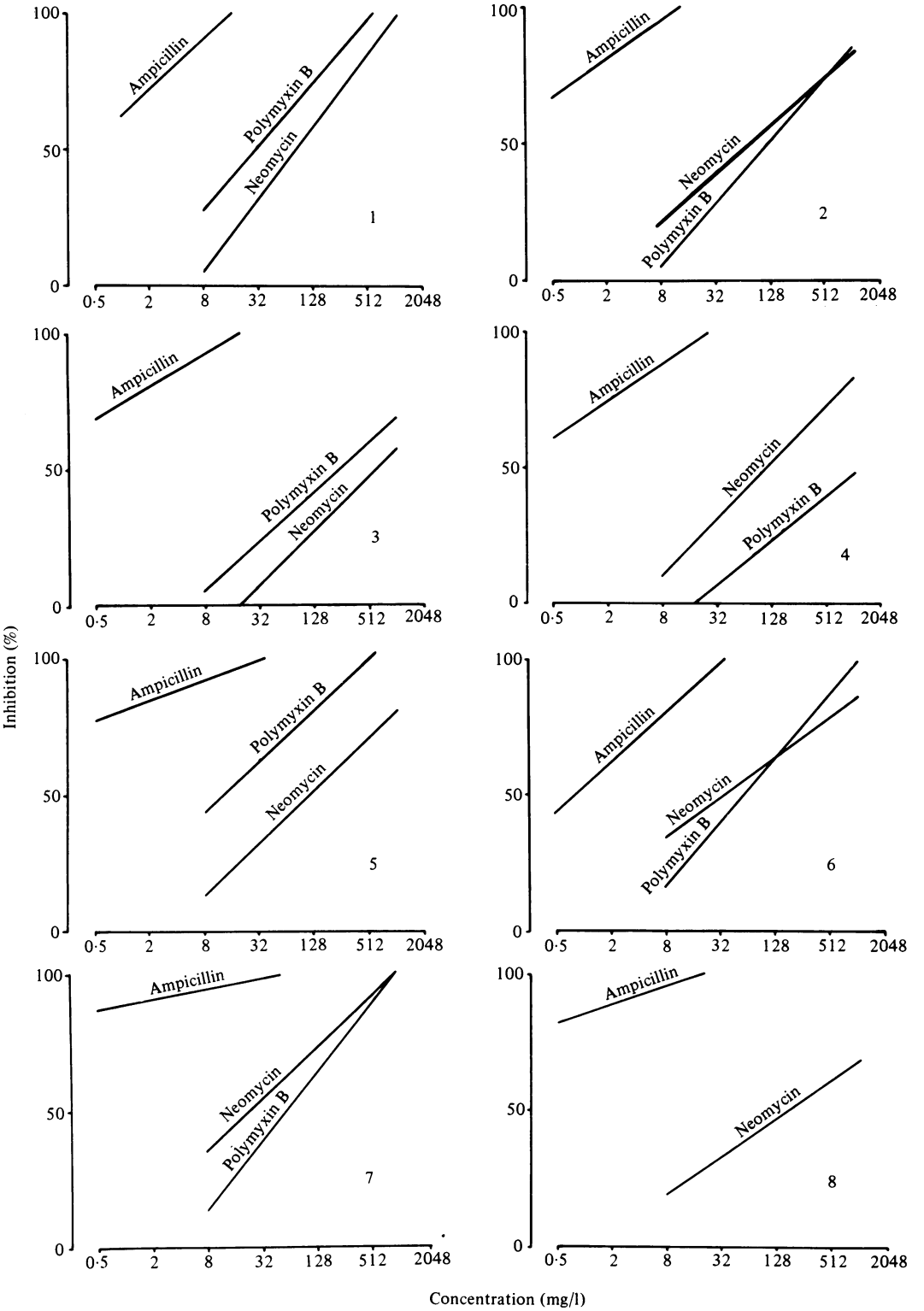

Fig. 1. Inhibitory effect of antibiotics in anaerobic medium (mg/l) on numbers of obligate anaerobes cultured from the human faecal flora of eight healthy subjects (1–8). Concentrations are given on a logarithmic scale. For each antibiotic the regression line was determined from at least six data, $r^2 > 0{\cdot}90$, $P < 0{\cdot}002$.

Table 1. *Minimal inhibitory concentrations (MIC) of antibiotics for* Escherichia coli *strains isolated from the faecal flora of eight healthy subjects*

		MIC* for *E. coli* strains†		
Subject	Antibiotic	1	2	3
1	Ampicillin	2	2	
	Neomycin	16	16	
	Polymyxin	0·5	0·5	
2	Ampicillin	16	—	—
	Neomycin	1	8	8
	Polymyxin	0·5	8	8
3	Ampicillin	8	4	
	Neomycin	32	16	
	Polymyxin	0·5	0·5	
4	Ampicillin	4		
	Neomycin	8		
	Polymyxin	1		
5	Ampicillin	2	8	
	Neomycin	8	8	
	Polymyxin	0·25	0·5	
6	Ampicillin	4	8	
	Neomycin	4	8	
	Polymyxin	0·5	0·25	
7	Ampicillin	2		
	Neomycin	4		
	Polymyxin	0·125		
8	Ampicillin	8		
	Neomycin	16		
	Polymyxin	—		

* mg/l.
† Strains were differentiated on colony form and haemolytic property.
— Indicates not done.

a diluted (*S. aureus*, 100×; *E. coli*, 1000×) overnight culture of the indicator organism. After some minutes the fluid was removed and the plates were dried. Three to six wells (diameter 6 mm) were filled with 50 μl of supernatant of premixed faeces (see below, binding of antibiotic to faeces) and six with various known concentrations of antibiotics dissolved in faecal supernatant. The supernatant of faeces was obtained by centrifugation (10000 ***g***) of faeces suspended in four volumes of water. Inhibition zones were measured after incubation for 16 h at 37 °C.

Binding of antibiotic to faeces

The differences between concentrations of antibiotics before and after incubation with faecal samples is referred to as the binding of antibiotic to faeces. For determination, one volume of the antibiotic dissolved in water was added to four volumes of 1:4 suspended faeces. After incubation for 1 h at room temperature the suspension was centrifuged (10000 ***g***) and the antibiotic concentration in the

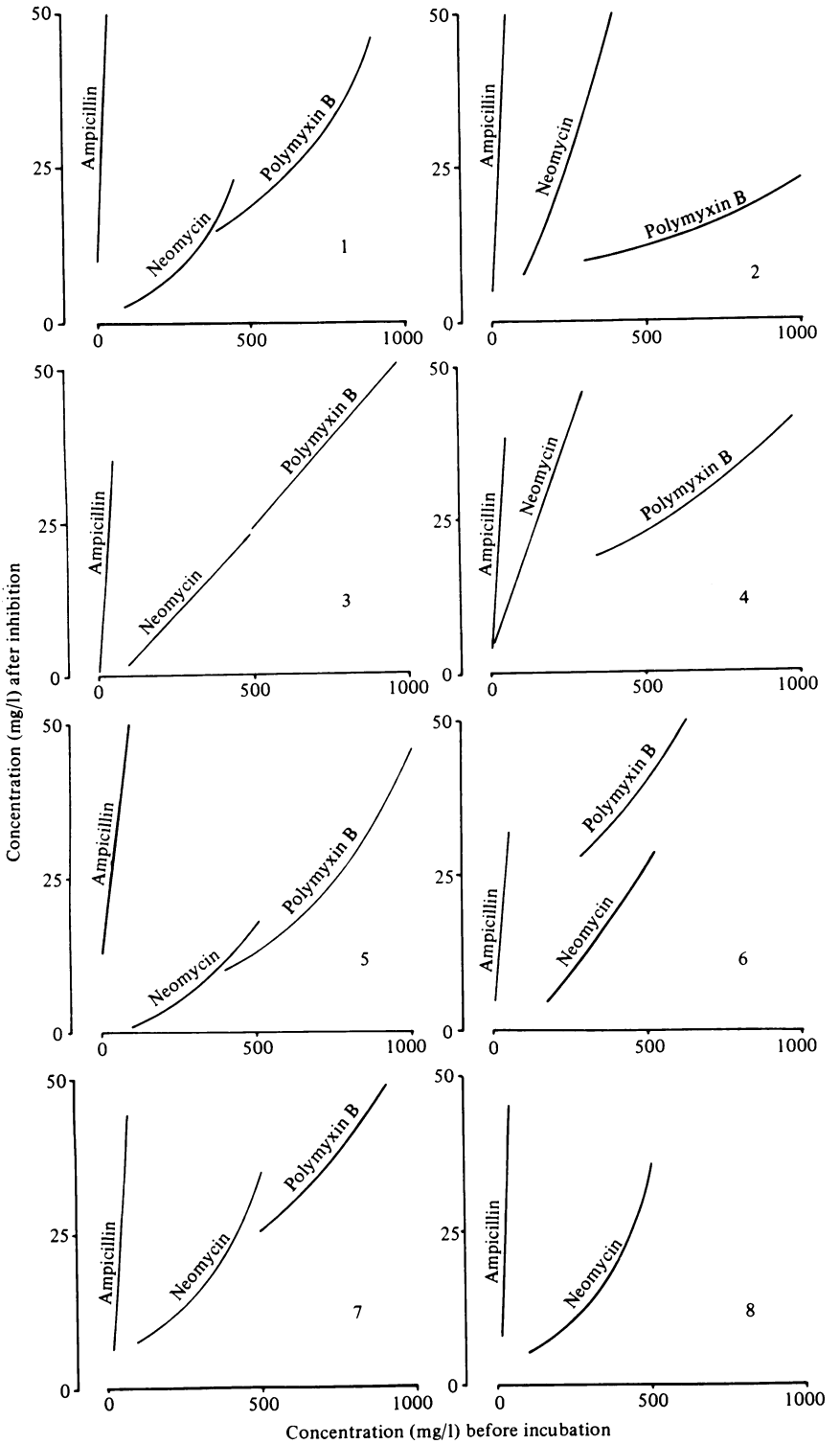

Fig. 2. Effect of incubation with diluted faeces of eight healthy subjects (1–8) on concentrations of antibiotics. For each antibiotic the regression line was determined from at least 6 data, $r^2 > 0{\cdot}90$, $P < 0{\cdot}002$.

supernatant determined. The antibiotics were not destroyed or inactivated by the supernatant fluid during 1 h at room temperature.

Statistical methods

Regression lines (least square method) and coefficients of determination (r^2) were calculated, either with the original data or with their logarithmic values. Probability values (P) were derived from two-tailed tests.

RESULTS

Effect of antibiotics on the faecal flora

The median total number of obligate anaerobes cultured per gram faeces ($\log_{10}$) was 10·35 (range 10·20–10·60). The inhibitory effect of antibiotics in the medium on the numbers of anaerobic bacteria in the flora is shown in Fig. 1. The anaerobic flora of all subjects was sensitive to ampicillin. Although small differences were found between individuals, 32 mg/l ampicillin always suppressed the anaerobic flora completely. The anaerobic flora was less sensitive to neomycin and polymyxin B and large differences were found between individuals.

The facultatively anaerobic Gram-negative rods isolated from the faecal flora were identified as strains of *E. coli*. Table 1 shows that the MIC for the strains varied for ampicillin (2–16 mg/l), neomycin (1–16 mg/l) and polymyxin B (0·125–8 mg/l).

Binding of antibiotics to faeces

Fig. 2 shows that ampicillin was not bound by faecal suspensions. In contrast, binding of polymyxin B to the solid part of diluted faeces was 90–98 % and for neomycin was 83–98 %.

DISCUSSION

The median number ($\log_{10}$) of anaerobes per gram faeces from healthy human subjects was 10·35. The yield can be considered sufficient when a detailed inventory is not intended (Wensinck *et al.* 1981). Wensinck *et al.* (1981) and Ruseler-Van Embden & Both-Patoir (1983) found that 100–150 colonies cultured from diluted faeces on a non-selective medium comprised the major anaerobic residents of the human intestinal flora such as *Eubacterium*, *Bifidobacterium*, *Peptostreptococcus*, *Bacteroides* and *Fusobacterium* species. Hazenberg, Bakker & Verschoor-Burggraaf (1981) showed that 150 colonies normalized germfree mice. Therefore, we assume that 100–150 colonies are representative for the anaerobic faecal flora.

The aim of SD is elimination of life-threatening facultatively anaerobic Gram-negative rods from the resident flora. Gram-positive facultative anaerobes may remain resident as they are considered as non-pathogenic; their MIC was not therefore determined. Despite the subculture of all colony forms of Gram-negative faculatively anaerobic rods, only strains of *E. coli* were isolated.

The obligately anaerobic human flora of all subjects was highly susceptible to ampicillin (50 % inhibition by 1 mg/l and 100 % inhibition by 32 mg/l) as compared with neomycin and polymyxin B. Ruseler-Van Embden & Both-Patoir (1983), using identical techniques, found that 15 % of colonies cultured from faeces of five

healthy subjects belonged to the *Bacteroides fragilis* group. Appelbaum & Chatterton (1978) studied 265 clinical isolates of anaerobic strains and concluded that the *B. fragilis* group was more resistant to ampicillin than any other taxon (16 mg/l: 60 % inhibition and 32 mg/l: 80 % inhibition). These studies indicate that 32 mg/l ampicillin can be expected to suppress the faecal flora by 90 % or more.

The results show that the obligate anaerobes and *E. coli* of all subjects were inhibited by low concentrations of ampicillin. On the other hand, a choice can be made between neomycin and polymyxin B for individual SD on the basis of the combination of low MIC for *E. coli* and low inhibition of the obligate anaerobes. These results extend previous findings using one faecal donor (Hazenberg *et al.* 1983*a*).

The present study shows that faeces of all donors bound polymyxin and neomycin but large individual differences were found. This binding explains the low recovery of neomycin (100–500 μg/g faeces) and polymyxin B (5–10 μg/g) in the EORTC study (1982) after daily oral dose of 0·5–1·0 g. On the basis of a daily production of 150 g faeces (Stephen & Cummings, 1980) more than 3000 μg/g would be expected.

Our results on binding of neomycin to faeces were compared with those of Wagman *et al.* (1974) who studied binding to canine faeces. The binding of neomycin, 5 mg/g faeces (wet weight), is in agreement with our results but the free (biologically active) concentration was higher (25 %) in their investigation than in our studies (10 %, median value in eight subjects). Hendriks (1980) showed that a considerable amount of orally administered neomycin was inactivated by faeces of monkeys. Hazenberg *et al.* (1983*b*) reported over 90 % binding of neomycin and polymyxin B by faeces of rats. From the results of Van der Waaij, Berghuis-De Vries & Korthals Altes (1974), it is concluded that a considerable part of neomycin was inactivated by faeces of patients and mice. Gotoff & Lepper (1965) found that 50 % of the activity of polymyxin E was lost when 1 mg was mixed with 1 g faeces. Our study shows that 1 g faeces binds a minimum of 90 % of 1 mg polymyxin B.

Wagman *et al.* (1974) reported that the binding to faeces is reversible. Further studies are required to clarify the relevance of this phenomenon for selective decontamination with neomycin and polymyxin B.

We are grateful to M. Goense and L. Lauwerijssen for the excellent technical assistance.

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