EFFECTS OF CEPHALEXIN, ERYTHROMYCIN AND CLINDAMYCIN ON THE AEROBIC GRAM-NEGATIVE FAECAL FLORA IN MAN

CHRISTINE L. HARTLEY, HELEN M. CLEMENTS AND K. B. LINTON
Department of Bacteriology, The Medical School, University of Bristol, Bristol BS8 1TD

PLATE VII

AEROBIC, Gram-negative bacilli comprise about 1% of the cultivable flora of the human faeces. *Escherichia coli* predominates with counts of $10^5$–$10^9$ organisms per gram of faeces. Other coliforms are generally present in very low numbers. Antibiotics may change the intestinal flora by direct action on the organisms or by changing any factor that affects the stability of the bacterial population. The effects on the bacteria in the colon of antibiotics that are active against the same organisms *in vitro* depend upon the dose given, the route of administration, the mode of action of the antibiotic and its degree of absorption from the upper gastrointestinal tract. Oral administration of antibiotics that are poorly absorbed in the upper gastrointestinal tract generally has the greatest effect on the faecal flora. Parenteral administration of antibiotics, however, may also alter the faecal flora because of biliary excretion. An antibiotic that is active against some of the intestinal aerobic organisms and is excreted at sufficiently high concentrations in the faeces may suppress or eliminate those organisms, or may exert a selective pressure in favour of the emergence of an antibiotic-resistant population.

Cephalexin is an antibiotic of the cephalosporin group that is well absorbed after oral administration (Gower and Dash, 1969). It is active against many Gram-positive cocci and enteric organisms, including some strains of *E. coli*, salmonellae and shigellae (Wick, 1967). Cephalexin is bactericidal at or near its minimal inhibitory concentration. Gower and Dash (1969) suggested that because absorption of cephalexin in healthy individuals approaches 100%, the antibiotic would have little or no effect on the intestinal flora. Gaya, Adnitt and Turner (1970), however, found that in hospital a significant number of patients acquired either *Pseudomonas* spp. or *Proteus* spp. in their stools.

Erythromycin is a macrolide antibiotic active against Gram-positive organisms. It is bacteriostatic in low concentrations and bactericidal in high concentrations. Excretion is mainly in the bile. Suppression of faecal *E. coli* by therapy with erythromycin has been reported, although *E. coli* is generally assumed to be resistant to erythromycin (Barker and Prescott, 1973).

Clindamycin is a semi-synthetic derivative of lincomycin and is particularly
active against Gram-positive cocci, certain *Clostridium* spp. and *Bacteroides* spp. (McGehee et al., 1968). Clindamycin is bactericidal and is rapidly absorbed when given by mouth; only c. 3% is excreted in the faeces. A common side-effect of clindamycin therapy is loose stools, but occasionally acute pseudomembranous colitis may follow therapy (Tedesco, Barton and Alpers, 1974; Viteri, Howard and Dyck, 1974). Finegold, Harada and Miller (1965) reported that lincomycin, which has the same spectrum of activity as clindamycin (McGehee et al., 1968), eliminated the anaerobic faecal flora but did not affect the aerobes.

In the present study the effects of cephalixin, erythromycin and clindamycin on the aerobic component of the human faecal flora were investigated, with particular reference to the emergence of resistant organisms and to any increase in the proportion of Gram-negative bacilli other than *E. coli* in the faecal flora.

**Materials and methods**

**Studies on faeces**

*Processing of faecal specimens.* Three grams of freshly-voided faeces were collected in a plastic container, refrigerated at 4°C, and examined within 4 h. Portions of c. 1 g were emulsified in physiological saline to give a 10% (w/v) suspension. Viable counts of coliforms (lactose-fermenting, bile-tolerant, Gram-negative bacilli) and non-lactose fermenting Gram-negative bacilli were determined by an adaptation of the method of Miles, Misra and Irwin (1938). After overnight incubation at 37°C, colony counts were made of the dilutions that gave rise to 20–50 colonies per drop on MacConkey Agar (Oxoid). Lactose-fermenting organisms of different colonial types and non-lactose fermenting organisms were estimated separately. For each faecal sample, 10 coliform colonies were picked at random from the counted colonies. Representative coliforms of different colonial types and non-lactose fermenting colonies were also picked. Each colony was seeded into nutrient broth and incubated at 37°C for 2 h.

*Identification of strains.* Lactose-fermenting organisms that produced indole in peptone water and acid and gas in MacConkey broth after overnight incubation at 44°C in a waterbath were considered to be *E. coli* faecal type 1 strains (Coli-Aerogenes Sub-Committee of the Society for Applied Bacteriology, 1956; Department of Health and Social Security, 1969). Lactose-fermenting organisms that were negative in either of these tests and non-lactose fermenting organisms were identified by the scheme of Cowan and Steel (1974).

*Determination of minimal inhibitory concentration (MIC).* MICs were determined on lyzed-blood agar plates containing a range of concentrations of the antibiotic under test. “Single-colony” inhibitory concentrations were determined by a multiple inoculation technique; 0·001 ml of 12 bacterial cell suspensions were applied to each plate and the plates were read after overnight incubation at 37°C. Three suspensions of different bacterial-cell densities were used for each test organism. The suspension that gave single colonies on a control plate without antibiotic was used in reading subsequent plates. The MIC was the lowest concentration that inhibited growth.

*Detection of cephalixin in faeces.* The assay was carried out by the agar-gel diffusion method described by Sukchotiratanat, Linton and Fletcher (1975). The sample of faeces was mixed with phosphate buffer to give a 10¹ (w/v) dilution and homogenised. The samples were stored at -20°C until tested. Standard solutions of cephalixin, with concentrations of 4–0·25 µg per ml were prepared in 0·2M phosphate buffer.

The method included a test to determine whether the zones of inhibition were produced by cephalixin or by non-specific inhibitory substances in the faeces. The enzyme used in this test was a crude preparation of type 1 β-lactamase derived from an *E. coli* strain (no. AD10/12/2) that possessed a strong chromosome-mediated resistance to cephalixin. One ml
of the enzyme preparation was added to 1 ml of each faecal suspension and to a range of cephalaxin standards; the mixtures were assayed after incubation at 37°C for 1 h.

Detection of erythromycin in faeces. The method of assay used for erythromycin was similar to that for cephalaxin. The assay organism was *Sarcina lutea* and the temperature of incubation 30°C. The standard series of antibiotic dilutions and the faecal homogenates were prepared in phosphate buffered saline. The erythromycin standard solutions contained 30-0.125 µg per ml.

**Subjects and procedures**

**Controls.** Thirteen healthy individuals who had not taken antibiotics for at least 1 year were examined. No antibacterial agents were taken by the control subjects during the course of study. The duration of the investigation was 34-66 days. Faecal samples were obtained at least twice a week.

**Cephalexin-treated group.** Cephalexin was administered as a 500-mg loading dose followed by 250 mg every 6 h for 4 days. The subjects (A1–A6) were six healthy males aged 20–25 years who had not taken antibiotics for at least 12 months. Faecal samples were collected for 2 weeks before therapy, during therapy, and for 3 weeks after therapy. Dilutions of faeces were seeded on to MacConkey agar plates with and without cephalexin 25 µg per ml. Twenty-five faecal samples were assayed for the presence of cephalexin. The MICs of cephalexin for *E. coli* strains present before, during and after therapy were determined over the range of antibiotic concentrations 0.5–500 µg per ml.

**Erythromycin-treated group.** Erythromycin stearate was administered as a 500-mg loading dose followed by 250 mg every 6 h for 4 days. The subjects (E1–E5) were five healthy adults (three males and two females) aged 20–40 years, who had not taken antibiotics for at least 12 months. Faecal samples were collected for 2 weeks before therapy, during therapy and at regular intervals for 5 weeks after therapy. Dilutions of faeces were seeded on to MacConkey agar plates with and without erythromycin 50 µg per ml. Twenty faecal samples were assayed for the presence of erythromycin. The MICs of erythromycin for the

---

![Graph](image-url)

**FIG. 1.**—The variation in the count of Gram-negative bacilli from the faeces of a typical subject in each of the four treatment groups. (a) Control group, subject 4. (b) Cephalexin group, subject A5. (c) Erythromycin group, subject E2. (d) Clindamycin group, subject C1. ●—● = *E. coli*; ○—○ = other Gram-negative bacilli; ▲▲▲ = period of antibiotic therapy.
E. coli strains from each subject isolated before, during and after therapy were determined over the range of concentrations 2·5–200 μg per ml.

Clindamycin-treated group. Clindamycin hydrochloride was administered as a 300-mg loading dose followed by 150 mg every 6 h for 4 days. The subjects (C1–C6) were six healthy males aged 20–25 years who had taken no antibiotics for at least 12 months. Faecal samples were collected before, during and for 4–5 weeks after therapy.

RESULTS

Fig. 1 shows the variation in the count of Gram-negative bacilli from a typical individual in each of the four treatment groups. Fig. 2 summarises the results of the various treatments on the total faecal coliform counts.

Controls

The total aerobic coliform count for the 13 control subjects was normally $10^4$–$10^9$ organisms per g of faeces. None of the samples from the controls gave...
HARTLEY, CLEMENTS AND LINTON

PLATE VII

ANTIBIOTICS AND THE HUMAN FAECAL FLORA

Fig. 3.—Specificity of the cephalexin assay. A = Cephalexin standard (0.5 μg per ml); B = dilution of faeces from a subject taking cephalexin; C = dilution of faeces as for B, but treated with type 1 β-lactamase.

Fig. 5.—Excretion of erythromycin in faeces. A, B, C = Erythromycin standards (10, 5 and 2.5 μg per ml respectively); D = faecal dilution from subject E1 during erythromycin therapy; E, F = faecal dilution from subject E1, 3 days and 1 week, respectively, after the end of therapy.

[facing page 128.]
a count of $>10^9$ organisms per g and only one sample gave a count of $<10^4$ organisms per g. The predominant faecal coliform was *E. coli*, which formed 90% or more of the aerobic flora in all subjects.

**Cephalexin treatment**

*Viable counts.* The total coliform count was unaffected by the short course of cephalexin therapy. Only one sample out of 105 gave a count of $>10^9$ organisms per g and one gave a count of $<10^4$ organisms per g. However, during cephalexin therapy Gram-negative bacilli other than *E. coli* emerged to form a much greater proportion of the count than was observed in these subjects at other times and in the control subjects.

*Faecal excretion of cephalexin.* The faecal excretion of cephalexin with the antibiotic regimen employed in this study is shown in table I. Cephalexin was not found in any sample from subjects A1–A6 either before therapy or more than 5 days after the end of therapy. No cephalexin was found in the 20 samples tested from six controls. All samples taken from 2 days after the commencement of cephalexin therapy until 1 day after the end of therapy contained cephalexin. In subjects A5 and A6 cephalexin excretion was demonstrated 4 days after the end of therapy. The concentration in all positive specimens was 3–6 µg per g faeces. Fig. 3 shows some typical assays that demonstrate the specificity of the cephalexin assay. The zone of inhibition of *Bacillus caldolactis* was due specifically to cephalexin because this zone was not produced by samples treated with the specific type 1 β-lactamase before assay. The addition of known concentrations of cephalexin to faecal suspensions showed binding of cephalexin to faecal protein. Therefore, the amount of cephalexin excreted in faeces is probably greater than that detected in this assay.

*Emergence of resistant organisms.* The total counts of cephalexin-resistant Gram-negative bacilli are shown in fig. 4. Only in subject A2 were these cephalexin-resistant *E. coli*. In subjects A1 and A6 the organisms belonged to

### Table I

*The excretion of cephalexin in faeces*

<table>
<thead>
<tr>
<th>Subject</th>
<th>1 day before therapy</th>
<th>at the stated intervals (days) during therapy</th>
<th>at the stated intervals (days) after therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 2 4</td>
<td>1 4 5 7</td>
<td></td>
</tr>
<tr>
<td>A1</td>
<td>0 3 3</td>
<td>3 0 0 0</td>
<td>0</td>
</tr>
<tr>
<td>A2</td>
<td>0 3 3</td>
<td>4 0 0 0</td>
<td>0</td>
</tr>
<tr>
<td>A3</td>
<td>0 4 4</td>
<td>3 0 0 0</td>
<td>0</td>
</tr>
<tr>
<td>A4</td>
<td>0 6 3</td>
<td>3 0 0 0</td>
<td>0</td>
</tr>
<tr>
<td>A5</td>
<td>0 3 3</td>
<td>3 3 0 0</td>
<td>0</td>
</tr>
<tr>
<td>A6</td>
<td>0 3 4</td>
<td>4 3 0 0</td>
<td>0</td>
</tr>
<tr>
<td>Six controls</td>
<td>0 0 0</td>
<td>0 0 0 0</td>
<td>0</td>
</tr>
</tbody>
</table>
the genus Enterobacter, in subject A4 to the genus Citrobacter, in subject A5 to both genera and in subject A3 to the genus Pseudomonas. In subjects A1 and A3 the proportion of resistant organisms never exceeded 0.2% of the total aerobic flora. In subjects A4, A5 and A6 the proportion was between 5% and 25% for a very short time during therapy. The resistant E. coli in subject A2 reached 100% of the total aerobic flora during therapy and persisted at lower levels for at least 10 days after the end of therapy. MIC determinations on 20 E. coli strains isolated from the six subjects before, during and after therapy showed a MIC of 4 μg per ml except in one instance. The resistant E. coli isolated from subject A2 had a MIC of 128 μg per ml. The organisms of the genera Enterobacter, Pseudomonas and Citrobacter isolated from subjects A1, A3, A5 and A6 had MICs of 128 μg per ml.

Erythromycin treatment

Viable count. Erythromycin therapy caused a rapid fall in the total count in all five subjects and E. coli was not detected in samples from subjects E1, E2, E3 and E5 at the end of therapy. The number of faecal samples that gave a coliform count of <10⁴ organisms per g in the erythromycin series and the
ANTIBIOTICS AND THE HUMAN FAECAL FLORA

control series was subjected to statistical analysis to determine the probability that the observed decrease in counts in the erythromycin series represented a normal fluctuation. A standard chi² test for this gave \( P < 0.01 \).

*Faecal excretion of erythromycin.* The approximate faecal excretion of erythromycin with the antibiotic regimen employed in this study is shown in table II. Erythromycin was not found in any faecal sample from the five subjects either before therapy or more than 6 days after the end of therapy, or in any of 20 samples from six control subjects. From 3 days after the

| TABLE II  
The excretion of erythromycin in faeces  

<table>
<thead>
<tr>
<th>Subject</th>
<th>Faecal concentration of erythromycin (µg per g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 days before therapy</td>
</tr>
<tr>
<td></td>
<td>2 4 1 4 5 7</td>
</tr>
<tr>
<td>E1</td>
<td>0 18 760 100 33 50 0</td>
</tr>
<tr>
<td>E2</td>
<td>0 0 600 50 0 0 0</td>
</tr>
<tr>
<td>E3</td>
<td>0 20 60 110 3 0 0</td>
</tr>
<tr>
<td>E4</td>
<td>0 80 200 30 100 2 0</td>
</tr>
<tr>
<td>E5</td>
<td>0 50 100 100 40 3 0</td>
</tr>
<tr>
<td>Six controls</td>
<td>0 0 0 0 0 0</td>
</tr>
</tbody>
</table>

beginning of therapy until 1 day after the end of therapy all samples taken contained erythromycin (fig. 5).

*Emergence of resistant organisms.* No growth of erythromycin-resistant Gram-negative bacilli occurred in any of the five subjects during the period of study. In samples taken at the end of the course of erythromycin and for the 5 succeeding days the count of faecal streptococci was very high, i.e., \( > 10^7 \) organisms per g of faeces. MIC determinations on 40 representative *E. coli* strains isolated before, during and after erythromycin therapy in the five subjects showed MICs of 10–30 µg per ml for all strains, except one from subject E5 that had a MIC of 40 µg per ml. Streptococci isolated from the faeces of all five individuals had MICs of \( > 200 \) µg per ml.

*Clindamycin treatment*

*Viable count.* Of the 82 samples examined from six individuals, 34% had a total count of \( > 10^9 \) organisms per g. Statistical analysis to test whether the increased coliform counts could result from normal fluctuations gave \( P = <0.01 \). In five of the six subjects the count of klebsiellae rose during therapy (fig. 6). In four of these five subjects the klebsiellae never formed \( > 40\% \) of the total count; however, in subject C3 klebsiellae formed 100% of the total count in a single sample.
FIG. 6.—The faecal counts of *Klebsiella* spp. in six subjects (C1–C6) treated with clindamycin. No *klebsiellae* were found in subject C5. ➤ = Period of antibiotic therapy.

**DISCUSSION**

The effect of the oral administration of certain antibiotics—especially those used in the treatment of urinary tract infection and against enteric pathogens—on the human faecal flora has been extensively investigated (Sompolinsky, Yaron and Alkan, 1967; Datta *et al.*, 1971; Grüneberg, Smellie and Leakey, 1973; Hirsh, Burton and Blenden, 1973). The emergence of coliforms carrying plasmids that mediate resistance to several antibiotics has been of special concern. However, other antibiotics that apparently do not bring about the selection of R-factor-bearing organisms may have profound effects on the intestinal flora. The human intestine provides the main reservoir of aerobic Gram-negative bacilli, organisms that have increased considerably in importance over the past 10 years as agents of nosocomial infection. Any factors, therefore, that affect the carriage and excretion of these organisms merit further investigation. Moreover, disturbances of the commensal intestinal flora as a consequence of antibiotic therapy may predispose a patient to infection by enteropathogens. Changes in the intestinal flora may also be implicated in the gastrointestinal side-effects of the administration of many chemotherapeutic agents. In the present study the effects of 5-day courses of oral cephalixin, erythromycin and clindamycin on faecal coliform excretion were examined.

The concentration of cephalixin excreted in the faeces appeared to reach
levels sufficiently high to influence the aerobic intestinal flora, contrary to the expectation of Gower and Dash (1969). The cephalexin concentration in the faeces of the subjects (3-6 μg per g) exceeded the MIC for the majority of intestinal strains of *E. coli*. However, no fall in the total *E. coli* count was observed. The duration of therapy was only 5 days and detectable changes in the *E. coli* flora might occur with a more prolonged course. Gaya *et al.* (1970) found that seven out of 12 patients treated with cephalexin “acquired” *Pseudomonas aeruginosa* in their stools, but in the present study a *Pseudomonas* sp. was detected in only one of six subjects and the organism formed <0.2% of the total count of Gram-negative bacilli. In four of the six subjects an *Enterobacter* sp. or *Citrobacter* sp., or both, formed a high proportion of the total coliform flora and in one subject a cephalexin-resistant *E. coli* strain became predominant. However, Gaya *et al.* studied a hospitalised population known to be regularly ingesting *Ps. aeruginosa* in food; it seems likely that small numbers of these organisms in the gut of treated individuals would be influenced by selection pressure during therapy. Differences in the effects of antibiotic treatment on individuals in the home and in hospital have been found by other workers (Sompolinsky *et al.*, 1967; Rose and Schreir, 1968; Winberg *et al.*, 1973).

The most obvious effect of even a very short course of erythromycin was the severe reduction of the aerobic coliform flora, an effect similar to that found with co-trimoxazole (Brumfitt and Pursell, 1972; Speller and Bruten, 1972). The faecal concentration of c. 400 μg per g was substantially greater than the MIC of 10–40 μg per ml for the normal intestinal *E. coli* population.

A short course of clindamycin was sufficient to bring about a significant increase in the total coliform count. All six subjects showed counts of >10⁹ organisms per g of faeces at the end of therapy and for a short time afterwards; klebsiellae formed a much higher proportion of the total coliform count than is normal although the count of *E. coli* also increased. None of the samples from the control subjects yielded total counts of >10⁹ organisms per g and klebsiellae formed <0.5% of the total. McGehee *et al.* (1968) reported that *Bacteroides* spp. are sensitive to clindamycin, and Finegold *et al.* (1965) observed that lincomycin eliminated the anaerobic intestinal flora. The removal of the clindamycin-sensitive anaerobic component of the intestinal flora may allow those aerobic organisms that possess intrinsic resistance to high concentrations of clindamycin to proliferate.

Thus, the antibiotics investigated exerted different effects on the Gram-negative faecal flora. Erythromycin considerably reduced the count of intestinal coliforms, but clindamycin caused an increase. Both cephalexin and clindamycin resulted in increased excretion of coliforms other than *E. coli*. The normal predominance of *E. coli* was very much reduced and other Gram-negative bacilli increased to abnormal levels.

**Summary**

The effects of 5-day courses of orally administered cephalexin, clindamycin and erythromycin on the Gram-negative, aerobic faecal flora of healthy adults
were examined. The concentration of cephalaxin reached in the intestine was high enough to cause the emergence of resistant Gram-negative bacteria; organisms belonging to the genera Enterobacter, Citrobacter and Pseudomonas increased to easily detectable levels. The faecal concentration of erythromycin was high and caused a severe reduction of the coliform flora. Clindamycin administration resulted in a considerable increase in the coliform count; the increase in the proportion of klebsiellae was especially marked.

This work was supported by the Medical Research Council (MRC Grant no. G970/107).

REFERENCES


