**Lactobacillus acidophilus, Bifidobacterium lactis and Lactobacillus F19 prevent antibiotic-associated ecological disturbances of Bacteroides fragilis in the intestine**

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**Objective:** The objective of this study was to compare the effect of clindamycin on the intestinal microflora in subjects ingesting yogurt with added probiotic microorganisms with the microflora in subjects ingesting placebo yogurt.

**Materials and methods:** Twenty-four healthy subjects were included in the study. All subjects received 150 mg clindamycin four times daily for 7 days and 250 mL yogurt twice daily for 14 days. Faecal samples were collected before, during and after administration of clindamycin.

**Results:** In the aerobic intestinal microflora, the numbers of enterococci increased after treatment in both groups, whereas other Gram-positive microorganisms decreased. In both groups, the numbers of *Escherichia coli* also decreased, whereas there was a concomitant increase in numbers of other Gram-negative bacilli. In the anaerobic microflora in subjects receiving yogurt with added microorganisms, the numbers of lactobacilli and bacteroides remained at the same levels throughout the study, whereas the numbers decreased in the placebo group. Other anaerobic bacteria decreased in both groups. The minimum inhibitory concentration of clindamycin against strains of bacteroides increased in both groups during the study.

**Conclusions:** The probiotic microorganisms evaluated in this study prevented ecological disturbances in the numbers of intestinal *Bacteroides fragilis* group species during clindamycin administration.

Keywords: probiotics, lactobacilli, clindamycin

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**Introduction**

Administration of antimicrobial agents is known to cause disruptions in the ecological balance of the normal microflora.¹ Emergence of antibacterial resistance frequently originates from the dense intestinal microbial population which predispose to the opportunities of genetic interchange through mobile genetic elements. The intestines are also an important source of eventual and potential pathogens.

Clindamycin is a lincosamide antibacterial agent that is excreted in bile, leading to high faecal concentrations, and ecological disturbances are seen mainly as a reduction in the numbers of intestinal anaerobic microorganisms.¹

The use of beneficial microorganisms, or probiotics, for prevention and treatment of gastrointestinal disturbances has been advocated.²³ Lactic acid-producing microorganisms like *Lactobacillus* and *Bifidobacterium* have most commonly been used.

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Administration of antimicrobial agent, probiotics and placebo

All volunteers received 150 mg clindamycin capsules (Dalacin, Pharmacia, Stockholm, Sweden) four times daily for 7 days. The subjects were randomized into two groups, the active and the placebo group with reference to the probiotic product. Twelve subjects received a yogurt product (250 mL) twice daily for 14 days containing $10^9$ cfu/mL of the strains *L. acidophilus* NCFB 1748, *B. lactis* Bb12 and *Lactobacillus paracasei* subsp. *paracasei* F19 (Arla Foods, Stockholm, Sweden). Individuals in the placebo group received a similar product but with no added microorganisms (Arla Foods, Stockholm, Sweden). The administration of the antimicrobial agent and the yogurts commenced on the same day.

Compliance

Signed diary cards that were returned at the end of the study checked the compliance. Detection of the probiotic microorganisms by culture with confirmation of suspect colonies by PCR and determinations of faecal concentrations of clindamycin were further verifications of compliance.

Sampling procedures

Stool specimens were taken before the administration of clindamycin and the yogurt products (days –3 and 0), during the clindamycin administration (days 2, 5 and 7) and after the administration (days 10, 14 and 21).

Assays of clindamycin concentrations

The concentration of clindamycin in faeces was determined by the agar diffusion method. The test medium was Antibiotic Medium No 1 (Difco, Detroit, MI, USA), with pH adjusted to 8.0, and the indicator strain was *Micrococcus luteus* ATCC 9341. The faecal samples were diluted 1:3 in phosphate buffer and centrifuged at 5000 rpm for 15 min. The samples were run in duplicate and on each plate a concomitant standard series (range 0.5–32.0 mg/L) was inoculated. The plates were incubated for 18 h at 37°C. Clindamycin concentrations were determined in relation to the diameters of the inhibition zones caused by the known concentrations from the standard series.

Microbiological procedures

The microbiological procedures were carried out according to Edlund et al. Different colony types were counted, isolated in pure cultures and identified to genus level according to Gram-staining, colony morphology and biochemical tests. Anaerobic bacteria were identified to genus level by gas–liquid chromatography of metabolites from glucose. The lower limit of detection was $10^7$ cfu/g faeces.

Assays of Clostridium difficile cytotoxin production

Isolated strains of *C. difficile* were tested for cytotoxin production on McCoy cells and an antitoxin kit for detection of toxin B in clinical specimens (TechLab, Blacksburg, VA, USA). *C. difficile* CCUG 19126 was used as the reference strain.

Identification of probiotic strains

Colony-forming units exhibiting colony and Gram-stain morphology similar to the probiotic strains were analysed with randomly amplified polymorphic DNA (RAPD)-PCR to verify the identification.

Antibiotic susceptibility tests

The clindamycin minimum inhibitory concentrations (MICs) for strains of *Bacteroides* spp. and *C. difficile* were determined by the agar dilution method according to NCCLS document M11-A5 for anaerobic microorganisms. Control strains were *Bacteroides fragilis* ATCC 25285 and *Bacteroides thetaiotaomicron* ATCC 29741.

Statistical analysis

The quantitative alterations were compared within groups between pre-treatment (mean of days –3 and 0) and during treatment (day 7) and between pre-treatment and after treatment (day 21) and were analysed by Wilcoxon’s signed rank test for paired samples. The MIC values for each species were compared within groups between pre-treatment and day 7 and between pre-treatment and day 21 by the Mann–Whitney U-test in order to detect significant decreases in susceptibility during and after the administration period. Differences between the groups in MIC values were likewise analysed by the Mann–Whitney U-test. $P$ values ≤ 0.05 were considered statistically significant and were adjusted for the multiple analysis.

Results

Concentrations of clindamycin in faeces

The mean concentration of clindamycin in faeces (mg/kg), standard deviation and range were: on day 2, 128.0, 79.8, 17.1–324.5; on day 5, 131.0, 87.2, 29.4–356.4; on day 7, 121.6, 95.0, 13.1–452.4; on day 10, 35.1, 59.7, 0–213.3; and on day 14, 0.7, 2.8, 0–13.8, respectively.

Impact of yogurt products on intestinal aerobic microflora during clindamycin administration

The numbers of enterococci increased after treatment in the placebo group ($P \leq 0.05$) and in the active group, whereas other Gram-positive microorganisms decreased. In both groups, the numbers of *Escherichia coli* decreased, in particular in the placebo group, and a concomitant increase in other Gram-negative bacilli occurred, mainly of *Klebsiella* spp.

Impact of yogurt products on intestinal anaerobic microflora during clindamycin administration

In the placebo group, the numbers of lactobacilli ($P < 0.05$), bifidobacteria ($P < 0.005$), eubacteria, veillonella and bacteroides ($P < 0.05$) decreased during treatment and increased again at the end of the study period (Figure 1). *Clostridium* spp. varied in numbers, some species disappeared and new species were detected during treatment. In subjects receiving yogurt with added microorganisms, the numbers of lactobacilli and bacteroides remained stable throughout the study period, whereas numbers of bifidobacteria ($P < 0.005$) and veillonella ($P < 0.05$) decreased (Figure 2). As in the placebo group, the numbers of *Clostridium* spp. varied during the study. *C. difficile* was isolated in six samples from four subjects in the placebo group on day 14 and on days 2 and 21 respectively in two of them. In the active group, *C. difficile* was isolated in three samples from three subjects on days 14 and 21. Strains isolated from two subjects in the placebo group and from three subjects in the active group produced cytotoxin.

Prevalence of probiotic strains

The numbers of *L. acidophilus* increased initially, decreased on day 7 and increased again until day 14. *Lactobacillus* F19 increased and remained at the same level during the administration of yogurt. In one subject, *Lactobacillus* F19 was detected before the administration. *B. lactis* was recovered from four samples of three subjects (range log$_2$: 3.0–6.6 cfu/g faeces).
In vitro activity of clindamycin against strains of *Bacteroides* species and *C. difficile* isolated from the intestinal microflora

The MIC50 and MIC90 of clindamycin against strains of *Bacteroides* increased significantly associated with the administration in both groups ($P < 0.0001$). Strains with decreased susceptibility before the start of administration were detected in both groups. Differences between the groups were not statistically significant.

Two of 11 *C. difficile* strains isolated from subjects in the placebo group and two of seven in the active group were resistant to clindamycin (MIC $\geq 8$ mg/L). The remaining isolates were intermediate resistant or susceptible to clindamycin.

**In vitro activity of clindamycin against the probiotic strains**

The MICs of clindamycin against *L. acidophilus*, *B. lactis* and *Lactobacillus* F19 were 2.0, 0.032 and 0.25 mg/L, respectively. Recovered strains of *L. acidophilus* isolated during and after administration of clindamycin ($n = 47$) all had MIC values $\leq 2.0$ mg/L and strains of *Lactobacillus* F19 ($n = 65$) had MIC values $\leq 0.25$ mg/L, respectively.

**Side effects**

One subject developed diarrhoea in connection with the study and one subject reported looser stools. Both subjects belonged to the...
active group and new stool samples were tested for *C. difficile*. In the sample from the first subject, no growth of *C. difficile* could be confirmed but the sample was cytotoxin positive. The individual was later treated with metronidazole to be relieved of the symptoms. In the second case, neither growth of *C. difficile* nor production of cytotoxin could be verified and the symptoms disappeared spontaneously.

**Discussion**

There were large individual variations in numbers of intestinal microorganisms associated with clindamycin administration, but quantitative changes in the numbers of *Bacteroides* species occurred to a greater extent in the placebo group than in the active group. At day 7, bacteroides was not detected in four subjects in the placebo group, whereas all subjects had detectable levels in the active group, even though two subjects had levels just above $10^2$ cfu/g faeces. However, the supplement did not prevent colonization with *C. difficile* and there was also one subject that developed *C. difficile*-associated disease (CDAD) in the active group. Risk factors for *C. difficile* infections are in particular broad-spectrum antibiotics and patients older than 65 years with severe underlying diseases. The subjects in our study were rather young but still the prevalence of *C. difficile* was over 30%. Similar high frequencies have been observed in an earlier study on the ecological effect of clindamycin.9

We can only speculate about possible interactive events occurring between lactobacilli and bacteroides leading to specific preservation of the numbers of *B. fragilis* species observed in this study. Mutual beneficial interactions might have occurred as complementation in degradation of nourishments or in creating a more favourable environment. Further studies are needed to elucidate the mechanisms of action of probiotic supplements.

In this study, there was a tendency for higher MIC values of clindamycin against bacteroides strains, although not statistically significant, in the active group. This phenomenon could be a reflection of the higher rate of resistance before the administration and also that bacteroides remained at higher levels in more subjects in this group.

In conclusion, the probiotic microorganisms tested in this study prevented ecological disturbances in the numbers of intestinal *B. fragilis* group species during clindamycin administration.

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**References**