Antibacterial Susceptibility of Intestinal Lactobacilli of Healthy Children

REET MÄNDAR[1], KRISTA LÕIVUKENE[2], PIRJE HÜTT[1], TÕNIS KARKI[1] and MARIKA MIKELSAAR[1]

From the [1]Department of Microbiology, University of Tartu, Tartu, Estonia and [2]United Laboratory, Tartu University Clinicum, Tartu, Estonia

We investigated the antibacterial susceptibility of intestinal lactobacilli of Estonian and Swedish children aged 1–2 y. Sixty isolates (10 species) of lactobacilli (29 Estonian and 31 Swedish strains) were tested against ampicillin, cefuroxime, cefoxitin, gentamicin, ciprofloxacin, tetracycline, vancomycin, metronidazole and erythromycin. We observed that intestinal lactobacilli do not display uniform susceptibility to antibiotics. None of the tested lactobacilli was resistant to ampicillin, gentamicin and erythromycin. Single strains were resistant to cefuroxime and tetracycline, about half of the strains to cefoxitin and ciprofloxacin and 73% of the strains to vancomycin. All studied strains were resistant to metronidazole. Most of the strains investigated were resistant to two or three antibiotics out of nine. Some differences in susceptibility were noted between strains belonging to different fermentation types. No differences in susceptibility were found between Estonian and Swedish isolates. Metronidazole, cefoxitin, vancomycin and ciprofloxacin seem to be safer for gastrointestinal lactoflora than other tested antibiotics in both countries.

R. Mändar, MD, Ravila 19, Department of Microbiology, University of Tartu, EE-50411 Tartu, Estonia. E-mail: reetm@med.ut.ee

INTRODUCTION

Lactobacilli are important components of healthy human microflora (1–3). Lactoflora can be found in different areas of the body, such as the mouth and the intestinal and urogenital tracts. Lactoflora contain various species of homo- and heterofermentative lactobacilli and differ between individuals. In the gastrointestinal tract, the most frequently observed species are Lactobacillus acidophilus, L. salivarius, L. casei, L. plantarum, L. fermentum and L. brevis (4–7).

Lactoflora also differ in terms of their antibiotic susceptibility patterns (8, 9). Lactobacilli of neonates can be seriously affected by several antibiotics (10). Some antibiotics, such as third-generation cephalosporins and fluoroquinolones, have been widely used for a comparatively shorter period in Estonia than in the Nordic countries (11). Thus, it can be supposed that microorganisms in Estonia and Sweden may harbor different susceptibility patterns.

Normal lactoflora are of great importance to the health of the host, protecting him/her from colonization or infection with pathogens. Moreover, instillation of lactobacilli may be useful therapeutically (12–16). The use of antibiotics disturbs the normal flora which may result in disease. Hence, it is advantageous to avoid the use of antibiotics that are highly active against lactobacilli. However, as there is little information in the literature concerning the susceptibility of lactobacilli to antibiotics it is difficult to follow this suggestion.

In this study we compared the antibacterial susceptibilities of intestinal lactobacilli in 1–2-y-old Swedish and Estonian children.

 ISSN 0036-5548

MATERIALS AND METHODS

Study group

The study group comprised 71 Estonian and 65 Swedish consecutively born 1–2-y-old children who participated in a prospective study of the appearance of allergic diseases. The study was approved by the Institutional Review Boards at Linköping and Tartu universities. Clinical data from these investigations have been reported elsewhere (17, 18).

Isolation and identification of lactobacilli

Fecal samples were serially diluted (10^{-2}–10^{-9}) under a stream of CO_2 in prereduced phosphate buffer (pH 7.2). The dilutions were seeded onto freshly prepared MRS agar (Oxoid; Unipath, Basingstoke, UK) which was incubated in a micro-aerophilic atmosphere (CampyGen, Oxoid) for 72 h.

Provisional identification of lactobacilli was determined by means of their ability to grow on the selective medium, Gram-stain reaction, cell morphology and negative catalase reaction. Isolated strains were grouped as obligately homofermentative (OHOL), facultatively (FHEL) and obligately heterofermentative (OHEL) lactobacilli, employing gas production from glucose and growth at 15°C (4, 19, 20). Finally, 60 isolates (from 29 Estonian and 31 Swedish randomly selected children) were identified to the species level using an API 50 CHL System (BioMerieux, Marcy l'Etoile, France).

Antibiotic susceptibility testing

Sixty clinical isolates and five collection strains (L. acidophilus ATCC 4356, L. paracasei DSM 6522 and DSM 20020, L. buchneri ATCC 4005 and L. reuteri DSM 20016) were tested. Ampicillin, cefuroxime, cefoxitin, gentamicin, ciprofloxacin, tetracycline and vancomycin MICs were tested by the E-test method. A thioglycollate broth for suspending bacteria (McFarland 0.5 turbidity standard), Wilkins–Chalgren (Oxoid) agar plates with 5% horse blood and E-test antibiotic strips (AB Biodisk, Solna, Sweden) were used. After 36 h of incubation at 37°C in a micro-aerophilic environment (CampyGen, Oxoid), the MICs and breakpoints

(susceptible/resistant) were determined in accordance with NCCLS guidelines (21).

Two antibiotics – metronidazole and erythromycin – were tested using the Kirby–Bauer disk diffusion method. BBL (Becton Dickinson, Cockeysville, MD) Sensi-Disc Susceptibility Test Discs (erythromycin 15 μg, metronidazole 5 μg) were used. The inoculum, agar plates and incubation conditions were similar to those used for MIC testing. Metronidazole was also tested under anaerobic conditions in a glove box (85% N_2, 10% CO_2, 5% H_2). Later, five selected strains from different species were tested using the E-test method as described above.

In the cases of cefuroxime and cefoxitin, the strains were additionally tested with the NCCLS agar dilution method (21) using the same inoculum, agar plates and incubation conditions as described above for MIC testing.

Statistical methods

The computer program "Whonet-4" (World Health Organization, Geneva) was used for calculating MIC_{50} and MIC_{90} and for scatterblot analysis. The Mann–Whitney U-test was used for comparing Estonian and Swedish strains.

RESULTS

The Estonian and Swedish lactobacilli tested belonged to all three fermentation types with fairly similar frequencies. However, the spectrum of species was broader in the case of the Estonian strains (Table I). We analyzed the susceptibility of lactobacilli on the strain, species and fermentation type levels.

Strain level

Most of the strains investigated were resistant to two or three antibiotics out of nine. Only one strain was susceptible to all the studied antibiotics, except metronidazole. An average Estonian strain (Est) was resistant to 3.1 and an average Swedish strain (Swe) to 2.7 antibiotics ($p > 0.05$).

No resistance was registered to ampicillin, gentamicin and erythromycin. Few strains (10% Est, 3% Swe) were resistant to tetracycline, 42% (48% Est, 39% Swe) to ciprofloxacin and 73% (76% Est, 71% Swe) to vancomycin. All studied strains were resistant to metronidazole, both anaerobically and micro-aerophilically. Single strains (7% Est, 3% Swe) were resistant to cefuroxime, but 60% (65% Est, 55% Swe) of strains were resistant to cefoxitin. The differences between Estonian and Swedish strains were not statistically significant ($p > 0.05$).

The MIC ranges for ampicillin, gentamicin and vancomycin were narrow: MIC_{50} and MIC_{90} were 0.125 and 0.75 μg/ml, respectively for ampicillin, 0.75 and 3 μg/ml, respectively for gentamicin and >512 and >512 μg/ml, respectively for vancomycin. The mean MICs were very low (<1 μg/ml) for ampicillin, cefuroxime, gentamicin and tetracycline, quite low (3.7 μg/ml) for ciprofloxacin and very high for vancomycin (62.5 μg/ml) and cefoxitin (84.6 μg/ml). The MIC ranges of five strains were 0.016–0.094 μg/ml for erythromycin and >256 μg/ml for metronidazole.

Species level

All cefuroxime-resistant strains appeared to be L. brevis but cefoxitin-resistant strains belonged to different species. None of the 16 L. paracasei subsp. paracasei strains were resistant to tetracycline and ciprofloxacin (Table II). Fourteen L. acidophilus strains out of 15 plus L. delbrueckii subsp. delbrueckii and L. crispatus were susceptible to vancomycin.

In the case of cephalosporins, the strains were additionally tested by the agar dilution method. Good concordance was observed between the results obtained by the two methods used; however, L. acidophilus strains which were susceptible to cefoxitin by the E-test were classified as intermediate by the agar dilution method.

Fermentation type level

FHEL tended to be more resistant to cefoxitin but more susceptible to ciprofloxacin (Table II). All vancomycin-susceptible strains belonged to OHOL. The susceptibility of the collection strains did not differ from that of the strains under investigation.

Table I. *Lactobacilli of Estonian and Swedish children tested for antibacterial susceptibility*

Fermentation type	No. of stains	Species	No. of strains	
			Estonian	Swedish
OHOL	18	L. acidophilus	6	9
		L. crispatus	1	0
		L. delbrueckii subsp. delbrueckii	1	0
		L. salivarius	1	0
FHEL	23	L. paracasei subsp. paracasei	5	11
		L. plantarum	7	0
OHEL	19	L. brevis	4	3
		L. fermentum	3	0
		L. buchneri	0	6
		L. coprophilus	1	2
Total	60		29	31

Table II. *Susceptibility of lactobacilli according to fermentation type and species*

Antibiotic	Fermentation type	Susceptible strains (%) Investigated strains ($n = 60$)	Collection strains ($n = 5$)	Susceptibility of five most frequently isolated species of lactobacilli Species	Resistant (%)	Intermediate (%)	Susceptible (%)
Cefuroxime	OHOL	100	100	L. acidophilus ($n = 15$)	0	0	100
	FHEL	96	100	L. paracasei subsp. paracasei ($n = 16$)	0	6	94
				L. plantarum ($n = 7$)	0	0	100
	OHEL	79	100	L. brevis ($n = 7$)	43	14	43
				L. buchneri ($n = 6$)	0	0	100
Cefoxitin	OHOL	61	100	L. acidophilus	27	20	53
	FHEL	0	0	L. paracasei subsp. paracasei	100	0	0
				L. plantarum	100	0	0
	OHEL	21	100	L. brevis	57	29	14
				L. buchneri	0	50	50
Ciprofloxacin	OHOL	17	0	L. acidophilus	87	0	13
	FHEL	70	100	L. paracasei subsp. paracasei	0	6	94
				L. plantarum	71	14	14
	OHEL	26	0	L. brevis	71	29	0
				L. buchneri	17	33	50
Tetracycline	OHOL	93	100	L. acidophilus	0	7	93
	FHEL	83	100	L. paracasei subsp. paracasei	0	0	100
				L. plantarum	29	29	43
	OHEL	63	50	L. brevis	14	57	29
				L. buchneri	17	17	67
Vancomycin	OHOL	89	100	L. acidophilus	7	0	93
	FHEL	0	0	L. paracasei subsp. paracasei	100	0	0
				L. plantarum	100	0	0
	OHEL	0	0	L. brevis	100	0	0
				L. buchneri	100	0	0

Scatterblot results of cephalosporins are presented in Fig. 1, which shows different scattering for different fermentation types.

DISCUSSION

We observed that intestinal lactobacilli do not display uniform susceptibility to antibiotics. Hamilton et al. (8) also found it difficult to predict their susceptibility pattern. We did not find differences in resistance between Estonian and Swedish lactobacilli when analyzed as whole groups. Thus, the previous different use of antimicrobial drugs in these countries (11) does not seem to be the reason for different susceptibility patterns of intestinal lactobacilli.

Beta-lactam antibiotics

Our results correspond to data from a number of previous studies showing that most strains of lactobacilli are susceptible to penicillins (8, 22–26).

The susceptibility of lactobacilli to two second-generation cephalosporins is curious. Second-generation cephalosporins are generally more active against aerobic Gram-negative microorganisms, cefoxitin being quite effective also against anaerobes (21). In our study cefuroxime expressed much better activity than cefoxitin. The possible explanation for this may be the involvement of other mechanisms of action, such as triggering of membrane-associated autolytic enzymes or inhibition of bacterial endopeptidase and glycosidase (21). Charteris et al. (26) did not observe beta-lactamase activity in lactobacilli and considered cell wall impermeability to be responsible for beta-lactam resistance. Some differences between fermentation types and species in our study support the idea that the resistance of lactobacilli is genuine in many cases. Other authors reported 38–100% of lactobacilli to be resistant to cefoxitin (22, 26); this discrepancy may reflect the different spectra of species studied. As cefoxitin is used for prophylaxis before surgery (27), moderate natural resistance of lactobacilli to it would seem to be reasonable.

Inhibitors of protein synthesis

We have found that all strains of lactobacilli are highly susceptible to erythromycin. This finding corresponds to the data of other investigators (25, 26, 28). At the same time most animal isolates have been found to be resistant to this antibiotic (29).

The majority of strains were susceptible to tetracycline, which is defined as a broad-spectrum antibiotic (21); similar results having also been reported previously (26). In the cases of erythromycin and tetracycline, plasmid-mediated resistance in lactobacilli has been described (26, 30).

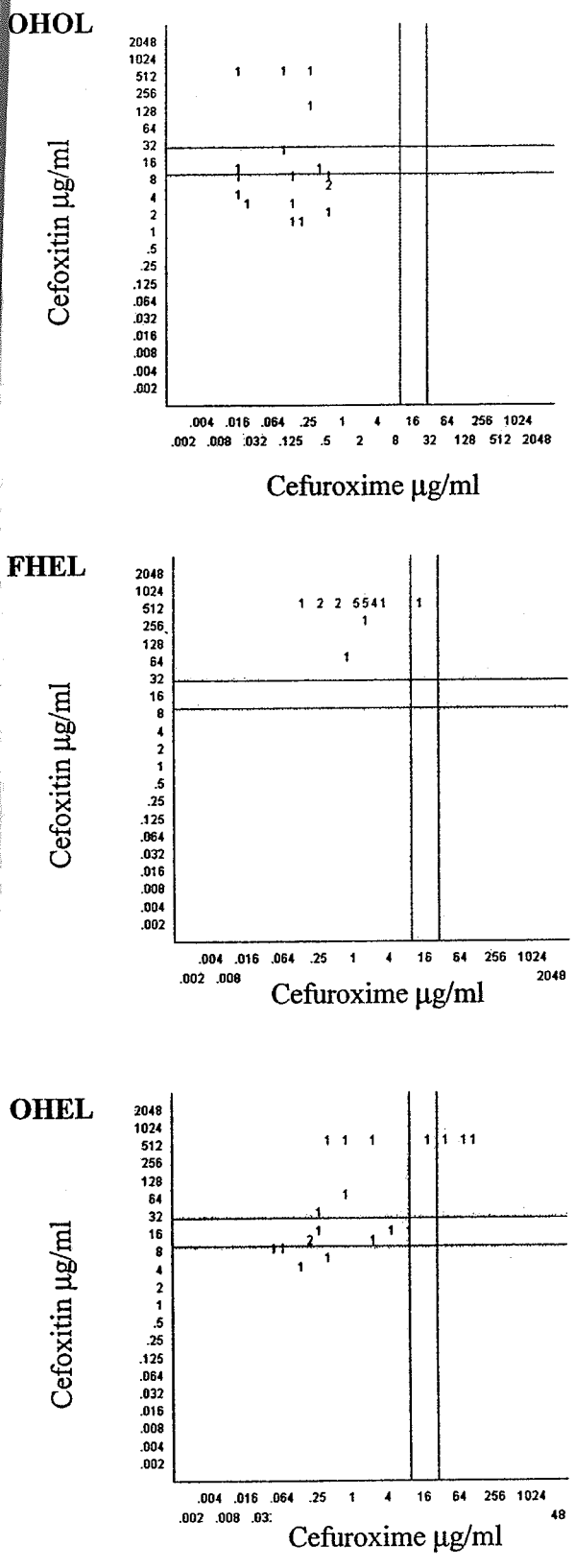

Fig. 1. Scatterblot analysis of susceptibility of lactobacilli (cefuroxime vs. cefoxitin), showing very uniform behavior of FHEL, scattered distribution of OHOL and double-scattered distribution of OHEL.

Surprisingly, gentamicin, which is primarily effective against aerobic Gram-negative bacteria and staphylococci (21), was also highly active against lactobacilli in micro-aerophilic milieu. In our previous study, 93% of intestinal lactobacilli from medical students were susceptible to gentamicin (24). Charteris et al. (26) have found 100% of lactobacilli to be gentamicin-resistant under anaerobic conditions.

Inhibitors of nucleic acid synthesis

Nearly half of the strains were resistant to ciprofloxacin. OHOL were significantly more resistant than the other groups. Controversial data suggesting that 40–100% of strains are susceptible to fluoroquinolones also exist in the literature (8, 22, 30). As many lactobacilli seem to be resistant to this antibiotic, it may be suggested for treatment of complicated urinary tract infections.

We have found all studied strains to be resistant to metronidazole, under both anaerobic and micro-aerophilic conditions. These data correspond to those obtained from our previous study (31) and also to the data of other authors (26, 32). However, susceptible strains have also been described (22, 33). The absence of hydrogenase activity in lactobacilli may be responsible for their resistance to metronidazole (34), which would seem to be reasonable, especially in local treatment of bacterial vaginosis, when combined with probiotics.

Vancomycin

Different investigators have found that many strains of lactobacilli are resistant to vancomycin (20, 23, 35). This was also revealed by our study, where nearly 74% of strains were resistant. In our previous study it was found that susceptibility to vancomycin can be observed by lactobacilli species: L. acidophilus, L. delbrueckii subsp. lactis and L. helveticus were susceptible to vancomycin, but L. delbrueckii subsp. delbrueckii, L. salivarius and all strains of FHEL and OHEL showed resistance to it (36). These data correspond to those of Hamilton-Miller and Shah (37) and Felten et al. (25). In the present study, all FHEL and OHEL were again resistant to vancomycin. Of the OHOL, two strains were resistant: L. salivarius and L. acidophilus. We suspect that the last strain may not in fact have been L. acidophilus. A similar problem occurred previously (38) where a strain initially identified as L. acidophilus was finally determined to be L. rhamnosus. In our case, repeat API testing once more identified the strain as L. acidophilus; the strain was also unable to ferment rhamnose and to grow at 15°C.

Vancomycin resistance has been included in the identification schemes of lactobacilli (20, 23). It has been found (39) that resistant species have cell wall peptidoglycan precursors that end in a depsipeptide D-alanine-D-lactate instead of the dipeptide D-alanine-D-alanine, the target for vancomycin activity. Lactobacilli have not been shown to carry plasmids determining vancomycin resistance.

L. paracasei spp. paracasei

All 16 L. paracasei subsp. paracasei strains behaved very uniformly: none of them were resistant to ampicillin, cefuroxime, gentamicin, erythromycin, ciprofloxacin or tetracycline, but all were resistant to cefoxitin, metronidazole and vancomycin. This may be helpful in identifying this species.

In conclusion, it is important for clinicians to know that metronidazole, cefoxitin, vancomycin and ciprofloxacin are safer for normal lactoflora than the other tested antibiotics in both countries.

ACKNOWLEDGEMENTS

We thank the Department of Public Health, University of Linköping, Sweden and the Department of Pediatrics, University Hospital, University of Linköping, Sweden for collaborating in this study. We are grateful to Dr. Paul Naaber and Dr. Epp Sepp and appreciate the excellent technical assistance of Eha-Mai Laanes, Silvi Truus and Agnes Laasimer. The study was supported by grants from Glaxo-Welcome, UK, Vårdal and The National Association for the Prevention of Asthma and Allergy, Sweden, the Swedish Medical Research Council (grant no. 7510) and the Estonian Science Foundation (grant no. 2749).

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Submitted July 6, 2000; accepted August 27, 2000