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Antimicrobial susceptibility of bifidobacteria

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***Objectives*: The aim of our study was to analyse the antibiotic susceptibility of various strains of *Bifidobacterium* spp. to a wide range of antimicrobial agents.**

***Methods*: Fifty strains belonging to eight species of bifidobacteria, isolated from humans, animals or probiotic products, were tested for susceptibility to 30 antibiotics by disc diffusion on Brucella agar supplemented with 5% laked sheep blood and vitamin K1 (1 mg/L). MICs of nine anti-anaerobe agents, including three new molecules (telithromycin, linezolid and gatifloxacin), were determined using the reference agar-dilution method.**

***Results*: All strains of bifidobacteria, whatever the species, were sensitive to penicillins: penicillin G, amoxicillin (MIC_{50} 0.06 mg/L), piperacillin, ticarcillin, imipenem and usually anti-Gram-positive antibiotics (macrolides, clindamycin, pristinamycin, vancomycin and teicoplanin). Susceptibility to cefalothin and cefotetan was variable. Most isolates (70%) were resistant to fusidic acid. As expected, high resistance rates were observed for aminoglycosides. Metronidazole, an agent known for its anti-anaerobe activity, was ineffective against 38% of the strains. The newly commercialized molecules, telithromycin, linezolid and gatifloxacin, were active with MIC_{50}s of 1 mg/L. The only variation in susceptibility observed among the different species concerned *Bifidobacterium breve*, which appeared to be generally more resistant. Potentially acquired resistance was only observed against tetracycline and minocycline, in 14% of the strains.**

***Conclusions*: With regard to a general concern about the safety of probiotics, such as potential transferability of resistance determinants, bifidobacteria, with their low natural and acquired resistance to 30 antibiotics, appear risk-free.**

Keywords: MICs, probiotics, anaerobes, tetracyclines, linezolid

Introduction

Bifidobacteria are anaerobic, Gram-positive, rod-like organisms with a characteristic Y- or V-shaped end. They inhabit mainly the intestinal and vaginal mucosa of humans and animals.[1] Bifidobacteria belong to the group of lactic acid bacteria (LAB) that includes probiotic strains of the *Lactobacillus*, *Bifidobacterium* and *Enterococcus* genera.[1] They are known as probiotic organisms due to their potential beneficial roles in the intestinal tract, especially in maintaining the intestinal microbial balance, by inhibiting the growth of potential pathogens.[2] Their reputation as health promoters has led to their use in microbial adjunct nutrition and in the prophylaxis and therapy of gastrointestinal infection and antibiotic-associated diarrhoea.[3–5]

The determination of antimicrobial susceptibility of a bacterial strain is an important prerequisite for its approval as a probiotic. Some authors claim that in cases of co-administration with antibiotics to prevent and treat intestinal disorders, probiotics should be resistant to certain antibiotics in order to survive in the gastrointestinal tract.[6] However, this opinion is controversial. Probiotics containing resistance traits may have negative consequences to human health. Risks relating to potential transfer of antibiotic resistance from probiotic strains to intestinal pathogens are a concern, especially after the report of a

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probiotic, *Enterococcus faecium*, which was found to be a possible recipient of the glycopeptide resistance *vanA* gene.[7] The presence of antibiotic-resistance genes in many LAB, and the transfer of plasmids and conjugative transposons to and from LAB, have been reported in *Enterococcus* and *Lactobacillus* species. To our knowledge, conjugative plasmids have not been described in bifidobacteria.[1] Bifidobacterial strains may, however, be pathogenic and cause gastrointestinal and extra-intestinal infections, even though these infections are rarely described in comparison with those associated with other probiotic species. Pathogenic bifidobacterial strains have been isolated from infections occurring at abdominal sites, in abscesses, at obstetric/gynaecological sites and in the chest.[8] *Bifidobacterium dentium* and *Bifidobacterium adolescentis* were responsible for half of these infections and were also implicated, as described in another study, in bacteraemia.[9]

The antimicrobial susceptibility of bifidobacteria has not been seriously questioned until now and the few data available in the literature are heterogeneous and contradictory. This study was carried out to determine precisely the susceptibility of strains of the genus *Bifidobacterium*, isolated from various origins, to several groups of common antimicrobial agents.

Materials and methods

Bacterial strains

Fifty strains of bifidobacteria, including seven reference strains obtained from the Pasteur Institute Collection (Paris, France), were studied. These strains, representing eight different species of bifidobacteria, were isolated from various habitats, with 15 strains from adult human faeces, 26 from newborn or infant faeces, five from probiotic products, two from infant intestine, one from animal faeces and one from human dental caries. The numbers of tested strains were divided as follows: *Bifidobacterium longum*, 14; *Bifidobacterium pseudocatenulatum*, 11; *Bifidobacterium bifidum*, 8; *Bifidobacterium animalis/Bifidobacterium lactis*, 8; *Bifidobacterium breve*, 6; *B. adolescentis*, 1; *Bifidobacterium angulatum*, 1; *B. dentium*, 1.

Clostridium perfringens ATCC 13124T, *Bacteroides fragilis* ATCC 25285 and *Bacteroides thetaiotaomicron* ATCC 29741 obtained from the American Type Culture Collection (Rockville, MD, USA) were used as control strains as suggested by the NCCLS.

Media

Broth containing tryptone peptone (30 g/L), glucose (5 g/L), yeast extract (20 g/L) and haemin (5 mg/L) was used for culture of strains in liquid medium (TGYH). Brucella agar (BBL, Becton Dickinson, Le Pont de Claix, France) supplemented with 5% laked sheep blood and vitamin K1 (1 mg/L) was used for culture of strains on solid medium and for antimicrobial susceptibility tests.

All the experiments were conducted in an anaerobic cabinet (Don Whitley, AES Laboratoire, Combourg, France) flushed with an O_2-free gas mixture ($CO_2/H_2/N_2$, 10/10/80).

Antimicrobial susceptibility tests

In the first part of the study, the susceptibility of bifidobacterial strains to antimicrobial agents was performed by the disc-diffusion method, according to the recommendations of the CA-SFM (Comité de l'Antibiogramme de la Société Française de Microbiologie).[10] Stock cultures were maintained at −80°C in cryotubes in brain–heart-infusion broth containing glycerol (15% v/v), and anaerobically transferred on Brucella agar at 37°C 48 h prior to assay. An inoculum was then prepared for each test organism by suspending cells in TGYH broth in order to achieve a turbidity equivalent to that of a 1.0 McFarland standard (3×10^8 cells/mL) and was delivered on the agar plate with a cotton swab in three directions. Standard discs of 32 antimicrobial agents were placed on the surface of the plates using a mechanical disc-dispensing apparatus (Bio-Rad, Marne-la-Coquette, France). Inhibition-zone diameters were measured after anaerobic incubation at 37°C for 48 h. Discs of antibiotics were obtained from Bio-Rad, Marne-la-Coquette, France, except for metronidazole (Neo-Sensitabs, Rosco, Denmark) and telithromycin (Oxoid, Dardilly, France).

In the second part of the study, MICs of nine antibiotics, i.e. penicillin G, amoxicillin, cefoxitin, tetracycline, erythromycin, telithromycin, linezolid, vancomycin and gatifloxacin were determined using the reference agar-dilution method recommended by the CA-SFM and the NCCLS.[10,11] Penicillin G, amoxicillin, cefoxitin, vancomycin, tetracycline and erythromycin were chosen in order to check the most frequently encountered mechanisms of acquired resistance by Gram-positive anaerobes. Telithromycin, linezolid and gatifloxacin were selected because these are new antibiotics. Telithromycin, gatifloxacin and linezolid were provided as standardized powders by, respectively, Aventis Pharmaceuticals (Romainville, France), Grunenthal (Paris, France) and Pharmacia-Upjohn (Guyancourt, France). Powders of known potency of penicillin G, amoxicillin, cefoxitin, tetracycline, erythromycin and vancomycin were obtained from commercial sources. Solutions of 512 mg/L of each antibiotic were prepared in the appropriate diluents and two-fold dilutions were carried out in distilled water before incorporation into Brucella agar supplemented with laked sheep blood and vitamin K1. All plates were used within 24 h of preparation. An inoculum was prepared for each test organism by suspending cells from a plate in TGYH broth in order to achieve a turbidity equivalent to that of a 1.0 McFarland standard (3×10^8 cells/mL) and was delivered by a Steers replicator onto the agar plates. At the beginning of each series of tests, a plate without antimicrobial agents was inoculated to determine the viability of the organisms and to serve as control for growth comparison. Plates were incubated in the anaerobic chamber at 37°C for 48 h. Because difficult endpoint readings may be encountered with anaerobes, MIC was determined as the lowest concentration of antimicrobial agent that resulted in either no growth, or a few colonies or a significant drop-off in the amount of growth, as recommended by the NCCLS.[12] Resistance rates were calculated according to French breakpoints (mg/L), which are mostly identical to those of the NCCLS and correspond to the following: penicillin G (>16), amoxicillin (>16), cefoxitin (>32), tetracycline (>8), erythromycin (>4), telithromycin (>2), linezolid (>4), vancomycin (>16) and gatifloxacin (>2). Each experiment was repeated three times in duplicates and included the control strains.

MIC_{50} was defined as the lowest concentration of antibiotic that inhibited 50% of the tested strains, and MIC_{90} as the lowest concentration that inhibited 90% of the tested strains.

Assays for β-lactamase activity by nitrocefin tests

Discs of the chromogenic cephalosporin, nitrocefin (bioMérieux, Marcy l'Étoile, France), were used to detect β-lactamase activity.[13] *B. fragilis* ATCC 25285 was used as a positive control.

Table 1. Susceptibility of bifidobacteria to antimicrobial agents using the disc diffusion method

Species (number of strains)	Inhibition zone diameter range (mm)														
	PEN 6 μg	AMX 25 μg	AMC 20/10 μg	TIC 75 μg	PIP 75 μg	OXA 5 μg	CEF 30 μg	FOX 30 μg	CTT 30 μg	CTX 30 μg	IPM 10 μg	KAN[a] 30 μg	GEN[a] 15 μg	TET 30 IU	MIN 30 IU
B. longum (14)	20–38	24–46	20–45	22–40	23–42	14–41	10–38	14–37	10–28	28–41	28–47	6	6–12	8–42	12–42
B. pseudocatenulatum (11)	22–42	26–45	26–45	31–50	21–46	22–45	17–35	20–42	24–36	16–41	30–52	6–8	6	8–42	10–45
B. bifidum (8)	24–46	26–45	25–48	20–44	20–44	23–38	11–36	20–37	15–30	28–42	34–43	6–10	6–10	9–32	14–32
B. animalis/lactis (8)	20–40	24–38	25–40	25–50	20–48	18–30	10–29	18–32	10–30	23–41	28–46	6	6	17–36	24–34
B. breve (6)	20–24	25–40	26–42	22–36	21–34	8–34	6–28	6–32	6–26	20–36	22–42	6–10	6–10	18–31	20–36
Other (3)	29–44	30–36	31–34	30–42	30–41	26–31	25–31	25–32	26–31	28–36	38–42	6–8	6	22–28	23–31
Percentage of resistant strains	0	0	0	0	0	10	16	4	26	0	0	100	100	14	14

Species (number of strains)	Inhibition zone diameter range (mm)														
	ERY 15 IU	SPI 100 μg	TEL 15 μg	CLI 2 IU	PRI 15 μg	LNZ 30 μg	VAN 30 μg	TEC 30 μg	NAL 30 μg	OFX 35 μg	GAT 5 μg	MTR 16 μg	CHL 30 μg	FA 10 μg	RIF 30 μg
B. longum (14)	28–45	24–40	31–50	31–46	29–44	28–48	21–36	18–32	6	9–28	19–41	10–44	25–39	6–36	6–44
B. pseudocatenulatum (11)	17–44	25–44	27–55	29–46	26–50	25–50	21–32	20–32	6	12–26	23–44	10–40	29–42	6–24	24–37
B. bifidum (8)	29–41	20–39	24–38	26–44	32–46	29–44	22–32	19–28	6	9–21	19–29	10–44	26–40	7–21	20–32
B. animalis/lactis (8)	21–45	20–48	30–51	16–51	30–50	29–45	20–36	18–34	6	6–25	19–28	10–44	22–46	6–32	20–40
B. breve (6)	31–38	29–40	31–46	28–44	30–44	34–42	20–34	25–32	6	17–26	18–26	10–42	28–38	7–12	6–44
Other (3)	32–41	33–38	30–46	38–41	41–44	33–42	26–28	20–24	6	17–20	25–28	10–25	25–35	7–15	27–33
Percentage of resistant strains	0	0	0	0	0	0	0	0	100	54	0	38	0	70	4

β-Lactams: PEN, penicillin; AMX, amoxicillin; AMC, co-amoxiclav; TIC, ticarcillin; PIP, piperacillin; OXA, oxacillin; CEF, cefalothin; FOX, cefoxitin; CTT, cefotetan; CTX, cefotaxime; IPM, imipenem. Aminoglycosides: KAN, kanamycin; GEN, gentamicin. Tetracyclines: TET, tetracycline; MIN, minocycline. Macrolides: ERY, erythromycin; SPI, spiramycin. Ketolide: TEL, telithromycin. Lincosamide: CLI, clindamycin. Streptogramin: PRI, pristinamycin. Oxazolidinone: LNZ, linezolid. Glycopeptides: VAN, vancomycin; TEC, teicoplanin. Quinolones: NAL, nalidixic acid; OFX, ofloxacin; GAT, gatifloxacin. Other: MTR, metronidazole; CHL, chloramphenicol; FA, fusidic acid; RIF, rifampicin.

[a]High concentrations of kanamycin (1000 μg) and gentamicin (500 μg) were also tested (see Results).

Table 2. Susceptibility of bifidobacteria to antimicrobial agents using the agar dilution method

Antibiotics	Species (number of strains)	MIC (mg/L)		
		MIC range	MIC_{50}	MIC_{90}
Penicillin G	*B. longum* (14)	≤0.06–1	0.25	0.5
	B. pseudocatenulatum (11)	≤0.06–0.5	≤0.06	0.25
	B. bifidum (8)	≤0.06–0.5	≤0.06	0.5
	B. animalis/lactis (8)	≤0.06–0.5	0.5	0.5
	B. breve (6)	≤0.06–0.5	0.25	0.5
	other (3)	≤0.06–0.5	≤0.06	0.5
	all (50)	≤0.06–1	0.25	0.5
Amoxicillin	*B. longum* (14)	≤0.06–1	0.25	0.5
	B. pseudocatenulatum (11)	≤0.06–0.25	≤0.06	0.25
	B. bifidum (8)	≤0.06–0.5	≤0.06	0.25
	B. animalis/lactis (8)	≤0.06–0.5	0.25	0.5
	B. breve (6)	≤0.06–1	0.25	0.5
	other (3)	≤0.06–0.5	≤0.06	0.5
	all (50)	≤0.06–1	≤0.06	0.5
Cefoxitin	*B. longum* (14)	0.25–64	4	32
	B. pseudocatenulatum (11)	0.25–2	0.5	2
	B. bifidum (8)	2–8	4	8
	B. animalis/lactis (8)	0.25–4	4	4
	B. breve (6)	0.25–32	8	16
	other (3)	2–8	2	8
	all (50)	0.25–64	4	16
Tetracycline	*B. longum* (14)	0.5–64	1	8
	B. pseudocatenulatum (11)	0.5–64	1	64
	B. bifidum (8)	1–64	1	16
	B. animalis/lactis (8)	4–8	8	8
	B. breve (6)	0.5–4	0.5	1
	other (3)	1–2	1	2
	all (50)	0.5–64	2	16
Erythromycin	*B. longum* (14)	≤0.06–0.5	≤0.06	0.25
	B. pseudocatenulatum (11)	≤0.06–0.25	≤0.06	≤0.06
	B. bifidum (8)	≤0.06	≤0.06	≤0.06
	B. animalis/lactis (8)	≤0.06–1	≤0.06	0.25
	B. breve (6)	≤0.06–0.25	≤0.06	0.25
	other (3)	≤0.06–1	≤0.06	≤0.06
	all (50)	≤0.06–1	≤0.06	0.25
Telithromycin	*B. longum* (14)	≤0.0015–0.125	≤0.0015	0.06
	B. pseudocatenulatum (11)	≤0.0015–0.125	≤0.0015	0.06
	B. bifidum (8)	≤0.0015–0.06	≤0.0015	≤0.0015
	B. animalis/lactis (8)	≤0.0015–0.125	≤0.0015	≤0.0015
	B. breve (6)	≤0.0015	≤0.0015	≤0.0015
	other (3)	≤0.0015–0.125	≤0.0015	≤0.0015
	all (50)	≤0.0015–0.125	≤0.0015	0.06
Linezolid	*B. longum* (14)	0.5–1	1	1
	B. pseudocatenulatum (11)	0.5–1	0.5	1
	B. bifidum (8)	0.5–1	1	1
	B. animalis/lactis (8)	0.5–1	0.5	1
	B. breve (6)	1	1	1
	other (3)	0.5–1	0.5	1
	all (50)	0.5–1	1	1
Vancomycin	*B. longum* (14)	0.25–1	0.5	1
	B. pseudocatenulatum (11)	0.25–1	0.5	1
	B. bifidum (8)	0.25–2	0.5	1
	B. animalis / lactis (8)	0.25–2	0.25	0.5
	B. breve (6)	0.25–1	0.5	0.5
	other (3)	0.5	0.5	0.5
	all (50)	0.25–2	0.5	1
Gatifloxacin	*B. longum* (14)	0.5–2	1	2
	B. pseudocatenulatum (11)	0.5–2	1	2
	B. bifidum (8)	0.25–2	1	2
	B. animalis / lactis (8)	0.5–2	1	2

Table 2. (*Continued*)

Antibiotics	Species (number of strains)	MIC (mg/L)		
		MIC range	MIC_{50}	MIC_{90}
	B. breve (6)	0.25–2	2	2
	other (3)	0.5–1	0.5	1
	all (50)	0.25–2	1	2

Assays for detection of tetracycline-resistance determinants by PCR

Total genomic DNA was extracted from all tetracycline-resistant strains and was used for amplification with degenerate primers that identify all known ribosome-protection-type Tc^r genes. Amplifications with primers specific to *tet*(M) and to *tet*(W) genes were also performed. PCR conditions and the primer sets were as described, respectively, by Barbosa *et al.*,[14] Roberts *et al.*[15] and Scott *et al.*[16] *E. faecium* UW7 was used as a positive control for *tet*(M) and degenerate *tet* PCRs, and *Butyrivibrio fibrisolvens* 1.230 was used as a positive control for *tet*(W) PCR.[14,17]

Results

The results of the antimicrobial disc-diffusion susceptibility tests on 50 strains of bifidobacteria are summarized in Table 1 and the MICs of nine antimicrobial agents, including the newly commercialized molecules, telithromycin, linezolid and gatifloxacin are shown in Table 2.

None of the bifidobacterial strains, whatever the species, were resistant to penicillin G, amoxicillin, amoxicillin/clavulanic acid, ticarcillin, piperacillin, cefotaxime and imipenem at tested concentrations of the antibiotics. Resistance to cefotetan was observed in 26% of the strains. Only one *B. longum* strain and one *B. breve* strain were resistant to cefoxitin, with MICs equal to 64 mg/L. Imipenem and cefotaxime produced very large inhibition zones on the tested organisms, denoting low MICs. β-Lactamase production by bifidobacterial strains was not detected.

All the strains were sensitive to usually anti-Gram-positive antibiotics. Erythromycin and telithromycin were highly active with MIC_{50}s, respectively, ≤0.06 and ≤0.0015 mg/L. None of the bifidobacterial strains was resistant to clindamycin or pristinamycin. Vancomycin and linezolid had MIC_{50}s ≤1 mg/L.

High resistance rates were observed for metronidazole and aminoglycosides with the disc-diffusion method. At the tested concentration, metronidazole was ineffective against 38% of the organisms tested. Low-level kanamycin (30 μg) and gentamicin (15 μg) were not active at all, with 100% of the strains showing resistance. However, tested strains were sensitive to high-level kanamycin (1000 μg) or gentamicin (500 μg).

All the strains were resistant to nalidixic acid. Ofloxacin showed variable inhibition zone sizes, with resistance reported in strains of each species. None of the bifidobacterial strains was resistant to gatifloxacin, a new fluoroquinolone with anti-anaerobe properties (MIC_{50} = 1 mg/L).

Fusidic acid at the tested concentration was not efficient, with only 30% of the strains being susceptible. Rifampicin was relatively active and inhibited 96% of bifidobacterial strains.

Broad-spectrum antibacterial agents, chloramphenicol and tetracycline, behaved differently. None of the strains was resistant to chloramphenicol, whereas tetracycline and minocycline showed different patterns toward the tested strains. Three strains of *B. pseudocatenulatum*, two of *B. longum* and two of *B. bifidum* were resistant to tetracycline, with MICs equal to either 16 or 64 mg/L. Resistance to tetracycline was, in all cases, associated with resistance to minocycline. The remaining strains were susceptible to this group of antibiotics. PCRs performed on the seven tetracycline-resistant strains revealed positive amplifications with the degenerate primers for *B. bifidum* R2, *B. longum* B36 and *B. pseudocatenulatum* R47. *Tet*(W) was detected in the same *B. bifidum* and *B. pseudocatenulatum* strains that gave positive results for the degenerate PCR (Figure 1, lanes C and E). No *tet*(M) gene was found in the tetracycline-resistant bifidobacteria tested in this study.

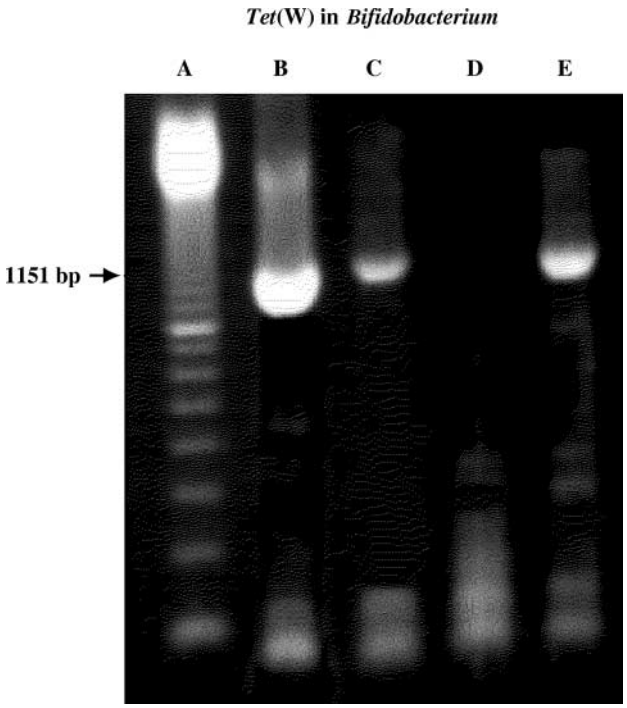

Figure 1. PCR detection of a *tet*(W) gene in *Bifidobacterium*. Lanes: A, 100 bp DNA ladder; B, *Butyrivibrio fibrisolvens* 1.230; C, *B. bifidum* R2; D, *B. longum* B36; E, *B. pseudocatenulatum* R47.

According to the reported results, the susceptibility profile did not seem to be specific to the species and a considerable inter-strain variation was observed. However, the species *B. breve* appeared to be generally more resistant to antibiotics.

Discussion

The susceptibility of bifidobacteria to antibiotics is of great concern, not only in those few cases where the organism is pathogenic and causes infections, but also in understanding the alteration of normal gut flora when antibiotics are taken and in selecting a bifidobacterial strain as a probiotic. It should be noted that the testing of antimicrobial susceptibility for this type of strictly anaerobic and slow-growing bacteria is difficult, sometimes requiring repetition of experiments.

β-Lactams, glycopeptides and erythromycin were highly active against tested bifidobacteria. New classes of antibiotics, i.e. ketolides and oxazolidinones, and new molecules with anti-anaerobe properties, i.e. gatifloxacin, were very effective. Cefalothin and cefotetan were less active than other β-lactams. Resistance to β-lactam antimicrobials may occur by a number of mechanisms, including production of a β-lactamase (penicillinase or cephalosporinase) that hydrolyses the antibiotic agent.[18] Such a mechanism of resistance was not observed in this study, suggesting alternative mechanisms of resistance to β-lactams, such as cell-wall impermeability or alteration of penicillin-binding proteins.[18] All tested bifidobacterial strains were intrinsically resistant to low-level aminoglycosides due to the lack of cytochrome-mediated drug transport among anaerobes.[19] No acquired resistance to aminoglycosides was observed in studied strains. Our results are in agreement with previous studies.[20–23] Charteris *et al.*[24] observed high levels of glycopeptide and cefoxitin resistance. This is in contrast with our observations and those of Citron *et al.*[25] where vancomycin ($MIC_{50} = 0.5$ mg/L in both studies) and cefoxitin ($MIC_{50} = 4$ mg/L and $MIC_{50} = 2$ mg/L, respectively) are clearly reported as highly active drugs against bifidobacterial strains. This discrepancy may be due to the limited reliability of the disc-diffusion method for the determination of vancomycin resistance, compared with the agar-dilution method. Another explanation may be the culture medium, since Charteris *et al.*[24] used trypticase phytone yeast (TPY) medium instead of laked blood brucella agar supplemented with vitamin K1.

The susceptibility of bifidobacteria to various antibiotics is of interest for the treatment of those rare cases where bifidobacteria are pathogenic agents.[8] Antimicrobials used under certain clinical conditions for treating anaerobic or mixed infections, such as amoxicillin (alone or with clavulanic acid), imipenem, clindamycin and cefoxitin are highly active against bifidobacteria. The administration of one of these compounds may lead to the elimination of these bacteria from the infection site. Whereas resistance to clindamycin occurred for 11% of non-sporulated Gram-positive anaerobic bacilli, as investigated by Mory *et al.*,[26] bifidobacterial strains were all inhibited by clindamycin in this study. The relative resistance of bifidobacteria to metronidazole is well highlighted in this study. Metronidazole's activity is due to the preferential reduction by the bacterial ferredoxin system of the parent compound, generating an intermediate product responsible for breaks in the double-stranded DNA.[27] Some strains of bifidobacteria lack the ferredoxin system responsible for the reduction of the parent compound and present a high percentage of resistance to metronidazole, an active drug against virtually all obligate anaerobes. No anaerobic strain resistant to linezolid was detected in our study, or the study of Behra-Miellet *et al.*,[28] which evaluated this antibiotic as a promising candidate for the treatment of infections caused by anaerobes. In this study, the most active agent appears to be telithromycin, showing the lowest MICs against all tested bifidobacterial strains.

The susceptibility of bifidobacteria to various antibiotics is of interest in understanding the alteration of normal intestinal microflora when antibiotics are taken. Bifidobacteria are dominant in the intestine and constitute part of the barrier effect that prevents gut colonization by pathogenic bacteria. Relative resistance to metronidazole might be beneficial in cases of co-administration of bifidobacteria with this antibiotic e.g. antibiotic-associated diarrhoea. From another point of view, the survival of bifidobacteria and lactobacilli—bacteria mainly present in the colon—is affected by the antibiotic concentration that reaches the distal part of the gastrointestinal tract. Since antibiotics are mainly absorbed in the ileum, the therapeutic dosage that reaches the colon might therefore be low compared with the initial dose. Hence, bifidobacteria and lactobacilli might survive better *in vivo* than *in vitro*.

The antimicrobial susceptibility of intestinal microorganisms is an important criterion for selecting an organism as a probiotic. Compared with lactobacilli, strains usually used as probiotics, bifidobacteria appear to be more susceptible to antibiotics. Indeed, lactobacilli are naturally resistant to glycopeptides, with a mechanism of resistance that is not closely related to *vanA*- and *vanB*-mediated enterococcal vancomycin resistance,[29] despite early work reporting the association of vancomycin resistance in *Lactobacillus acidophilus* to a transferable plasmid.[30] In addition, a recent study reported the existence of probiotic lactobacilli strains resistant to tetracycline (29.5%), chloramphenicol (8.5%) and erythromycin (12%).[31] In the present study, only potentially acquired resistance to tetracycline and minocycline was observed in a proportion of 14% of tested bifidobacterial strains. These phenotypic results on tetracycline resistance are similar to those observed by Lim *et al.*[20] We identified, for the first time, *tet*(W) as the gene responsible for tetracycline resistance in *B. pseudocatenulatum* and *B. bifidum*. The *tet*(W) gene was previously found in a human *B. longum* and in three genera of rumen obligate anaerobes, suggesting inter-generic transfer of this resistance gene between anaerobic bacteria.[16]

Since there has been a significant rise in the consumption of probiotic products, it is important that probiotics designed especially for consumers' health are well documented regarding antibiotic resistance. Continuous attention should be paid to the selection of probiotic strains free of transferable antibiotic-resistance determinants. With regard to general concern on the safety of probiotics, i.e. potential transferability of antibiotic-resistance determinants, bifidobacteria, with their low natural and acquired resistance to antibiotics, appear safe for use in the general healthy population.

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