Eur J Clin Microbiol Infect Dis (2007) 26:287–289
DOI 10.1007/s10096-007-0274-8

BRIEF REPORT

Antimicrobial susceptibility of human blood culture isolates of *Lactobacillus* spp

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Published online: 23 February 2007

The clinical significance of *Lactobacillus* is a subject of debate, although it is evident that this genus has been implicated in various types of infections, especially in immunocompromised patients [1, 2]. Few published studies have included a sufficient number of human clinical isolates of individual *Lactobacillus* species in order to facilitate comparisons of antimicrobial sensitivities [2, 3]. The study presented here aimed at characterizing several *Lactobacillus* isolates using 16S rRNA gene sequencing for species identification, PFGE for strain identification, and susceptibility testing of 20 antimicrobial agents, including several newer antimicrobial agents.

Twenty-three *Lactobacillus* strains were obtained from the blood culture samples of 23 patients in Århus County, Denmark, between 1997 and 2004. Six of these strains have been characterized previously in some detail [4]. Species identification was performed using partial 16S rRNA analysis. Identification revealed one isolate of *L. curvatus*, one isolate of *L. delbrueckii*, one isolate of *L. gasseri*, five isolates of *L. paracasei*, four isolates of *L. plantarum*, ten isolates of *L. rhamnosus* and one isolate of *L. salivarius*. Thus, a high prevalence of *L. rhamnosus* (43%), *L. paracasei* (22%), and *L. plantarum* (17%) was observed in accordance with previous studies [1]. The *L. plantarum* species identity was confirmed using a species-specific PCR [5].

The strains were typed using pulsed-field gel electrophoresis (PFGE). Cluster analysis was performed using GelCompar II ver. 2.5, and the strains were compared in the database of Chr. Hansen A/S, which consists of several hundred strains; mainly food isolates and industrial starter cultures. Ten isolates of *L. rhamnosus* could be separated into two subgroups containing seven and three isolates, respectively. Each subgroup was not completely homogeneous, as the isolates showed variation of one to two bands. None of the PFGE fingerprints in this study resembled the fingerprint of the probiotic strain *L. rhamnosus* GG (data not shown). A Finnish study found that 22 of 46 *L. rhamnosus* bacteremia isolates had PFGE fingerprints identical to the GG strain [3] although they were phenotypically different [6]. The five isolates of *L. paracasei* had unique fingerprints, with one isolate being similar to ATCC 25598. Three of the four *L. plantarum* strains had similar fingerprints, with two being identical and the third strain differing by only two of 17 bands.

Antimicrobial susceptibility to a total of 20 antimicrobial agents was determined using E-tests (AB Biodisk, Solna, Sweden) on ISO-sensitest agar (Oxoid, Basingstoke, UK) according to the manufacturer's recommendation. The MIC ranges (mg/l) of antimicrobial agents for which no differences were observed between *Lactobacillus* spp were as follows: ampicillin (0.125–4), chloramphenicol (1–8), clindamycin (0.032–1), erythromycin (0.032–2), synercid (0.25–2) and linezolid (0.5–4). The MICs of the antimicrobial agents for which variation among the species was observed are presented in Table 1.

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Table 1 Distribution of MICs for antimicrobial agents showing variation amongst species and/or strains of *Lactobacillus* spp

Antimicrobial agent	MIC (mg/l) against each species						
	L. curvatus	*L. delbrueckii* [a]	*L. gasseri*	*L. paracasei*	*L. plantarum*	*L. rhamnosus*	*L. salivarius*
CIP	2	>32	>32	2–4	>32	1–4	8
FSA	16	>256	>256	>256	4–12	>256	1
GEN	1	2	4	2–8	0.38–1.5	4–6	>256
KAN	ND	ND	ND	32–>256	ND	32–128	ND
RIF	0.064	0.125	0.125	0.125–0.25	0.5	0.125–>*256* [b]	0.25
STR	16	4	4	8–32	16–32	4–16	>256
TET	1	0.5	0.5	0.5–1	16–32	0.5–*8* [b]	2
VAN	>256	0.5	2	>256	>256	>256	>256
TMP	8	>32	>32	0.25–0.5	0.25–1	>32	1
MOX	0.25	2	>32	0.25–0.5	1–4	0.25–0.5	0.5
GMF	0.064	0.25	4	0.125	0.5–1	0.064–0.125	0.25
DAP	1	1	8	2–4	1	1–4	1
CFX	>32	0.125	1	16–>32	0.25–0.5	>32	1
MPN	4	0.032	0.5	1–8	0.064	4–>32	0.25
n	1	1	1	5	4	10	1

[a] subsp. *lactis*
[b] Numbers in *italics* indicate possible acquired resistance
CIP ciprofloxacin, *FSA* fusidic acid, *GEN* gentamicin, *KAN* kanamycin, *RIF* rifampicin, *STR* streptomycin, *TET* tetracycline, *VAN* vancomycin, *TMP* trimethoprim, *MOX* moxifloxacin, *GMF* gemifloxacin, *DAP* daptomycin, *CFX* cefotaxime, *MPN* meropenem

Several isolates were tested for susceptibility towards antimicrobial agents that had already been administered to the patients from whom the isolates originated. Thus, one *L. curvatus*, one *L. debrueckii* subsp. *lactis*, one *L. gasseri*, two *L. paracasei*, one *L. plantarum* and two *L. ramnosus* isolates originated from patients treated with ampicillin prior to the time of bacteriological diagnosis. In addition, one *L. delbrueckii* subsp. *lactis*, one *L. plantarum* and three *L. rhamnosus* isolates originated from patients treated with gentamicin, one *L. gasseri* and two *L. paracasei* isolates were from patients treated with ciprofloxacin, and one *L. rhamnosus* isolate was from a patient treated with erythromycin. In all of these cases the isolate was susceptible towards the antimicrobial agents administered, except for the *L. gasseri* isolate, which was resistant to ciprofloxacin. One PFGE type 1 isolate of *L. rhamnosus* that showed resistance towards rifampicin was isolated from a patient who had been administered this antimicrobial agent. This patient died as a result of bacteremia. One PFGE type 2 isolate of *L. rhamnosus* showed markedly diminished susceptibility towards tetracycline, although the level of tetracycline resistance (8 mg/l) might not be clinically significant. This isolate originated from a patient who had been administered ceftriaxone, cefuroxime and erythromycin but not tetracycline. In support of previously published findings [1], this study shows that the presence of acquired resistance appears to be rare.

The difficulties associated with identifying lactobacilli to the species level mean they are often regarded as one group, from a clinical viewpoint, despite clear differences in the susceptibility patterns of the various species [7]. High or intrinsic resistance related to individual *Lactobacillus* species was observed against vancomycin, ciprofloxacin, tetracycline, fusidic acid, kanamycin, cefotaxime, trimethoprim and meropenem. Intrinsic resistance to metronidazole and sulphonamides has also been described [3]. The high level of resistance to meropenem observed in some isolates is interesting considering the susceptibility to imipenem reported previously [3]. Finally, many of the isolates had a linezolid MIC of 4 mg/l, which would be characterized as intermediate resistance [8].

It is generally recommended that *Lactobacillus* infections be treated with a β-lactam (penicillin or ampicillin) and an aminoglycoside (typically gentamicin) for synergy [1, 9] and with erythromycin and/or clindamycin as therapeutic alternatives [1]. Other antimicrobial agents identified in this study as possible choices include gemifloxacin and synercid. Most of the 23 infections reported here were treated with ampicillin in combination with other antimicrobial agents.

The mortality rate for *Lactobacillus* bacteremia is considered to be close to 30% [1]. To our knowledge, four (17%) patients included in this study died. One 65-year-old male was infected with *L. rhamnosus* and was treated with vancomycin and metronidazole (both ineffective against *L. rhamnosus*), amphotericin (antifungal agent) and rifampicin. The strain was either initially resistant to rifampicin or it developed resistance to this agent. The three other patients were all infected with *L. plantarum* isolates with similar fingerprints: They had been hospitalized in 2000

and 2001 at three different hospitals and suffered from different diseases. These data suggest that the *L. plantarum* strain that caused these infections is particularly virulent. Further studies are needed to illuminate this issue.

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