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Antimicrobial Susceptibilities of *Lactococcus lactis* and *Lactococcus garvieae* and a Proposed Method To Discriminate between Them

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The MICs of antimicrobial agents contained in the SCEPTOR Streptococcus MIC panels (Becton Dickinson Microbiology Systems) were determined for *Lactococcus lactis*, *L. garvieae*, and unknown *Lactococcus* species. Several isolates had reduced susceptibilities to many of the antimicrobial agents contained in the panel. For *L. garvieae*, the MICs of penicillin and, possibly, cephalothin were higher than for *L. lactis*, and unlike *L. lactis*, *L. garvieae* was resistant to clindamycin, indicating that knowledge of the *Lactococcus* species causing an infection might influence the choice of antimicrobial therapy. Susceptibility to clindamycin can also be used to differentiate between *L. lactis* and *L. garvieae*.

Lactococcus spp. have only recently been reported to cause infections in humans (5, 15, 23). The clinical information in these reports indicates that these bacteria cause a variety of different types of infections similar to the types caused by enterococci. In fact, before lactococci were recognized as human pathogens, we often identified them as variants of established *Enterococcus* spp., including *Enterococcus faecium*, *E. faecalis*, *E. durans*, and *E. hirae*. There are seven recognized *Lactococcus* species and subspecies that can be identified by physiologic reactions (4, 7–10, 12, 21). The majority of human source isolates that we have received have been either *Lactococcus lactis* subsp. *lactis* or *L. garvieae* (5).

Distinguishing between these two species solely on the basis of their physiologic characteristics is very difficult. We have used molecular tests such as whole-cell protein analysis (5, 7) and DNA or RNA analysis (1–3, 13, 14, 17–20, 22) to help in the identification of recent isolates. The value of molecular techniques for the differentiation between *L. lactis* and *L. garvieae* is demonstrated by our reevaluation of isolates that we had previously identified by substrate utilization only. Molecular results indicated that some of the isolates we had classified as *L. lactis* were actually *L. garvieae* and that a few that were identified as *L. lactis* were *L. garvieae* (5, 9).

The importance of confirming the identification of *L. lactis* and *L. garvieae* by doing expensive and labor-intensive molecular tests in a clinical microbiology laboratory is unknown. No information is available about possible differences in antimicrobial susceptibility between these species. Such differences might have an impact on disease therapy and would justify additional effort in species identification. In this study, we tested the antimicrobial susceptibilities of *L. lactis* and *L. garvieae* to determine if there are differences in antimicrobial susceptibility between these species.

Six isolates of *L. lactis* and 13 of *L. garvieae*, all from humans, were recovered from the culture collection at the Centers for Disease Control and Prevention. This is a small group of bacteria because of the rarity of infections caused by these bacteria, but their identity is without question, which makes this group more significant. Species identity was confirmed by molecular techniques that included whole-cell protein analysis and DNA and RNA analysis. The type strains for *L. lactis* (ATCC 19435) and *L. garvieae* (ATCC 43921) were also confirmed by molecular techniques and included in our tests. Nine isolates that are presumed to be lactococci but could not be assigned to a species were also tested.

Antimicrobial susceptibility testing was done by a broth dilution procedure. Bacteria were grown on Tryticase soy agar–5% sheep blood plates (Becton Dickinson Microbiology Systems, Cockeysville, Md.) for 18 h and suspended in Mueller-Hinton broth to an optical density equivalent to a 0.5 McFarland turbidity standard. Ten microliters of this suspension was inoculated into 10 ml of cation-adjusted Mueller-Hinton broth supplemented with 5% lysed horse blood (16). SCEPTOR Streptococcus MIC panels (Becton Dickinson Microbiology Systems) were inoculated by using the automated BD SCEPTOR preparation station. MIC panels were incubated for 18 h at 37°C in a CO_2 incubator. Growth was read visually, and the MIC was defined as the lowest concentration of a drug that inhibited growth. *E. faecalis* (ATCC 29212 and ATCC 51299), *Staphylococcus aureus* (ATCC 29213), and *Escherichia coli* (ATCC 35218) were included as controls (16).

The MICs are listed in Table 1. All MICs for the control bacteria were within the ranges specified by the National Committee for Clinical Laboratory Standards (16). In addition to being resistant to clindamycin, *L. garvieae* appeared to be somewhat less susceptible to penicillin and cephalothin than did *L. lactis*. For some members of both species, the MICs of several other antimicrobial agents in the panel, including amoxicillin-clavulanic acid, ampicillin, and norfloxacin, were higher than those for members of the same species. One of the unknown *Lactococcus* spp. had susceptibility results similar to those of *L. garvieae*, and eight had results similar to those of *L. lactis*.

L. lactis and *L. garvieae* differed in susceptibility to clindamycin. *L. garvieae* was always more resistant to clindamycin

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TABLE 1. Antimicrobial susceptibilities of *L. lactis* and *L. garvieae*

Antimicrobial agent(s)	MIC range (μg/ml) for strains of:	
	L. lactis	*L. garvieae*
Vancomycin	≤1.0	≤1.0
Amoxicillin-clavulanic acid	≤0.12/0.06–1.0/0.48	0.48/0.24–1.0/0.48
Ampicillin	≤0.12–0.24	0.48
Penicillin	0.12–0.24	1.0[a]
Chloramphenicol	2.0–4.0	2.0–4.0
Clindamycin	≤0.12	≥8.0[b]
Erythromycin	≤0.12	≤0.12
Cephalothin	≤1.0–4.0	4.0–8.0
Cefuroxime	≤4.0	≤4.0
Ciprofloxacin	≤1.0–4.0	≤1.0–2
Norfloxacin	≤4.0–16.0	≤4.0–16.0
Trimethoprim-sulfamethoxazole	≤1.0/19	≤1.0/19
Tetracycline	≤2.0	≤2.0–≥8.0

[a] One isolate required an MIC of penicillin of 0.24 μg/ml.
[b] One isolate required an MIC of clindamycin of ≥2.0 μg/ml.

(MIC, ≥2 μg/ml) than was *L. lactis*, and *L. lactis* was always susceptible (MIC, ≤0.12 μg/ml). We used this information to determine if the two species could be differentiated by a Kirby-Bauer-like test using clindamycin disks. The test was similar to the vancomycin disk procedure that is used to identify *Leuconostoc* and *Pediococcus* spp. (6).

Several colonies of bacteria were spread on a Tryticase soy agar–5% sheep blood plate, and a clindamycin disk (2 μg) was added to the plate. The diameter of the growth inhibition zone was measured after 24 h of incubation at 37°C in a CO_2 incubator.

All of the bacteria that we confirmed as *L. lactis* had zones of inhibition around the clindamycin disk of ≥20 mm (average, 24 mm). All of the *L. garvieae* isolates had no zones of inhibition. The unknown lactococci that were susceptible to clindamycin (eight isolates) by MIC determination had zones of inhibition that were similar to that of *L. lactis*. The other unknown lactococci had zones of inhibition similar to that of *L. garvieae*. We have incorporated the clindamycin disk test in our identification procedures to differentiate between *L. lactis* and *L. garvieae*.

Our results indicate that there are differences in antimicrobial susceptibility between *L. lactis* and *L. garvieae*. These differences may have an impact on the choice of antimicrobial therapy, especially if clindamycin is being considered. For both species, and other human isolates of unidentified lactococci, the MICs of several of the antimicrobial agents included in the SCEPTOR Streptococcus MIC panel are higher, but these organisms are as susceptible to these antimicrobial agents as are the enterococci (16, 24), the group of bacteria most often confused with lactococci. Resistance to vancomycin was not observed in this group of lactococci. Although previous investigators have reported that vancomycin resistance does occur in lactococci (11), subsequent reevaluation of the identity of these bacteria at the Centers for Disease Control and Prevention revealed that they were not *Lactococcus* species but *Pediococcus* species. Vancomycin resistance, therefore, has yet to be found in bacteria that can be confirmed as *Lactococcus* species.

The antimicrobial susceptibility results support the importance of differentiating between *L. lactis* and *L. garvieae*. Differentiation between these species can be simplified by incorporating sensitivity to clindamycin into an identification scheme. This test must be limited to differentiation between *L. lactis* and *L. garvieae*, since nearly all of the unknown *Lactococcus* isolates had clindamycin susceptibilities that were similar to that of *L. lactis* and one unknown isolate had a susceptibility similar to that of *L. garvieae*. However, if the physiologic reactions indicate that the isolate is either *L. lactis* or *L. garvieae*, a clindamycin disk test result or the MIC of clindamycin can be used to differentiate between these two species.

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