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Letters to the Editor

nimE Gene in a Metronidazole-Susceptible *Veillonella* sp. Strain

Metronidazole (MTZ) is widely used for the prophylaxis and the treatment of infections caused by anaerobes. These infections are mainly due to *Bacteroides fragilis*, but several reports identified *Veillonella* spp. as pathogens in severe infections (5, 7). Despite the extensive use of MTZ, resistance was maintained at a very low level for a long time and was first described for *B. fragilis* in 1978 (4). In contrast, an increasing level (3 to 5%) of *B. fragilis* group strains with decreased susceptibility to MTZ was observed (2). The presence of nitroimidazole (*nim*) resistance genes was first reported in 1989 (3), and five resistance determinants, *nimA* to *nimE*, were characterized either on plasmids or chromosomes (9). Only the location of *nimE* was not reported when the gene was described for *B. fragilis* group strains (9). More recently, the *nimF* gene, sharing 78% identity in sequence with *nimD*, has been reported in a *B. fragilis* group strain (S. Behrendtz, H. Fang, M. Hedberg, and C. Edlund, Abstr. 3rd World Congr. Anaerob. Bact. Infect., abstr. 6.003, 2003).

We have studied MTZ susceptibility and presence of *nim* genes for 116 clinical isolates of *Veillonella* spp. These strains were recovered either in pure culture or in aerobic-anaerobic polymicrobial cultures from various samples in 106 different patients hospitalized or consulting at the Montpellier University Hospital between March 1999 and July 2001. MTZ MICs were determined by the NCCLS reference agar dilution method (8). According to the NCCLS breakpoints, all the *Veillonella* sp. strains were susceptible to MTZ (Table 1). A PCR method using universal primers was used to detect *nim* genes after extraction of total genomic DNA according to the rapid procedure proposed by Trinh and Reysset (10). An amplification product was obtained for one isolate for which the MTZ MIC was 4 mg/liter. The 458-bp amplified fragment obtained was sequenced and gave the maximum identity (99.8%) with the *nimE* gene sequence of *B. fragilis* ARU 6881 (GenBank accession number AJ244018). To search for a possible chromosomal location of this *nimE* gene, DNA electrophoresis of I-CeuI restriction fragments, Southern blotting, and hybridization with a *nim* digoxigenin-labeled probe were performed (1). None of the I-CeuI-generated fragments hybridized with the probe. A *Bacteroides* strain known to harbor a chromosomal copy of the *nim* gene was used as a positive control. On the other hand, plasmid purification was performed by using a Quantum Prep plasmid miniprep kit (Bio-Rad Laboratories). Plasmid DNA was apparently not detectable when analyzed by agarose gel electrophoresis, but plasmid extraction yielded positive *nim* amplification. Taken together, these results suggested that *nimE* was probably located on a plasmid present in low copy number in the *Veillonella* strain, and this could be related to the low MTZ MIC observed for this strain.

TABLE 1. Repartition of the MTZ MICs obtained by the agar dilution method for the 116 *Veillonella* sp. clinical isolates

MTZ MIC (mg/liter)	No. of *Veillonella* sp. strains for each MIC value
8	1
4	8[a]
2	70
1	35
0.5	1
0.25	1

[a] Includes the *nimE* gene-harboring strain isolated from a pressure ulcer of the sacrum in mixed aerobic-anaerobic cultures.

nim genes were mainly described in *B. fragilis* group isolates, and *nimA* is the only one presently recovered in other anaerobic bacteria: *Propionibacterium* spp., *Actinomyces odontolyticus*, *Prevotella bivia*, and *Clostridium bifermentans* (6). We report the first detection of a *nim* gene in a *Veillonella* sp. It is also the first description of the *nimE* gene outside the *B. fragilis* group. As previously described for the genus *Bacteroides*, the presence of *nim* genes did not lead systematically to the expression of MTZ resistance, but the isolation of this *Veillonella* sp. strain extends the list of *nim* gene-harboring bacteria.

Nucleotide sequence accession number. A partial sequence of 448 bp for the *Veillonella* sp. nitroimidazole resistance protein (*nimE*) gene has been deposited in GenBank under accession number AY575922.

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REFERENCES

1. **Bonnet, R., H. Marchandin, C. Chanal, D. Sirot, R. Labia, C. De Champs, E. Jumas-Bilak, and J. Sirot.** 2002. Chromosome-encoded class D β-lactamase OXA-23 in *Proteus mirabilis*. Antimicrob. Agents Chemother. **46:**2004–2006.
2. **Breuil, J., C. Burnat, O. Patey, and A. Dublanchet.** 1989. Survey of *Bacteroides fragilis* susceptibility patterns in France. J. Antimicrob. Chemother. **24:**69–75.
3. **Breuil, J., A. Dublanchet, N. Truffaut, and M. Sebald.** 1989. Transferable 5-nitroimidazole resistance in the *Bacteroides fragilis* group. Plasmid **21:**151–154.
4. **Ingham, H. R., S. Eaton, C. W. Venables, and P. C. Adams.** 1978. *Bacteroides fragilis* resistant to metronidazole after long-term therapy. Lancet **1:**214.
5. **Liu, J. W., J. J. Wu, L. R. Wang, L. J. Teng, and T. C. Huang.** 1998. Two fatal cases of *Veillonella* bacteremia. Eur. J. Clin. Microbiol. Infect. Dis. **17:**62–64.
6. **Lubbe, M. M., K. Stanley, L. J. Chalkley.** 1999. Prevalence of *nim* genes in anaerobic/facultative anaerobic bacteria isolated in South Africa. FEMS Microbiol. Lett. **172:**79–83.
7. **Marchandin, H., H. Jean-Pierre, C. Carrière, F. Canovas, H. Darbas, and E. Jumas-Bilak.** 2001. Prosthetic joint infection due to *Veillonella dispar*. Eur. J. Clin. Microbiol. Infect. Dis. **20:**340–342.
8. **NCCLS.** 2001. Methods for antimicrobial susceptibility testing of anaerobic bacteria, 5th ed. Approved standard M11–A5. NCCLS, Wayne, Pa.
9. **Stubbs, S. L., J. S. Brazier, P. R. Talbot, and B. I. Duerden.** 2000. PCR-restriction fragment length polymorphism analysis for identification of *Bacteroides* spp. and characterization of nitroimidazole resistance genes. J. Clin. Microbiol. **38:**3209–3213.
10. **Trinh, S., and G. Reysset.** 1996. Detection by PCR of the *nim* genes encoding 5-nitroimidazole resistance in *Bacteroides* spp. J. Clin. Microbiol. **34:**2078–2084.

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