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Low-Level Vancomycin Resistance in *Clostridium innocuum*

FRANCINE MORY,[1]* ALAIN LOZNIEWSKI,[1] VERONIQUE DAVID,[2] JEAN PHILIPPE CARLIER,[3] LUC DUBREUIL,[4] AND ROLAND LECLERCQ[2]

Laboratoire de Bactériologie, Hôpital Central, Centre Hospitalier et Universitaire, 54035 Nancy Cedex,[1] Laboratoire de Bactériologie-Virologie, Centre Hospitalier et Universitaire Henri Mondor, 94010 Créteil Cedex,[2] Centre National de Référence des Anaérobies, Institut Pasteur, 75724 Paris Cedex 15,[3] and Laboratoire de Microbiologie, Faculté de Pharmacie, 59006 Lille Cedex,[4] France

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Low-level vancomycin resistance was observed for 28 clinical *Clostridium innocuum* isolates and *C. innocuum* NCIB 10674, whereas teicoplanin was active. DNA from three clinical isolates and the type strain could not be amplified by PCR with primers specific for the genes *vanA*, *vanB*, and *vanC*, suggesting that *C. innocuum* is intrinsically resistant to vancomycin.

Members of the genus *Clostridium* are a major part of the anaerobic microflora of humans and are a potential cause of human infections. *Clostridium innocuum* belongs to the normal intestinal flora of human infants and adults and is one of the species which have been reported to cause human infections, such as intra-abdominal sepsis, bacteremia, and endocarditis (5, 9, 12). However, its identification by commercial kits remains unsatisfactory and requires, therefore, the use of gas-liquid chromatography and additional conventional tests (1). Clostridia are usually considered susceptible to vancomycin and teicoplanin (8). Unexpectedly, we observed that most *C. innocuum* strains isolated from clinical specimens examined in the Laboratory of Bacteriology, University Hospital Center, Nancy, France, had diminished inhibition zone diameters (range, 14 to 16 mm) with vancomycin disks (30 μg) as determined by the agar diffusion method. Thus, we wished to determine the in vitro activities of vancomycin and teicoplanin against these strains.

We studied 28 *C. innocuum* strains consecutively isolated form 28 patients hospitalized at the University Hospital Center of Nancy between 1993 and 1995 (blood, $n = 3$; intra-abdominal pus, $n = 25$). All these isolates have been identified as *C. innocuum* on the basis of morphological and biochemical characteristics. Identification of three blood culture isolates was confirmed by determination of the nucleotide sequence of a portion of the 16S rRNA sequence. From genomic DNA, 311-bp fragments were amplified by PCR with universal 16S rRNA oligonucleotides and sequenced (10). The nucleotide sequences of the three clinical isolates and of *C. innocuum* NCIB 10674 displayed 99% identity and were distantly related to those of 33 other *Clostridium* species determined by Collins et al. (3). Strains were stored at −80°C in brucella broth supplemented with 15% glycerol prior to assay. *Clostridium perfringens* ATCC 13124, *Clostridium difficile* ATCC 9689, and *C. innocuum* NCIB 10674 were included as controls. In addition, the in vitro activities of teicoplanin and vancomycin against 37 other clinical *Clostridium* isolates, including *C. perfringens* ($n = 12$), *C. difficile* ($n = 11$), *Clostridium clostridioforme* ($n = 5$), *Clostridium butyricum* ($n = 4$), *Clostridium tertium* ($n = 3$), and *Clostridium ramosum* ($n = 2$), were determined.

MICs of vancomycin (Lilly, St. Cloud, France) and teicoplanin (Marion Merrell Dow, Levallois-Perret, France) were determined, as recommended by the National Committee for Clinical Laboratory Standards (11), by the agar dilution method on Wilkins-Chalgren agar (Difco Laboratories, Detroit, Mich.). A final inoculum of 10^5 CFU per spot was delivered with a multipoint inoculator. Cultures were then incubated at 35°C for 48 h in an anaerobic chamber (Don Whitley Scientific Ltd., Shipley, United Kingdom). MICs were defined as the lowest concentrations of each antibiotic that inhibited visible growth on agar and were interpreted in accordance with the guidelines of the Comité de l'Antibiogramme de la Société Française de Microbiologie (4).

A PCR assay was used to detect the presence of *vanA*, *vanB*, *vanC1*, and *vanC2* resistance genes in the three clinical *C. innocuum* strains isolated from blood as well as in *C. innocuum* NCIB 10674. The PCR assay was performed as described previously for the detection of glycopeptide resistance genotypes in enterococci (6). *Enterococcus faecium* BM 4147 (*vanA*), *Enterococcus faecalis* V 583 (*vanB*), *Enterococcus gallinarum* BM 4174 (*vanC1*), and *Enterococcus casseliflavus* ATCC 25788 (*vanC2*) were used as controls.

Teicoplanin showed excellent activity against the *C. innocuum* strains tested, inhibiting all isolates at concentrations ranging from 0.25 to 1 μg/ml (MIC at which 90% of the isolates are inhibited [MIC_{90}], 0.5 μg/ml). In contrast, the MICs of vancomycin ranged from 8 to 16 μg/ml (MIC_{90}, 16 μg/ml), classifying all isolates as intermediately resistant to this antibiotic, although 46% (13 of 28) of strains had inhibition zone diameters as determined by the disk diffusion method which were ≥17 mm. This confirms the poor correlation existing between inhibition zone diameters and MICs for glycopeptide susceptibility testing in gram-positive bacteria (2, 14). Vancomycin was at least eight times more active against the other *Clostridium* strains tested (Table 1).

No amplification product was observed with DNA from the four *C. innocuum* strains tested by PCR, suggesting that the low-level vancomycin resistance may be due to genetic determinants other than those identified in enterococci.

Few previous studies have reported *Clostridium* species to be intermediately resistant or even strongly resistant to vancomycin (1, 7, 13). In two of these studies (7, 13), varied clostridial species were tested as one group of uniform organisms, i.e., *C. innocuum* was not distinguished from the other species.

* Corresponding author. Mailing address: Laboratoire de Bacteriologie, Hôpital Central, 29, Avenue du Maréchal de Lattre de Tassigny, 54035 Nancy Cedex, France. Phone: (33) 3-83-85-14-34. Fax: (33) 3-83-85-26-73.

TABLE 1. Comparative susceptibilities of *C. innocuum* and other *Clostridium* species to vancomycin

Organism (no. of isolates)	Vancomycin MIC (μg/ml)		
	Range	50%	90%
C. innocuum (28)	8–16	16	16
C. perfringens (12)	0.25–1	0.5	1
C. difficile (11)	0.5–2	1	2
C. clostridioforme (5)	0.5–2	NA[a]	NA
C. butyricum (4)	0.5–2	NA	NA
C. tertium (3)	0.5–1	NA	NA
C. ramosum (2)	2	NA	NA

[a] NA, not applicable because fewer than 10 strains were tested.

Alexander et al. (1) have noted that 50 and 90% of the 21 strains of *C. innocuum* that they tested were inhibited by 4 and 8 μg of vancomycin per ml, respectively. However, these authors did not compare the inhibitory activities of vancomycin and teicoplanin. Except for those for some strains which appeared to be susceptible to vancomycin (MICs = 2 to 4 μg/ml), their results are similar to ours since the difference in MIC_{90}s was less than 2 dilutions. These differences could be explained in part by the use of different susceptibility testing media.

Thus, to our knowledge, this report is the first description of low-level vancomycin resistance without cross-resistance to teicoplanin in *C. innocuum*. The fact that this phenomenon was observed in all isolates tested suggests the presence of intrinsic low-level vancomycin resistance, the genetic basis and biochemical mechanism of which remain to be investigated. Moreover, documenting low-level resistance to vancomycin may aid in characterization of this species, identification of which may be difficult (1).

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