

## Penicillin Resistance in *Veillonella*

*Veillonella* species are gram-negative anaerobic cocci that are part of the indigenous human flora, particularly in the oropharynx and the intestinal tract. These organisms play an important role in the bacterial ecology of the oral cavity and may contribute to the pathogenesis of gingival and periodontopathic processes (6, 9). *Veillonella* species have been isolated as the only etiologic agent in some cases of serious infectious processes such as osteomyelitis and endocarditis (2, 3, 8, 11).

Penicillin has been suggested as the treatment of choice for infections with *Veillonella* species as the etiologic agent (1, 2, 8). However, in the few cases mentioned in the literature *Veillonella* strains have not shown uniform penicillin susceptibility (4, 5, 7).

Forty clinical *Veillonella* strains (identified, according to established criteria [12], by microscopy and biochemical tests, including determination of metabolic end products) obtained from 40 different patients, from 1992 through 1996, were studied for penicillin susceptibility by using the Epsilon Test (E-test) (AB Biodisk, Solna, Sweden) with blood-supplemented Brucella Agar (Biomedics, Valdemoro, Spain). Thirty were retested by the standard agar dilution method (10). To also evaluate the possible importance of the outer membrane barrier in the resistant phenotype, the tests were run simultaneously in two series of blood-supplemented Wilkins and Chalgren agar (Oxoid, Basingstoke, United Kingdom) plates, with and without polymyxin B nonapeptide (50 µg/ml). For 32 strains, other β-lactams were also studied by the E-test. All plates were incubated in an anaerobic atmosphere at 35°C for 48 h.

Table 1 shows the distribution of the *Veillonella* strains tested by the E-test. Eighty-five percent (E-test) or 80% (agar dilution test) of the strains required penicillin concentrations over 2 µg/ml (breakpoint for susceptibility according National Committee for Clinical Laboratory Standards [NCCLS] criteria [10]) to be inhibited. Most of the discrepancies between methods (30%) would have disappeared if the presence of a double zone of fuzzy growth or discrete colonies scattered inside an otherwise clear inhibition zone had been ignored. This phenomenon was observed again when colonies (three to seven) reisolated from the internal growth were retested with new strips.

The mechanism involved in high-level penicillin resistance of

*Veillonella* remains to be elucidated. Our strains consistently tested negative for β-lactamase by the nitrocefin test. The lack of any modification of the agar dilution penicillin MICs in the presence of polymyxin B nonapeptide suggests that the outer membrane is not relevant to the reduced penicillin susceptibility of our *Veillonella* strains. Therefore, it seems probable that penicillin-binding proteins with low β-lactam affinity may account for the poor activity of penicillin against *Veillonella* species. This may be supported by the fact that the strains for which penicillin MICs are >8 µg/ml (more resistant) are also less susceptible to ampicillin (MIC, >0.25 µg/ml) or cefoxitin (MIC, >1 µg/ml), with *P* values (Yates correction) of 0.019 and 0.0002, respectively. Nevertheless, and according to NCCLS criteria, all but one of our tested strains were susceptible to ampicillin and amoxicillin-clavulanate (MICs, <2 µg/ml) and cefoxitin (MICs, <16 µg/ml). MICs at which 50% of the isolates were inhibited (MIC<sub>50</sub>s) and MIC<sub>90</sub>s of penicillin, ampicillin, and cefoxitin were 16 and 64, 0.5 and 1, and 2 and 4 µg/ml, respectively. The in vitro advantage of ampicillin or amoxicillin-clavulanate over penicillin G should be considered when treating *Veillonella* infections.

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TABLE 1. Distribution of *Veillonella* strains according to their susceptibilities to β-lactam antibiotics

MIC (µg/ml) <sup>a</sup>	No. (%) of strains susceptible to:			
	Penicillin [40] <sup>b</sup>	Ampicillin [32]	Amoxicillin-clavulanate [32]	Cefoxitin [40]
0.12	0	5 (15.6)	5 (15.6)	1 (2.5)
0.25	0	6 (18.8)	7 (21.9)	0 (0)
0.50	0	15 (46.9)	14 (43.8)	8 (20.0)
1	2 (5.0)	5 (15.6)	5 (15.6)	5 (12.5)
2	4 (10.0)	0	0	20 (50.0)
4	3 (7.5)	1 (3.1)	1 (3.1)	4 (10.0)
8	7 (17.5)	0	0	2 (5.0)
16	12 (30.0)	0	0	0
32	7 (17.5)	0	0	0
64	3 (7.5)	0	0	0
>64	2 (5.0)	0	0	0

<sup>a</sup> Determined by E-test.

<sup>b</sup> Numbers in brackets indicate the number of strains tested.