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# Antimicrobial Susceptibilities of Clinical *Desulfovibrio* Isolates

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**The antimicrobial susceptibilities of 16 clinical isolates of *Desulfovibrio* spp. were determined. All or most isolates were susceptible to imipenem ($MIC_{90}$ [MIC at which 90% of the isolates tested were inhibited], 0.5 μg/ml), metronidazole ($MIC_{90}$, 0.25 μg/ml), clindamycin ($MIC_{90}$, 4 μg/ml), and chloramphenicol ($MIC_{90}$, 16 μg/ml) but were resistant or intermediate to penicillin G ($MIC_{90}$, 64 μg/ml), piperacillin ($MIC_{90}$, 256 μg/ml), piperacillin-tazobactam ($MIC_{90}$, 256 μg/ml), cefoxitin ($MIC_{90}$, >256 μg/ml), and cefotetan ($MIC_{90}$, 64 μg/ml). Among isolates with decreased susceptibility to β-lactams ($n = 15$), only six were β-lactamase positive and susceptible to amoxicillin-clavulanate and ticarcillin-clavulanate.**

*Desulfovibrio* spp. belong to a group of nonsporing, gram-negative, dissimilatory sulfate-reducing, anaerobic bacteria. These organisms can be isolated from various environmental sources and from the intestinal tract of humans and animals. *Desulfovibrio* spp. have been reported only infrequently as causes of human infections, including bacteremia and brain and liver abscesses (4–6, 8, 9). Thus, published data on the in vitro susceptibilities of these bacteria to antibiotics are scarce and have always been obtained from a single isolate (3–6, 8, 9). However, despite the small numbers of strains tested, resistance to β-lactams and/or ciprofloxacin has been found. Thus, consistent data regarding the susceptibilities of *Desulfovibrio* spp. to antibiotics currently used for prophylaxis or empirical treatment of anaerobic infections in which these organisms may be involved are required.

In the present study, we determined the susceptibilities to β-lactams, metronidazole, clindamycin, chloramphenicol, and ciprofloxacin of 16 strains (D1 to D16) of *Desulfovibrio* spp. isolated consecutively from 16 patients hospitalized at the University Hospital Center of Nancy (Nancy, France) between 1992 and 1999 (blood, $n = 3$; liver abscess pus, $n = 1$; intra-abdominal pus, $n = 11$; brain abscess pus, $n = 1$) and of 2 reference strains, *D. desulfuricans* ATCC 27774 (isolated from sheep rumen) and *D. desulfuricans* ATCC 29577 (isolated from a tar-sand mixture). *Bacteroides fragilis* ATCC 25285 and *Bacteroides thetaiotaomicron* ATCC 29741 were included as control organisms. Strains were stored at −80°C in brucella broth supplemented with 15% glycerol prior to assay.

MICs were determined by the agar dilution method on brucella agar (BD Difco, Le Pont De Claix, France) supplemented with 5% defibrinated sheep blood, 5 μg of hemin (Sigma, St. Louis, Mo.) per ml, and 1 μg of vitamin $K_1$ (Sigma) per ml, as recommended for anaerobic bacteria by the National Committee for Clinical Laboratory Standards (NCCLS) (7). Antimicrobial agents used were obtained from their respective manufacturers. Penicillin G, amoxicillin, ticarcillin, piperacillin, cefoxitin, cefotetan, cefotaxime, imipenem, metronidazole, clindamycin, chloramphenicol, and ciprofloxacin were tested at concentrations ranging from 0.06 to 256 μg/ml. β-Lactamase inhibitors were tested at fixed concentrations (clavulanate, 2 μg/ml; tazobactam, 4 μg/ml; sulbactam, 8 μg/ml; cloxacillin [class C β-lactamase inhibitor], 10 and 25 μg/ml) in combination with β-lactams. As recommended for fastidious anaerobic organisms (7), fresh *Desulfovibrio* cultures grown for 72 h on supplemented brucella blood agar were suspended in reduced brucella broth and inoculated onto the test medium (approximately $10^5$ CFU per spot) with a multipoint inoculator (Denley, Billingshurst, United Kingdom). Inoculated plates were then incubated at 35°C in an anaerobic chamber (Don Whitley Scientific Ltd., Shippley, United Kingdom). MIC results were read after 48 h of incubation. MICs were defined as the lowest concentration of each antimicrobial agent used alone or in combination with a β-lactamase inhibitor and were interpreted in accordance with the guidelines of the NCCLS (7). To evaluate the reproducibility of this method for *Desulfovibrio* spp., three strains (ATCC 29577, D3, and D11) were independently tested five times for each antimicrobial agent. β-Lactamase production was assessed by the nitrocefin disk method (Cefinase; bioMérieux, Marcy-l'Etoile, France) after 1 h of incubation at 35°C (2). Preliminary studies (unpublished data) have shown that this method is more sensitive than the standard method recommended by the manufacturer for the detection of *Desulfovibrio* β-lactamase.

All isolates were susceptible to imipenem and metronidazole, whereas penicillin G, piperacillin (even when combined with tazobactam), and cefoxitin were devoid of significant antimicrobial activity against all strains (Table 1). Most of the clinical isolates were susceptible to clindamycin and chloramphenicol. Ciprofloxacin was also active against most of the strains tested. The reproducibility of the MIC determinations was good, since, for the three strains repeatedly tested, the MICs of each antimicrobial agent were identical or varied only by a twofold dilution. The MICs obtained for the control organisms varied by no more than 1 twofold dilution and were similar in range to the NCCLS reference values (7). Regarding β-lactam susceptibility, all of the strains tested can be distributed into three groups (Table 2). The first group is composed of strains with the highest susceptibility to β-lactams (ATCC 27774, ATCC 29577, and D1). For these strains, amoxicillin,

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TABLE 1. In vitro activities of 16 antimicrobial agents against 16 clinical isolates of *Desulfovibrio* spp.

| Agent | MIC[a] (μg/ml) | | | No. of isolates[b] | | |
|---|---|---|---|---|---|---|
| | 50% | 90% | Range | S | I | R |
| Penicillin G | 16 | 64 | 1–64 | 0 | 1 | 15 |
| Amoxicillin | 32 | 128 | 0.25–128 | ND | ND | ND |
| Amoxicillin-clavulanate | 32 | 64 | 0.25–64 | 7 | 0 | 9 |
| Ticarcillin | 256 | 256 | 2–256 | 5 | 0 | 11 |
| Ticarcillin-clavulanate | 128 | 256 | 2–256 | 7 | 0 | 9 |
| Piperacillin | 256 | 256 | 64–256 | 0 | 1 | 15 |
| Piperacillin-tazobactam | 128 | 256 | 64–256 | 0 | 4 | 12 |
| Cefotaxime | 128 | 128 | 1–128 | 7 | 0 | 9 |
| Cefotaxime-sulbactam | 128 | 128 | 0.06–128 | ND | ND | ND |
| Cefoxitin | 128 | >256 | 32–>256 | 0 | 1 | 15 |
| Cefotetan | 64 | 64 | 4–128 | 5 | 0 | 11 |
| Imipenem | 0.125 | 0.5 | 0.125–1 | 16 | 0 | 0 |
| Metronidazole | 0.125 | 0.25 | 0.125–1 | 16 | 0 | 0 |
| Clindamycin | 0.5 | 4 | 0.25–4 | 12 | 4 | 0 |
| Chloramphenicol | 8 | 16 | 4–16 | 14 | 2 | 0 |
| Ciprofloxacin | 0.5 | 64 | 0.5–64 | ND | ND | ND |

[a] 50% and 90%, MICs at which 50 and 90% of the isolates tested, respectively, were inhibited.
[b] Categorization as susceptible (S), intermediate (I), or resistant (R) was performed using NCCLS-recommended breakpoints (for testing of anaerobic bacteria). ND, not determined.

ticarcillin, and cefotaxime showed rather good antimicrobial activities. A second group, comprising six isolates (D2 to D7), showed a positive nitrocefin test. Against these strains, the inhibitory activities of amoxicillin and ticarcillin were significantly lower than those observed against isolates belonging to the first group but were restored by clavulanate. This suggests production of at least a class A β-lactamase (1). Among strains of the second group, the MICs of cefotaxime ranged from 4 to 16 μg/ml. Neither clavulanate nor sulbactam restored the activity of cefotaxime. Thus, the production of an extended-spectrum β-lactamase cannot be excluded. The fact that cloxacillin was unable to potentiate the activity of cefotaxime does not favor the production of a class C β-lactamase. A third group, comprising nine strains (D8 to D16), also resulted in high amoxicillin, ticarcillin, and cefotaxime MICs compared to the first group. However, for these strains the β-lactamase inhibitors were unable to enhance the activity of any antibiotic tested in combination.

Although the incidence of human infections caused by *Desulfovibrio* spp. is unknown, these organisms are potential pathogens for humans and should be taken into account for empirical antibiotic therapy. *Desulfovibrio* spp. can be resistant to various antimicrobial agents, including drugs commonly used to treat mixed infections, such as β-lactams combined with β-lactamase inhibitors, cefotetan, and cefoxitin. However, none of the strains tested were resistant to imipenem or metronidazole; these should therefore be considered the drugs most suitable for treating infections caused by *Desulfovibrio* spp. Finally, it appears that a class A β-lactamase may be involved in the resistance of some strains to β-lactams; however, for other β-lactam-resistant strains the mechanism of resistance is obviously more complex and remains to be determined.

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TABLE 2. MICs of amoxicillin, ticarcillin, and cefotaxime, alone and combined with β-lactamase inhibitors, for 16 clinical *Desulfovibrio* isolates, *D. desulfuricans* ATCC 27774, and *D. desulfuricans* ATCC 29577

| Agent(s)[a] | MIC (μg/ml) for indicated strain (clinical source)[b] | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ATCC 27774 | ATCC 29577 | D1 (BL) | D2[c] *(IP)* | D3[c] (BA) | D4[c] (BL) | D5[c] (IP) | D6[c] (IP) | D7[c] (LA) | D8 (IP) | D9 (IP) | D10 (IP) | D11 (BL) | D12 (IP) | D13 (IP) | D14 (IP) | D15 (IP) | D16 (IP) |
| AMZ | 0.25 | 0.25 | 0.25 | 128 | 128 | 8 | 8 | 8 | 8 | 32 | 64 | 64 | 32 | 64 | 32 | 32 | 32 | 32 |
| AMZ + CLA | 0.25 | 0.25 | 0.25 | 1 | 1 | 0.25 | 0.5 | 0.25 | 0.25 | 32 | 64 | 64 | 32 | 64 | 32 | 32 | 32 | 32 |
| TIC | 2 | 2 | 2 | 128 | 128 | 16 | 16 | 16 | 16 | 256 | 256 | 256 | 256 | 256 | 256 | 256 | 256 | 256 |
| TIC + CLA | 2 | 2 | 2 | 4 | 4 | 2 | 2 | 2 | 2 | 256 | 256 | 256 | 256 | 256 | 256 | 256 | 256 | 128 |
| CTX | 2 | 2 | 1 | 16 | 16 | 4 | 8 | 4 | 4 | 128 | 128 | 128 | 128 | 128 | 128 | 128 | 128 | 128 |
| CTX + SUL | 0.06 | 0.06 | 0.06 | 8 | 8 | 2 | 4 | 2 | 2 | 128 | 128 | 128 | 128 | 128 | 128 | 128 | 128 | 128 |
| CTX + CLA | 1 | 1 | 1 | 8 | 8 | 2 | 8 | 4 | 2 | 128 | 128 | 128 | 128 | 128 | 128 | 128 | 128 | 128 |
| CTX + $CLO_{10}$ | 2 | 2 | 1 | 16 | 16 | 4 | 8 | 4 | 4 | 128 | 128 | 128 | 128 | 128 | 128 | 128 | 128 | 128 |
| CTX + $CLO_{25}$ | 2 | 2 | 1 | 16 | 16 | 4 | 8 | 4 | 4 | 128 | 128 | 128 | 128 | 128 | 128 | 128 | 128 | 128 |

[a] Abbreviations: AMZ, amoxicillin; AMZ + CLA, amoxicillin plus clavulanate at a fixed concentration (2 μg/ml); TIC, ticarcillin; TIC + CLA, ticarcillin plus clavulanate at a fixed concentration (2 μg/ml); CTX, cefotaxime; CTX + SUL, cefotaxime plus sulbactam at a fixed concentration (8 μg/ml); CTX + CLA, cefotaxime plus clavulanate at a fixed concentration (2 μg/ml); CTX + $CLO_{10}$, cefotaxime plus cloxacillin at 10 μg/ml; CTX + $CLO_{25}$, cefotaxime plus cloxacillin at 25 μg/ml.
[b] BL, blood; IP, intra-abdominal pus; BA, brain abscess pus; LA, liver abscess pus.
[c] β-lactamase-positive strain.

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