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Susceptibilities of 23 *Desulfovibrio* Isolates from Humans[▽]

Ken-ichi Nakao,[1,2] Kaori Tanaka,[2]* Suguru Ichiishi,[2] Hiroshige Mikamo,[3] Toshiyuki Shibata,[1] and Kunitomo Watanabe[2]

Department of Oral and Maxillofacial Sciences, Graduate School of Medicine, Gifu University, 1-1 Yanagido, Gifu 501-1194, Japan[1]; Division of Anaerobe Research, Life Science Research Center, Gifu University, 1-1 Yanagido, Gifu 501-1194, Japan[2]; and Department of Infection Control and Prevention, Aichi Medical University, 21 Karimata, Yazako, Nagakute-cho, Aichi-gun, Aichi 480-1195, Japan[3]

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Antimicrobial susceptibilities of 23 strains of *Desulfovibrio* spp. were tested by Etest. Generally, *Desulfovibrio* spp. were highly susceptible to sulbactam-ampicillin, meropenem, clindamycin, metronidazole, and chloramphenicol: MIC_{90}s of 6, 4, 0.19, 0.25, and 8 μg/ml, respectively. In addition, these strains generally showed high MICs to piperacillin and piperacillin-tazobactam. *Desulfovibrio fairfieldensis* (eight strains) was the species least susceptible to most agents, especially β-lactams, and was the only species resistant to fluoroquinolones. *Desulfovibrio desulfuricans* strain Essex 6 isolates were less susceptible to β-lactams than *D. desulfuricans* strain MB isolates.

Desulfovibrio spp. are gram-negative anaerobes and a type of dissimilatory sulfate-reducing bacteria. Most established species of *Desulfovibrio* are distributed in the environment, but some *Desulfovibrio* spp. reside in oral cavities and intestinal tracts of animals, including humans (1, 17). In 1996, Tee et al. first reported the isolation of *Desulfovibrio* species from a blood culture of a patient with cholecystitis and suggested that *Desulfovibrio* species might act as an opportunistic pathogen (16). Since then, several case reports that suggested *Desulfovibrio* may be the causative organism have been published (5, 7, 8, 10, 11, 14, 15). In the published case reports, most *Desulfovibrio* strains were isolated from the blood of patients who suffered from a brain abscess, appendicitis, intra-abdominal abscess, or abdominal wall abscess, while some were isolated from peritoneal fluid or the pus from abscesses of various origins. Gibson et al. have also reported that *Desulfovibrio* spp. might be associated with ulcerative colitis (4). Furthermore, Langendijk et al. found that some *Desulfovibrio* spp. may be associated with the early onset of periodontitis, rapidly progressive periodontitis, adult periodontitis, and refractory periodontitis (6).

Presently, four *Desulfovibrio* spp. (*D. fairfieldensis*, *D. desulfuricans*, *D. piger*, and *D. vulgaris*) are recognized to be associated with humans (5, 8). They are slow growers and relatively difficult to isolate from clinical specimens by a conventional approach. Identification to the species level without molecular techniques is considerably difficult. Lozniewski et al. tested the susceptibility of 16 clinical isolates of *Desulfovibrio* spp. and showed the broad MIC range of some antimicrobial agents (9). However, they did not identify their isolates to the species level. Furthermore, Warren et al. tested 18 clinical *Desulfovibrio* isolates, which were identified to the species level; however, only scattered strains of *D. desulfuricans* were included in their study (18). Therefore, the information available regarding the antibiogram of human *Desulfovibrio* isolates is extremely limited and incomplete.

Considering the present situation, it is important and necessary to obtain additional information concerning the antibiogram of *Desulfovibrio* spp. for the empirical treatment of anaerobic infections in which *Desulfovibrio* spp. might be involved. Our laboratory has collected *Desulfovibrio* strains from various human specimens, both clinical and nonclinical, over the past several years. After molecular identification by 16S rRNA sequencing, 13 isolates of *D. desulfuricans* strains Essex 6 and MB were included in our collection. Therefore, in this study, we performed the Etest to obtain additional knowledge regarding the antimicrobial susceptibilities of *Desulfovibrio* species.

Twenty-three strains of *Desulfovibrio* spp. were tested. These strains were isolated from human specimens and identified by classical phenotypic and molecular methods such as 16S rRNA gene sequencing. Specimens from which isolates were obtained and the number of isolates are as follows: mucosal swab of a patient with pouchitis, 6; stool specimens,7; tongue coating, 6; appendicitis, 3; and blood culture, 1. The following four *Desulfovibrio* strains were used as reference strains: *D. fairfieldensis* ATCC 700045, *D. desulfuricans* Essex 6 (ATCC 29577^T), *D. desulfuricans* MB (ATCC 27774), and *D. piger* ATCC 29098^T. *Bacteroides fragilis* ATCC 25285^T was used as the quality control strain. A special agar medium for *Desulfovibrio*, which was formulated and named "*Desulfovibrio* agar" (DA) by the author, was also used as a susceptibility test medium. The ingredients of DA were as follows: polypeptone, 15 g; soya-peptone, 7.5 g; yeast extract, 7.5 g; beef extract, 7.5 g; L-cysteine HCl, 0.75 g; ferric ammonium citrate, 0.75 g; dextran sodium sulfate, 10 g; and agar powder, 15 g per liter of distilled water (pH 7.0). In our preliminary experiment, it was confirmed that *Desulfovibrio* spp. grew more rapidly on the surface of DA than on the surface of *Brucella* blood agar (BBA) and formed a blackish or black halo around the colonies on this

* Corresponding author. Mailing address: Division of Anaerobe Research, Life Science Research Center, Gifu University, Yanagido, Gifu 501-1194, Japan. Phone: 81-58-230-6555. Fax: 81-58-230-6551. E-mail: kktb@gifu-u.ac.jp.

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medium due to H_2S production followed by FeS formation. DA was also used for a pour plate method as described below.

Nine antianaerobic antimicrobial agents were used: sulbactam-ampicillin, piperacillin, piperacillin-tazobactam, cefoxitin, cefotaxime, meropenem, clindamycin, chloramphenicol, and metronidazole. Eleven non-antianaerobic antimicrobial agents were also included in this study: ampicillin, cefoperazone, sulbactam-cefoperazone, cephalothin, ceftazidime, cefpirome, erythromycin, minocycline, ciprofloxacin, levofloxacin, and sulfamethoxazole-trimethoprim.

The Etest was first performed according to a commonly used protocol. Namely, the bacterial suspension was inoculated on the surface of BBA by swabbing. The bacterial suspension was adjusted to McFarland no. 1 standard solution. However, this protocol is not always appropriate for testing *Desulfovibrio* species because achieving confluent growth of the inoculated organisms is difficult on the surface of BBA. Then we used the DA medium. Although *Desulfovibrio* spp. grew better on the surface of this medium, it was still difficult to achieve confluent growth by swab inoculation. Finally, we used the pour plate method described by Wilkins et al. (19). Briefly, 100 μl of bacterial suspension (McFarland no. 1 standard) was mixed with 20 ml DA medium, which was maintained at 50°C and then poured into petri dishes. When this pour plate method was used, a clear transparent elliptical zone of growth inhibition was observed within 48 h of incubation, with another zone of blackened medium surrounding this clear zone due to H_2S formation. After 48 h of anaerobic incubation (atmosphere of 80 to 85% N_2, 5 to 10% CO_2, and 10% H_2), the MIC was read as the concentration at which the border of the elliptical zone of growth inhibition intersected the scale in the Etest strips. Results for reference strains using the DA agar method were comparable to those of the standard BBA method (data not shown).

β-Lactamase production was tested using the nitrocefin hydrolysis test (Cefinase; Becton-Dickinson Co., Ltd.) according to the manufacturer's instructions.

Regardless of the species, all of the *Desulfovibrio* spp. tested in this study were susceptible to five antianaerobic agents with low MIC_{90}s, i.e., sulbactam-ampicillin (6 μg/ml), clindamycin (0.19 μg/ml), meropenem (4 μg/ml), metronidazole (0.75 μg/ml), and chloramphenicol (8 μg/ml). On the other hand, these strains showed high MIC_{90}s toward the other antianaerobic agents: piperacillin (>256 μg/ml), piperacillin-tazobactam (>256 μg/ml), cefoxitin (>256 μg/ml), and cefotaxime (>256 μg/ml) (Table 1). The high susceptibilities of *Desulfovibrio* spp. to clindamycin, metronidazole, chloramphenicol, and imipenem have been noted in previously published reports (9, 18). In this study, one *D. fairfieldensis* strain that was intermediately resistant to meropenem was recognized. With regard to the susceptibility of *Desulfovibrio* spp. to carbapenems, previous reports have demonstrated their uniform susceptibility to imipenem (9, 18). However, another report demonstrated that *D. fairfieldensis* strains were less susceptible to ertapenem, with fairly high MIC_{90} (>32 μg/ml) and MIC_{50} (>32 μg/ml) values (18). Further studies are required to determine the susceptibilities of *D. fairfieldensis* strains to an array of carbapenems, which are now very often chosen as empirical treatment for serious infections.

Fifteen *Desulfovibrio* strains, including 8 *D. fairfieldensis* strains and 7 *D. desulfuricans* Essex 6 isolates, were highly resistant to both piperacillin and cefoxitin: MICs of >256 μg/ml for piperacillin and 64 to >256 μg/ml for cefoxitin, respectively. *D. desulfuricans* MB isolates were susceptible or intermediately susceptible to piperacillin and cefoxitin, except for one resistant isolate. Most of the *D. fairfieldensis* strains were cefotaxime resistant (MIC range, >265 μg/ml), but the other species showed low MICs to cefotaxime (MIC range, 0.5 to 6 μg/ml).

All of the *Desulfovibrio* strains tested showed relatively low MIC_{90}s to erythromycin (MIC_{90}, 1.5 μg/ml), slightly higher MIC_{90}s to ampicillin (MIC_{90}, 8 μg/ml) and minocycline (MIC_{90}, 24 μg/ml), and consistently higher MIC_{90}s to sulfamethoxazole-trimethoprim (MIC_{90}, >32 μg/ml). Their susceptibilities to the remaining seven non-antianaerobic agents were strain dependent with broad MIC ranges.

Eight *D. fairfieldensis* strains were obviously highly resistant to four cephems (cephalothin, cefoperazone, ceftazidime, and cefpirome), sulbactam-cefoperazone, and two fluoroquinolones (ciprofloxacin and levofloxacin). This study, together with the previous report, demonstrated that among three species of the genus *Desulfovibrio*, *D. fairfieldensis* was the species least susceptible to antimicrobial agents. *D. fairfieldensis* strains from our collections were highly resistant to narrow- and broad-spectrum cephems (cephalothin, cefoxitin, cefoperazone, cefotaxime, ceftazidime, and cefpirome) with no detectable β-lactamase by the nitrocefin tests. The mechanism underlying the trend of resistance of this species to β-lactams remains to be elucidated. *D. desulfuricans* Essex 6 isolates showed relatively lower MICs to fluoroquinolones (0.19 to 3 μg/ml). *D. desulfuricans* Essex 6 isolates were more resistant to β-lactam antibiotics than were *D. desulfuricans* MB isolates. Morin et al. have demonstrated that some *D. desulfuricans* strains possess a β-lactamase gene (bla_{DES-1}) and suggested that the gene is associated with a certain subtype of *D. desulfuricans*, although they did not discriminate *D. desulfuricans* Essex 6 from *D. desulfuricans* MB isolates in their study (12). In our study, it is notable that all four β-lactamase producers from among the *D. desulfuricans* strains were the *D. desulfuricans* Essex 6 isolates. Therefore, the existence of bla_{DES-1} in our *D. desulfuricans* strains should be investigated.

The conventional Etest method is simple and easy to perform and may be suitable for all anaerobes, including some slow growers (2, 3). *Desulfovibrio* spp. have a tendency to form tiny, viscous, and pitting colonies on the agar surface: some modification was required to obtain a good performance of the Etest. We proposed to use the DA agar medium and to incorporate the inocula in the pour plate instead of swabbing them. In these bacterium-impregnated agar plates, after only 2 days of incubation, all of the *Desulfovibrio* strains grew homogeneously and formed clear, black-edged, elliptical zones of inhibition. The reproducibility of the MIC determinations by this method was well acceptable, since the MICs were identical or varied within a twofold dilution range for several strains tested, including for the reference strains. The MICs obtained for the quality control strains (*B. fragilis* ATCC 25285) were within the ranges of the CLSI reference values (13; data not shown).

It is essential to increase research efforts to isolate *Desulfovibrio* strains from clinical specimens in order to establish a more useful antibiogram of the *Desulfovibrio* species. However, in the present

TABLE 1. Susceptibilities of *Desulfovibrio* isolates from humans to 20 antimicrobial agents

Species and antimicrobial agent (no. of isolates)	MIC (μg/ml)[a]		
	Range	50%	90%
Desulfovibrio spp. (n = 23)			
Antianaerobic			
Sulbactam-ampicillin	0.064–6	0.38	6
Piperacillin	32–>256	>256	>256
Piperacillin-tazobactam	16–>256	>256	>256
Cefoxitin	16–>256	>256	>256
Cefotaxime	0.047–>256	1.5	>256
Meropenem	0.023–12	0.19	4
Clindamycin	0.016–0.25	0.125	0.19
Chlorampenicol	1.5–12	4	8
Metronidazole	<0.016–1.5	0.125	0.25
Others			
Ampicillin	0.19–24	0.75	8
Cefoperazone	12–>256	>256	>256
Sulbactam-cefoperazone	1–>256	4	>256
Cefalothin	16–>256	>256	>256
Ceftazidime	2–>256	6	>256
Cefpirome	0.5–>256	4	>256
Erythromycin	0.064–4	0.5	1.5
Minocycline	0.19–24	6	24
Ciprofloxacin	0.125–>32	0.5	>32
Levofloxacin	0.094–>32	0.75	>32
Sulfamethoxazole-trimethoprim	>32	>32	>32
D. fairfieldensis (n = 8)[b]			
Antianaerobic			
Sulbactam-ampicillin	4–6		
Piperacillin	>256		
Piperacillin-tazobactam	>256		
Cefoxitin	64–>256		
Cefotaxime	0.047–>256		
Meropenem	2–12		
Clindamycin	0.032–0.19		
Chloramphenicol	3–6		
Metronidazole	<0.016–0.38		
Others			
Ampicillin	4–8		
Cefoperazone	>256		
Sulbactam-cefoperazone	24–>256		
Cefalothin	>256		
Ceftazidime	>256		
Cefpirome	>256		
Erythromycin	0.5–4		
Minocycline	4–24		
Ciprofloxacin	>32		
Levofloxacin	>32		
Sulfamethoxazole-trimethoprim	>32		
D. desulfuricans Essex 6 (n = 7)[c]			
Antianaerobic			
Sulbactam-ampicillin	0.125–1.5		
Piperacillin	>256		
Piperacillin-tazobactam	16–>256		
Cefoxitin	>256		
Cefotaxime	0.047–6		
Meropenem	0.064–0.25		
Clindamycin	0.094–0.25		
Chloramphenicol	1.5–12		
Metronidazole	0.064–1.5		
Others			
Ampicillin	0.19–2		
Cefoperazone	16–>256		
Sulbactam-cefoperazone	3–>256		
Cefalothin	3–>256		
Ceftazidime	3–>256		
Cefpirome	0.5–32		
Erythromycin	0.38–0.75		
Minocycline	2–12		
Ciprofloxacin	0.19–2		
Levofloxacin	0.25–3		
Sulfamethoxazole-trimethoprim	>32		
D. desulfuricans MB (n = 6)[d]			
Antianaerobic			
Sulbactam-ampicillin	0.064–0.25		
Piperacillin	32–>256		
Piperacillin-tazobactam	32–96		
Cefoxitin	16–>256		
Cefotaxime	0.5–1.5		
Meropenem	0.023–0.064		
Clindamycin	0.094–0.19		
Chloramphenicol	1.5–4		
Metronidazole	0.016–0.38		
Others			
Ampicillin	0.19–0.38		
Cefoperazone	12–24		
Sulbactam-cefoperazone	1–3		
Cefalothin	2–6		
Ceftazidime	2–4		
Cefpirome	0.5–2		
Erythromycin	0.38–1		
Minocycline	3–8		
Ciprofloxacin	0.125–0.38		
Levofloxacin	0.25–0.5		
Sulfamethoxazole-trimethoprim	>32		
D. piger (n = 2)[e]			
Antianaerobic			
Sulbactam-ampicillin	0.5–1		
Piperacillin	>256		
Piperacillin-tazobactam	>256		
Cefoxitin	16		
Cefotaxime	1–1		
Meropenem	0.032–0.19		
Clindamycin	0.016–0.016		
Chloramphenicol	1.5–3		
Metronidazole	0.032–0.5		
Others			
Ampicillin	12–32		
Cefoperazone	12–32		
Sulbactam-cefoperazone	1–1.5		
Cefalothin	96–>256		
Ceftazidime	3–3		
Cefpirome	0.75–4		
Erythromycin	0.016–0.125		
Minocycline	0.19–1		
Ciprofloxacin	0.125–0.19		
Levofloxacin	0.095–0.125		
Sulfamethoxazole-trimethoprim	>32		

[a] 50% and 90%, MIC_{50} and MIC_{90}, respectively.
[b] One isolate from pouchitis, five from stool specimens, and two from tongue coating.
[c] One isolate from appendicitis, two from stool specimens, and four from tongue coating.
[d] One isolate from blood, three from pouchitis, and two from appendicitis.
[e] Both isolates from pouchitis.

situation, in addition to the published information concerning the antimicrobial susceptibilities of *Desulfovibrio*, the findings of this study may also be useful for clinicians in treating patients with *Desulfovibrio* bacteremia and patients with mixed infections associated with endogenous *Desulfovibrio* spp.

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