Susceptibility of Dysgonic Fermenter 2 to Antimicrobial Agents In Vitro

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Dysgonic fermenter 2 (DF-2) is a fastidious, gram-negative organism well recognized as a cause of fulminant septicemia in patients without spleens or patients with alcoholic cirrhosis. In vitro antibiotic susceptibility testing of eight strains with a Schaedler broth dilution technique revealed DF-2 to be susceptible to all of the antibiotics tested except aztreonam. Previous reports that DF-2 is aminoglycoside resistant were based on disk diffusion or agar dilution assays that may be less reliable given the slow growth of the organism and its requirement for CO₂ incubation. Penicillin is commonly used as prophylaxis after dog bites and has excellent activity against DF-2.

Dysgonic fermenter 2 (DF-2) is a recognized cause of serious infection following dog bites in splenectomized patients or patients with liver cirrhosis (1, 7). The clinical picture of splenectomized patients can be one of fulminant septicemia and disseminated intravascular coagulation; examination of the buffy coat of blood has revealed the organism in several cases. The mortality of infection with DF-2 is about 25% (7).

The organism is fastidious in its growth requirements, and systematic studies on antibiotic susceptibilities using several strains have not been performed. We tested MICs and MBCs of various antibiotics for eight strains of DF-2 with a Schaedler broth dilution method to develop recommendations on appropriate agents for therapy of DF-2 infections.

MATERIALS AND METHODS

Bacterial isolates. Nine strains of DF-2 from clinical isolates submitted to the Centers for Disease Control, Atlanta, Ga., were used, in addition to a clinical isolate from a patient with DF-2 sepsis from the Veterans Administration Medical Center, Johnson City, Tenn. All strains were oxidase and catalase positive, produced acid fermentatively from glucose, lactose, and maltose, and were definitively identified as DF-2 by the Meningitis and Special Pathogens branch of the Centers for Disease Control. Strains were maintained on chocolate agar (Scott Laboratories, Inc., Fiskeville, R.I.).

Antibiotics. Standard reagent grade powders of antimicrobial agents provided by the manufacturers were used to prepare fresh solutions for testing. The antibiotics used were methicillin, vancomycin, ciprofloxacin, gentamicin, tobramycin, piperacillin, penicillin, ticarcillin, rifampin, aztreonam, imipenem, cefotaxime, chloramphenicol, ceftazidime, cefazolin, cefoperazone, cefamandole, amikacin, erythromycin, clindamycin, and trimethoprim-sulfamethoxazole.

Susceptibility testing was done by broth dilution with Schaedler broth (no. 3900-3821, Mata formulation; Scott Laboratories). Preliminary experiments showed that Schaedler broth was superior to Mueller-Hinton medium in supporting growth of DF-2. Several strains failed to grow in Mueller-Hinton broth. Among the strains that did grow in Mueller-Hinton broth, growth rates (assessed by serial dilu-

tion and plating of samples from the broth at 24-h intervals) were lower than when Schaedler broth was used. An inoculum of 10⁷ CFU or greater was necessary to sustain growth consistently in Schaedler broth. The inoculum was prepared from a 72-h growth on chocolate agar and suspended in Schaedler broth. A barium sulfate standard was used to produce a stock concentration of 1.5 × 10⁸ CFU/ml, from which a 10-fold dilution was made. Each tube contained 1 ml of DF-2 suspension (1.5 × 10⁷/ml) and 1 ml of broth; the final concentration of DF-2 in each tube was 0.75 × 10⁷/ml (confirmed by serial dilution and plating). The MIC was defined as the lowest concentration of antibiotic that inhibited visible growth in Schaedler broth after 72 h of incubation in 5% CO₂ at 35°C. The MBC, determined by plating of 0.025 ml from clear tubes onto chocolate agar, was defined as the lowest concentration at which fewer than five colonies of DF-2 grew.

Experiments were performed to study the effect of Schaedler broth and prolonged CO₂ incubation on antimicrobial agent activity. *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), and *Pseudomonas aeruginosa* (ATCC 27853) were used, and susceptibility testing was performed as described above, except that the final concentration of bacteria per tube was 2.0 × 10⁵ CFU/ml in (i) Mueller-Hinton broth, (ii) Schaedler broth, and (iii) Schaedler broth to which the antimicrobial agents were added and allowed to incubate in CO₂ for 48 h. The bacterial inoculum was then added, and the tubes were incubated in CO₂ for another 24 h.

RESULTS

Results of experiments comparing Mueller-Hinton broth with Schaedler broth with and without CO₂ incubation using American Type Culture Collection strains of *S. aureus*, *E. coli*, and *P. aeruginosa* are shown in Table 1. The MICs of most antibiotics were unchanged when Schaedler broth was used. However, Schaedler broth with CO₂ incubation tended to increase the MICs. With the exception of imipenem, piperacillin, and trimethoprim, the difference in MICs between Mueller-Hinton broth and Schaedler broth with CO₂ was 1 tube dilution.

Two strains of DF-2 consistently failed to grow in Schaedler broth, although they did grow slowly on chocolate

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TABLE 1. Comparison of Mueller-Hinton broth, Schaedler broth, and Schaedler broth with CO₂ incubation on antimicrobial susceptibility testing

Organism and antibiotic	MIC (μg/ml) ^a		
	Mueller-Hinton broth	Schaedler broth	Schaedler broth and CO ₂ incubation ^b
<i>S. aureus</i>			
Gentamicin	0.48	0.48	0.48
Amikacin	2.0	2.0	4.0
Tobramycin	0.24	0.24	0.24
Ceftazidime	8.0	8.0	8.0
Vancomycin	1.0	1.0	2.0
Clindamycin	0.125	0.125	0.125
Rifampin	0.03	0.03	0.03
Erythromycin	0.5	0.5	1.0
Methicillin	0.5	0.5	0.5
<i>E. coli</i>			
Gentamicin	0.24	0.24	0.24
Amikacin	2.0	2.0	4.0
Tobramycin	0.24	0.24	0.24
Piperacillin	1.0	4.0	8.0
Cefazolin	1.0	1.0	1.0
Imipenem	0.03	0.125	1.0
Trimethoprim-sulfamethoxazole	0.5/9.5	0.5/9.5	1.5/9.5
<i>P. aeruginosa</i>			
Gentamicin	2.16	2.16	2.16
Amikacin	2.0	2.0	4.0
Tobramycin	1.44	1.44	1.44
Cefazolin	1.0	1.0	1.0
Ceftazidime	1.0	2.0	4.0
Aztreonam	2.0	2.0	2.0

^a Each organism was tested in duplicate against each antimicrobial agent; results are expressed as the mean of two observations.

^b Antibiotics were incubated in Schaedler broth with 5% CO₂ for 48 h before addition of bacteria.

agar. The MICs and MBCs for the remaining eight strains are shown in Table 2. With the sole exception of aztreonam, DF-2 was susceptible to all of the antibiotics tested. Tolerance, defined as an MBC/MIC ratio of 32 or greater, was not found.

Because the susceptibility of all of our strains to aminoglycosides is in contrast to all previous reports on DF-2, we redetermined the MICs of the aminoglycosides with cation supplementation of the Schaedler broth and found that this did not affect the MICs. With a Kirby-Bauer disk diffusion assay, all strains appeared resistant to the aminoglycosides. This was not a standardized Kirby-Bauer assay, since incubation in CO₂ was necessary to sustain growth of the organism and the incubation period was considerably longer than the 16 to 18 h used as endpoints for most bacterial strains. Interestingly, three strains demonstrated apparent heterotypic resistance to the aminoglycosides, with a zone of inhibition around the disk within which there was faint growth. Subcultures from this zone, when replated again, showed susceptibility and resistance phenotypes.

DISCUSSION

An estimated 55 million dogs are kept as pets in the United States, and approximately 500,000 animal bites occur annually (4). Since *Pasteurella multocida* can be cultured from the mouths of 55% of dogs (1), it is common practice for physicians to administer penicillin after dog bites. DF-2 is susceptible to penicillin (and a number of other antibiotics); clinical infection would probably be more common were it not for the frequent use of antibiotics after dog bites. In addition, normal serum is bactericidal for DF-2, and the organism is likely to produce infection only in patients with quantitative or qualitative abnormalities of complement (7).

Butler et al. performed antibiotic susceptibility testing by using an agar dilution method and CO₂ incubation (1). They used Schaedler medium, since the organism grows so slowly on other media that inhibition is difficult to define. Their isolates were susceptible to all of the antibiotics tested, with the exception of the aminoglycosides and colistin. Schoen et al. (10) used a nonstandardized disk diffusion assay using

TABLE 2. MIC and MBC for eight strains of DF-2

Antibiotic	Mean ± SEM	
	MIC (μg/ml)	MBC (μg/ml)
Penicillin G	0.04 ± 0.01	0.08 ± 0.03
Methicillin	0.49 ± 0.22	1.59 ± 0.59
Erythromycin	0.48 ± 0.24	0.50 ± 0.24
Ticarcillin	1.13 ± 0.13	1.38 ± 0.18
Piperacillin	1.13 ± 0.13	1.25 ± 0.16
Vancomycin	0.29 ± 0.06	0.71 ± 0.47
Clindamycin	0.05 ± 0.01	0.05 ± 0.00
Cefazolin	0.52 ± 0.21	0.89 ± 0.19
Cefamandole	0.30 ± 0.06	0.41 ± 0.06
Cefoperazone	0.50 ± 0.00	0.63 ± 0.00
Cefotaxime	0.23 ± 0.01	0.27 ± 0.03
Ceftazidime	0.39 ± 0.09	1.53 ± 0.40
Gentamicin	0.57 ± 0.23	0.95 ± 0.25
Amikacin	1.50 ± 0.37	1.50 ± 0.37
Chloramphenicol	0.39 ± 0.09	0.66 ± 0.10
Rifampin	0.06 ± 0.00	0.06 ± 0.00
Aztreonam	12.66 ± 4.80	18.00 ± 4.41
Imipenem	0.13 ± 0.00	0.17 ± 0.00
Ciprofloxacin	0.37 ± 0.25	0.44 ± 0.47
Trimethoprim-sulfamethoxazole	8.20 ± 1.20/152.40 ± 4.10	8.20 ± 2.25/76.00 ± 2.80

sheep blood agar plates and found no zones of inhibition with methicillin, gentamicin, and kanamycin in the two strains tested. Waters et al. (11) and Hinrichs and Dunkelberg (8), also using a disk diffusion method, found their DF-2 strain to be aminoglycoside resistant (4).

Our study used a broth (as opposed to agar) dilution method with Schaedler broth. In contrast to previous reports, we found all DF-2 strains to be susceptible to the aminoglycosides. Additional experiments were performed to confirm this finding. Cation supplementation of the Schaedler broth did not alter our results; this was done because calcium and magnesium are known to affect the susceptibility of *Pseudomonas* species to aminoglycosides (3). No zones of inhibition were noted around the aminoglycosides on Kirby-Bauer disk diffusion assay (although we did note heterotypic resistance). Disk diffusion assays are probably unreliable for testing of DF-2, since the time of incubation (3 to 5 days) and the conditions of incubation (CO₂ environment) are not standard. Cooper's theory of critical time (2) (one of the basic concepts of disk diffusion susceptibility testing) states that a certain time is required for the inoculum to reach a critical density, after which it grows in concentrations of the antibiotics many times the MIC for the organism. The critical time for most organisms is 2 to 6 h of incubation but is much longer for a slow-growing organism like DF-2.

Several investigators have shown that incubation in CO₂ causes a fall in the pH of the medium and decreases the zone size and increases the MICs (for organisms other than DF-2) of the aminoglycosides (5, 9). Therefore, CO₂ incubation is not desirable for routine susceptibility testing. When CO₂ incubation is unavoidable (as with DF-2), then broth dilution methods are probably superior since there is less surface area in contact with the atmospheric CO₂ than is the case with the agar dilution or diffusion method. Our comparison of Mueller-Hinton broth with Schaedler broth and CO₂ incubation (using three nonfastidious organisms) revealed increased amikacin, but not tobramycin or gentamicin, MICs as a result of CO₂ incubation. Other antibiotics whose MICs were raised by CO₂ incubation in Schaedler medium were vancomycin, erythromycin, trimethoprim, piperacillin, and imipenem. With the exception of imipenem, piperacillin, and trimethoprim, the increased MICs were a matter of 1 tube dilution, which is often regarded as within the limits of experimental error (6). Despite the technique we used (which could have made DF-2 appear resistant), all DF-2 strains tested were susceptible to the aminoglycosides.

In a patient with fulminant septicemia following a dog bite, DF-2 should be strongly suspected. Examination of a Gram

stain of the buffy coat, always of value in patients with splenectomy, is particularly useful in DF-2 infections, since the organism grows slowly and several days may pass before it is identified by the microbiology laboratory (7). DF-2 is susceptible to most antibiotics (with the exception of aztreonam) in vitro when measured by a broth dilution technique, and penicillin appears to be the drug of choice, with numerous other acceptable alternatives. Prompt therapy for established cases, prophylaxis for dog bite wounds (especially in high-risk individuals), and increasing awareness of DF-2 may decrease the morbidity and mortality from infections with this organism.

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