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NOTES

In Vitro Antibiotic Susceptibilities of *Burkholderia mallei* (Causative Agent of Glanders) Determined by Broth Microdilution and E-Test

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In vitro susceptibilities to 28 antibiotics were determined for 11 strains of *Burkholderia mallei* by the broth microdilution method. The *B. mallei* strains demonstrated susceptibility to aminoglycosides, macrolides, quinolones, doxycycline, piperacillin, ceftazidime, and imipenem. For comparison and evaluation, 17 antibiotic susceptibilities were also determined by the E-test. E-test values were always lower than the broth dilution values. Establishing and comparing antibiotic susceptibilities of specific *B. mallei* strains will provide reference information for assessing new antibiotic agents.

Burkholderia mallei is the causative agent of glanders, a rare disease of equines that is primarily of veterinary concern. In humans, glanders is rarely encountered and is seen in only a few parts of the world (5, 6, 16). *B. mallei* offers additional interest due to its possible misuse as a biological threat agent (12, 15). Few antibiotic susceptibility studies for *B. mallei* have been performed. The first dates back to 1948 (13), and more recent studies have been reported in the Iraqi and Russian scientific literature (1, 2, 3, 9, 10, 17). The most recent susceptibility study, which compared *B. mallei, Burkholderia pseudomallei*, and *Burkholderia cepacia*, indicated that ceftazidime, ciprofloxacin, doxycycline, imipenem, and piperacillin were the most effective agents against these organisms (8). Russian reports, however, have reported resistance to flouroquinolones and tetracyclines (11, 17). Because of the scattered information on MICs and the lack of specific data on type strains, we report specific MIC and E-test data for a number of strains. This information would prove useful in the event of a wartime or terrorist release as well as laboratory-acquired infections in individuals working with one of these organisms. The evaluation of E-test data relative to accepted MIC standard methods may provide a more convenient and safer susceptibility testing method for these threat agents in the field.

The *B. mallei* strains used in this study were NCTC 120, NCTC 10248, NCTC 10229, NCTC 10260, NCTC 10247, ATCC 23344, NCTC 3708, NCTC 3709, ATCC 10399, ATCC 15310, and GB15-2. GB15-2 is a reisolate of ATCC 23344 after passage through Syrian hamsters. This reisolation was accomplished by the transfer of spleen material from a primary intraperitoneally infected animal to a second animal, followed by inoculation of spleen material from that animal to a third animal. Most antibiotics were obtained from U.S. Pharmacopoeia; Rockville, Md. Ceftriaxone was obtained from Sigma Chemical Co., St. Louis, Mo. Quinupristin-dalfopristin (Synercid) was kindly provided by Rhone-Poulenc Rorer, Collegeville, Pa. Stock solutions at 5 mg/ml in the appropriate solvent for each drug were prepared based on the current National Committee for Clinical Laboratory Standards (NCCLS) recommendations (14) and stored at −70°C until use. Amoxicillin-clavulanate (2:1) stock was 5 mg of amoxicillin and 2.5 mg of clavulanate per ml. Co-trimoxazole was 5 mg of sulfamethoxazole and 0.26 mg of trimethoprim per ml (19:1). E-test strips were obtained from AB Biodisk and stored at −70°C until use. MICs were determined by the microdilution method in 96-well plates. Antibiotics were serially diluted twofold in 50 μl of cation-adjusted Mueller-Hinton broth (CAMHB). The antibiotic range was 64 to 0.03 μg/ml based on a final well volume of 100 μl after inoculation. The inocula were prepared from actively growing bacteria in 10 ml of CAMHB restarted with 1 ml of an overnight broth culture. Strains growing in log phase were diluted with CAMHB to a bacterial cell density of 10^6 CFU/ml (conversion factor of 5×10^8 CFU/ml per unit of optical density at 660 nm). To each well of the 96-well plate, 50 μl of this dilution was added for a final inoculum of approximately 5×10^4 CFU/well. After overnight incubation (18 to 24 h) at 37°C, the MICs were determined both visually and by reading the plates at 630 nm (MR5000 microplate reader; Dynex). All MICs were determined in triplicate. For E-test determinations, strips were placed on the surface of Mueller-Hinton agar plates previously seeded with 100 μl of the 10^6-CFU/ml prepared inoculum. Plates were incubated for 18 to 24 h at 37°C, and MICs were determined by reading the intersection of the elliptical growth inhibition zone with the E-test strip. Quality control of antibiotic stocks and E-test strips was established by using *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, and *Escherichia coli* ATCC 25922 according to NCCLS guidelines (14) and AB Biodisk quality control ranges (packet insert). All work was carried out under biosafety level 3 laboratory conditions.

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TABLE 1. Broth dilution and E-test MICs

Antibiotic	MIC (μg/ml)[a] determined by:							
	Broth dilution				E-test			
	All strains tested			ATCC 23344	All strains tested			ATCC 23344
	Range	50%	90%		Range	50%	90%	
Amikacin	0.5–4	1	2	1	0.25–1	0.5	0.5	0.5
Gentamicin	0.25–1	0.5	0.5	0.5	0.047–0.125	0.064	0.094	0.064
Netilmicin	0.25–2	0.5	0.5	0.5				
Streptomycin	2–8	2	4	2				
Tobramycin	0.25–16	0.25	0.5	0.25				
Azithromycin	0.25–1	0.5	1	0.5	0.094–0.75	0.25	0.5	0.25
Clarithromycin	4–16	4	16	4				
Ofloxacin	0.5–8	1	8	1	0.023–3	0.5	1	0.75
Ciprofloxacin	<0.03–4	0.5	1	1	0.008–0.5	0.125	0.25	0.25
Amoxicillin-clavulanate (2:1)	1–4	2	4	4	0.125–0.5	0.25	0.25	0.19
Amoxicillin	>64	>64	>64	>64				
Ampicillin	32–64	64	64	32	2–16	4	6	8
Piperacillin	1–8	4	8	4	0.125–1	0.25	0.38	0.19
Imipenem	0.12–1	0.12	0.25	0.12	0.064–0.19	0.094	0.125	0.094
Ceftazidime	1–6	2	4	2	0.125–1	0.250	0.5	0.125
Cefotaxime	4–6	16	16	8				
Cefotetan	16–>64	32	32	32	2–32	8	16	6
Cefuroxime	32–64	64	64	64	1.5–16	3	6	2
Cefazolin	32–>64	>64	>64	>64				
Ceftriaxone	16–64	32	32	16	1–32	3	12	1
Aztreonam	32–>64	32	32	32	2–32	4	12	2
Sulfamethoxazole	0.25–>64	4	16	4				
Co-trimoxazole	0.25–64	16	32	16	0.003–0.25	0.032	0.125	0.032
Trimethoprim	1–32	8	16	16				
Doxycycline	<0.03–0.5	<0.03	0.12	<0.03	<0.016–0.094	0.023	0.032	0.032
Rifampin	2–16	4	8	2				
Chloramphenicol	4–64	16	32	32	0.25–24	3	8	8
Quinupristin-dalfopristin	1–32	16	32	16				

The broth dilution MIC data presented in Table 1 suggest many possible alternative treatments for *B. mallei* infections that could be evaluated for efficacy in appropriate animal models. The aminoglycosides may be of use for treating infections. However, since we have observed in our laboratory that *B. mallei* may be a primarily intracellular pathogen (D. L. Fritz and D. M. Waag, unpublished data), the aminoglycosides may be ineffective due to their poor penetration into cells. The macrolides may prove to be a more effective treatment for *B. mallei*, as they show good cell penetration and azithromycin demonstrated adequate in vitro activity. Our findings agree with those of Kenny et al. (8) regarding the activities of ciprofloxacin, piperacillin, imipenem, ceftazidime, and doxycycline. Based on our data, ofloxacin should also be considered as a possibility for treatment, as 70% of the strains were sensitive at 2 μg/ml and 80% were sensitive at 4 μg/ml. The amoxicillin-clavulanate results suggest that β-lactam–β-lactamase-inhibitor combinations could be further investigated. Recent experience with a single human case of laboratory-acquired glanders corroborated in vitro data with in vivo efficacy for the *B. mallei* ATCC 23344 strain when a combination of intravenous doxycycline plus imipenem followed by oral doxycycline plus azithromycin successfully controlled a disseminated infection (4). Animal passage and reisolation of *B. mallei* (strain GB15-2) did not appreciably change its antibiotic sensitivity. Twelve antibiotic MICs were unchanged, and another 12 were shifted by one dilution. Three antibiotics shifted sensitivity by two dilutions, with the animal reisolate becoming more sensitive to piperacillin and less sensitive to imipenem and sulfamethoxazole. Only doxycycline sensitivity was greatly altered, with the MIC shifting from <0.03 μg/ml in ATCC 23344 to 0.25 μg/ml in GB15-2. This change in the doxycycline MIC in GB15-2 may reflect a tolerance in response to low levels of tetracycline inadvertently introduced in the animal feed by livestock by-products. It seems unlikely that the shift was due to changes in the cell envelope structure or content, as none of the other antibiotics tested had significant shifts in MICs. If this is an induced tolerance effect, it raises concerns that eventual true resistance could emerge. It is significant that the E-test results did not reflect this difference in the two strains. For most strains and antibiotics, the E-test MICs were 10-fold lower then those observed with the broth dilution method. The co-trimoxazole MIC differences were the most striking, with the E-test results 100- to 1,000-fold lower than those from the broth dilution method. These results are consistent with previous reports that give lower values for the E-test than for other MIC determinations, particularly the broth method (7). The E-test, however, was much easier to perform and required fewer manipulations, which is desirable when working with biosafety level 3 organisms, and it has been recommended for this reason. However, given the wide discrepancy in MICs, we do not recommend the E-test as a means of determining susceptibilities of *B. mallei*. The establishment of MICs for a number of defined and archived strains of *B.*

mallei will be helpful to serve as references in future testing. *B. mallei* ATCC 23344 has susceptibility patterns representative of those of the strains used in this study (Table 1) and of those in other reports (8, 10). ATCC 23344 would serve well as a reference strain for antibiotic resistance monitoring of new strains as well as for new antibiotic susceptibility testing.

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