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Susceptibility of *Acinetobacter* Strains Isolated from Deployed U.S. Military Personnel[▽]

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The susceptibilities of 142 *Acinetobacter baumannii-calcoaceticus* complex isolates (95 from wounded U.S. soldiers deployed overseas) to 13 antimicrobial agents were determined by broth microdilution. The most active antimicrobial agents (≥95% of isolates susceptible) were colistin, polymyxin B, and minocycline.

The U.S. military has noted an increase in the number of *Acinetobacter baumannii-calcoaceticus* complex (ABC) infections among military personnel injured while deployed to Iraq (Operation Iraqi Freedom [OIF]) and Afghanistan (Operation Enduring Freedom [OEF]) (1). ABC is noted for highly resistant isolates that limit therapeutic options (10). In this study, we determined the susceptibilities of a collection of ABC isolates, recovered primarily from U.S. military personnel injured in Iraq and Afghanistan, to several antimicrobial agents of potential interest.

Brooke Army Medical Center receives wounded soldiers evacuated from areas of hostilities and also provides emergency medical care to local military and civilian populations. We selected 142 nonduplicate ABC isolates (from 142 inpatients and outpatients) available in our clinical microbiology laboratory between October 2003 and November 2005. Isolates were identified using the VITEK system (bioMerieux, Inc., Durham, NC). They included isolates from urine (1 isolate), blood (18 isolates), surface wounds (23 isolates), lower respiratory samples (39 isolates), and deep wounds (61 isolates). Prior evaluations of Brooke Army Medical Center ABC isolates have revealed numerous strains causing disease (7). To rule out clonality, we randomly assessed 25 isolates for strain similarity by ribotyping using the endonuclease EcoRI (Qualicon RiboPrinter; DuPont, Wilmington, DE). We found 12 unique strain patterns (data not shown).

The antimicrobial agents included ampicillin-sulbactam, sulbactam alone, imipenem, ticarcillin-clavulanate, ceftazidime, colistin sulfate, polymyxin B, amikacin, gentamicin, azithromycin, doxycycline, minocycline, and tigecycline.

Broth microdilution (BMD) panels were prepared using a Quick Spense plate dispenser (Sandy Spring Instrument Co., Germantown, MD) and were stored at −70°C. Frozen isolates were subcultured twice prior to testing using the Clinical and Laboratory Standards Institute (CLSI) BMD susceptibility method with cation-adjusted Mueller-Hinton broth, a target inoculum concentration of 5×10^5 CFU/ml, and incubation at 35°C for 20 to 24 h (4). MICs were interpreted using the criteria of the CLSI (5), except for sulbactam, azithromycin, and tigecycline (due to a lack of CLSI interpretative criteria). Trailing MIC end points have been described with BMD testing of β-lactam antimicrobial agents and *Acinetobacter* (11). Because the clinical significance of this phenomenon is unknown, two MIC readings were recorded with these drugs: one defining the MIC as the concentration inhibiting all visible bacterial growth (no-growth end point) and a second considering the MIC as the concentration at which growth was reduced ≥80% (trailing end point).

Blood culture isolates underwent minimal bactericidal concentration (MBC) testing according to the CLSI guidelines (3). Log-phase inocula were prepared by transferring colony material from a blood plate at 20 to 24 h of incubation to a tube of tryptic soy broth, which was incubated at 35°C until slight turbidity appeared. The broth was then adjusted to a 0.5 McFarland standard prior to inoculation of microdilution panels. Aliquots of 0.01 ml removed from each clear well were subcultured onto blood agar plates after 20 to 24 h of incubation at 35°C (3). Antimicrobial agents examined in MBC testing were ampicillin-sulbactam, sulbactam, imipenem, colistin, polymyxin B, minocycline, and tigecycline. The MBC was defined as a 99.9% reduction in the initial inoculum density of each isolate tested (3).

Quality control (QC) organisms were *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 29213 (American Type Culture Collection, Manassas, VA). QC ranges for sulbactam are not available. QC strains were tested with each set of patient isolates and were always within acceptable limits (5).

Statistical analysis was performed using SSPS, version 11.5.1 (SPSS Inc., Chicago, IL) using a Pearson chi-square test for the categorical variable. Significance was defined as a P value of <0.01.

There was broad antimicrobial resistance among the isolates tested, and deployed patients' isolates were more resistant than nondeployed patients' isolates (Table 1). Over time, there were slight changes in resistance patterns; however, only resistance to imipenem was statistically increased at the end of the study period in comparison to the beginning (87% versus 56% of isolates susceptible [$P < 0.01$]). None of the blood culture

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TABLE 1. MICs of 13 antimicrobial agents against 142 isolates of *Acinetobacter baumannii-calcoaceticus* complex from deployed and nondeployed U.S. military patients[a]

Drug[b]	Result for:							
	Deployed personnel (n = 95)				Nondeployed personnel (n = 47)			
	MIC (μg/ml)[c]			% Susceptible[d]	MIC (μg/ml)[c]			% Susceptible[d]
	Range	50%	90%		Range	50%	90%	
Ampicillin-sulbactam								
No-growth end point	2–>128	32	128	16*	2–>128	16	128	47*
Trailing end point	2–128	16	64	38*	≤1–>128	8	32	60*
Sulbactam								
No-growth end point	≤2–>64	16	64	—[e]	≤2–>64	8	64	—
Trailing end point	≤2–32	8	32	—	≤2–32	4	32	—
Imipenem								
No-growth end point	≤0.5–>16	2	>16	63*	≤0.5–>16	1	>16	87*
Trailing end point	≤0.5–>16	1	>16	66*	≤0.5–>16	≤0.5	>16	87*
Ticarcillin-clavulanate								
No-growth end point	8–>256	>256	>256	5*	4–>256	64	>256	26*
Trailing end point	≤2–>256	>256	>256	8*	≤2–>256	32	>256	34*
Ceftazidime								
No-growth end point	8–>64	64	>64	9*	4–>64	>64	64	34*
Trailing end point	2–>64	32	>64	33	2–>64	32	>64	45
Colistin	0.25–8	1	1	99	0.5–8	1	2	96
Polymyxin B	0.5–4	1	2	99	0.5–4	1	2	98
Amikacin	≤0.5–>64	16	>64	50	1–>64	8	>64	51
Gentamicin	≤0.5–>8	>8	>8	7*	≤0.5–>8	>8	>8	38*
Azithromycin	<1–>8	>8	>8	—	2–>8	>8	>8	—
Doxycycline	≤0.25–>16	8	>16	40	≤0.25–>16	4	>16	62
Minocycline	≤0.12–16	1	4	97	≤0.12–8	0.5	4	98
Tigecycline	0.12–>8	2	8	—	0.12–>8	1	2	—

[a] Deployed personnel are those who received care after suffering an injury while deployed in support of OIF or OEF (the current war in Afghanistan). Nondeployed personnel are those inpatients receiving care without a history of deployment in support of OIF/OEF.
[b] No-growth end point, MIC defined as the concentration that inhibited all visible bacterial growth; trailing end point, MIC defined as the concentration at which growth was reduced ≥80%. With a trailing end point, colonies or subtle growth patterns occurred above the determined MIC.
[c] 50 and 90%, MICs at which 50 and 90% of isolates are inhibited, respectively.
[d] According to CLSI criteria, except where otherwise indicated. *, $P \leq 0.01$ for difference between isolates from deployed and nondeployed personnel.
[e] —, no interpretive criteria available.

isolates fulfilled the definition of "tolerance" to the bactericidal effects of the drugs (a 32-fold difference between the MIC and the MBC [3]). Colistin and polymyxin B had distinct MICs based on the lack of visible bacterial growth (which was used for interpretation of MICs), but for 35 isolates, scant growth was detected in wells with antimicrobial concentrations higher than the recorded MIC. For 20 of these isolates, MICs of colistin or polymyxin B were ≥4 μg/ml; these 20 isolates would be identified as resistant if the scant growth was taken into consideration. The 35 isolates were retested, and the phenomenon reoccurred for 9 of them when they were retested twice.

Acinetobacter is an increasingly important nosocomial pathogen (12, 13), and an increasing number of U.S. military casualties are infected with ABC (1). The most active agents against ABC isolates obtained from injured military personnel returned from overseas deployments were colistin, polymyxin B, and minocycline. Imipenem was active against only 63% of isolates, and the tigecycline MIC ranged from 0.12 to more than 8 μg/ml. This multidrug-resistant nature of ABC is consistent with previous reports, although our isolates appeared consistently more resistant (2, 8, 11).

For our population, colistin and polymyxin B were expected to be highly active. Susceptibility to minocycline was common, higher than that found in previous studies describing tetracyclines (2, 11). Tigecycline, a glycylcycline designed to overcome the major tetracycline resistance mechanisms (6), was less active than minocycline in our study. Unfortunately, no specific breakpoints have yet been established for this drug for *Acinetobacter* spp.

The phenomenon of skip wells with scant growth after a clearly defined point of no growth with colistin and polymyxin B is of unclear origin. Trailing has been reported with β-lactams, but the skip pattern noted for colistin or polymyxin B is not consistent with the trailing phenomenon and has not been reported previously (11). Without guidance from studies of clinical outcomes or CLSI guidelines for interpretation, the clinical importance of the trailing phenomena or skip wells is unclear. The skip wells may represent a type of heteroresistance or the selection of resistant mutants during testing (9).

The reason for the different levels of resistance among isolates from deployed patients versus nondeployed patients is not clear. Determining the source of acquisition of ABC by OIF/OEF patients might help elucidate this issue; there is evidence supporting a role for nosocomial transmission during transport back to the United States (7; unpublished data).

It is also unclear what role drug usage pressure played in the resistance of this pathogen. Imipenem was previously administered as the prophylactic agent of choice for war wounds in theaters due to concern about ABC infections. Empirical use

of this agent has now been discouraged, but it is still used for management of proven or suspected ABC infection.

Of the antimicrobial agents we identified as the most active, minocycline is a static agent, and there are limited data on its use in the management and outcome of war wounds. The other agent, colistin, has traditionally been thought to have significant toxicity. This has not been our experience, however, and colistin appears to be effective (data not shown).

At this time, there are few choices for therapy of ABC infection. ABC and its associated resistance patterns have the ability to greatly impact future medical care and health care resources, necessitating a continued focus on the development of alternative treatment regimens.

The views expressed herein are those of the authors and do not reflect the official policy or position of the Department of the Army, the Department of Defense, or the U.S. government.

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