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Increased Antimicrobial Susceptibility Profiles among Polymyxin B-Resistant *Acinetobacter baumannii* Clinical Isolates

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SENTRY ANTIMICROBIAL SURVEILLANCE

ABSTRACT

Objective: Infections caused by multidrug-resistant (MDR) *Acinetobacter baumannii* have become a major treatment challenge, requiring the re-introduction of polymyxins into clinical practice. Given this situation, the emergence of polymyxin B resistance is expected. Recently, increased antimicrobial susceptibility among *in vitro* passaging of colistin-resistant *A. baumannii* isolates was observed when compared with the parent colistin-susceptible strains. The aim of this study was to compare the susceptibility profile between polymyxin B-resistant and -susceptible *A. baumannii* patient isolates submitted to the SENTRY Antimicrobial Surveillance Program.

Methods: A collection of 3,707 *A. baumannii* clinical isolates was susceptibility tested by the CLSI broth microdilution method and the susceptibility rates of epidemiologically unrelated polymyxin B-resistant isolates were compared to those of polymyxin B-susceptible isolates. Susceptibility rates were analyzed by χ^2 test using the Epi Info™ Version 3.4.1 software package. *P* values <0.05 were considered to be statistically significant.

Results: The majority of the antimicrobials tested showed limited spectrums of activity (susceptibility rates, ≤55.4%) against the polymyxin B-susceptible *A. baumannii* group, except for imipenem (73.2%), meropenem (76.5%) and some tetracyclines. Doxycycline, minocycline and tigecycline showed the highest susceptibility rates 74.7, 91.7 and 97.0%, respectively. Overall, the polymyxin B-resistant group showed a higher susceptibility rate for the vast majority of antimicrobials tested. This shift was not observed among those antimicrobial agents with higher activity against the polymyxin B-susceptible group (i.e. carbapenems and tetracyclines). Statistically significant differences were observed among most drugs showing lower activity, including ampicillin/sulbactam (susceptibility rate 3x higher), aztreonam (4x), cefoxitin (20x), ceftriaxone (2x) and cefuroxime (8x). Tigecycline was highly active regardless the susceptibility to polymyxin B.

Conclusions: Polymyxin B-resistant *A. baumannii* clinical isolates showed higher susceptibility rates when compared to polymyxin B-susceptible isolates for the majority of antimicrobials tested. These findings suggest that possible lipopolysaccharide modifications among polymyxin B-resistant bacterial cells may increase permeability to the antimicrobial agents. These data may provide additional insights for combination therapeutic options and also for possible novel antimicrobial agents in drug development.

INTRODUCTION

Acinetobacter baumannii is an opportunistic pathogen found in many health care environments. This microorganism often harbors a wide range of resistance determinants. Consequently, infections caused by multidrug-resistant (MDR) *A. baumannii* have become a major treatment challenge, requiring the re-introduction of polymyxins into clinical practice. Given this situation, the emergence of a polymyxin B resistance phenotype would be expected.

Recently, increased antimicrobial susceptibility among colistin-resistant *A. baumannii* isolates selected by serial passaging was observed when compared with the parent colistin-susceptible strains (Li et al, Clin Infect Dis 2007; 45: 594-8). The aim of this study was to compare the susceptibility test profiles between polymyxin B-resistant and -susceptible *A. baumannii* isolates submitted to the SENTRY Antimicrobial Surveillance Program (2001-2007).

MATERIALS AND METHODS

Bacterial isolates. A total of 3,707 *A. baumannii* clinical isolates collected as part of the global SENTRY Program during the 2001-2007 period were included in this study. These isolates were consecutively collected from hospitalized patients with one isolate per patient included in the study. Species identification was confirmed by standard biochemical tests and use of the Vitek System (bioMerieux, Hazelwood, MO), when necessary.

Antimicrobial susceptibility testing. Isolates were tested for antimicrobial susceptibility using the broth microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS; M7-A7, 2006). Cation-adjusted Mueller-Hinton broth was used in validated panels manufactured by TREK Diagnostics (Cleveland, OH). For comparison purposes, geographically unrelated polymyxin B-resistant isolates were included in this evaluation and the resistance phenotype was confirmed by Etest (AB BIODISK, Solna, Sweden). Quality control (QC) was performed using *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 27853. All QC results were within ranges as published in CLSI documents.

Categorical interpretations. Categorical interpretations for antimicrobials were those found in the M100-S18 document (CLSI, 2008) for *Acinetobacter* spp., except for aztreonam, cefoxitin and cefuroxime, which were interpreted according to the Enterobacteriaceae breakpoints. Tigecycline MIC results were interpreted according to the Enterobacteriaceae breakpoints approved by the United States Food and Drug Administration (USA-FDA; breakpoint for susceptibility, ≤2 mg/L; breakpoint for resistance, ≥8 mg/L).

Statistical analysis. Susceptibility rates of 24 polymyxin B-resistant isolates were compared to those obtained from 3,683 polymyxin B-susceptible isolates by the χ^2 test using the Epi Info™ Version 3.4.1 software package (Centers for Disease Control and Prevention, Atlanta, USA). *P* values < 0.05 were considered to be significant.

RESULTS

- Most drugs demonstrated limited spectrums of activity against the polymyxin B-susceptible *A. baumannii* group (susceptibility rates ≤53.8%), except for the carbapenems (imipenem [71.6% susceptible] and meropenem [68.0% susceptible]), tetracyclines (doxycycline [74.6% susceptible], minocycline [88.4% susceptible] and tigecycline [97.0% inhibited at ≤2 mg/L]; Table 1).
- Overall, the polymyxin B-resistant group showed higher susceptibility rates for the majority of antimicrobials (16 of 22) when compared to those from the polymyxin B-susceptible group. The differences were most apparent in the polymyxin B-resistant group among those drugs showing lower susceptibility rates (≤48.8%) and were statistically significant for the ß-lactams ampicillin/sulbactam, aztreonam, cefoxitin, cefepime, ceftriaxone, and cefuroxime ($P < 0.05$; Table 1).
- Antimicrobials with better activity, such as imipenem, meropenem, tobramycin, minocycline and tigecycline, showed similar or slightly higher activity against the polymyxin B-susceptible isolates when compared to those that were polymyxin B-resistant.

Table 1. MIC_{50}, antimicrobial susceptibility profile and comparative evaluation of susceptibility rates between 3,683 polymyxin B-susceptible *A. baumannii* and 24 polymyxin B-resistant *A. baumannii.*

	MIC_{50} (mg/L)/ % of susceptibility[a]			
Antimicrobial agents	Polymyxin-susceptible phenotype[a]	Polymyxin-resistant phenotype[a]	*P*[b]	OR (95 CI %)[b]
Ampicillin/sulbactam	16/45.4	4/70.8	.013	2.92 (1.14-7.76)
Piperacillin	>64/21.0	64/33.3	.138	1.88 (0.74-4.69)
Piperacillin/tazobactam	>64/33.2	32/45.8	.187	1.71 (0.71-4.07)
Ticarcillin/clavulanate	128/29.3	64/33.3	.761	1.14 (0.54-2.83)
Aztreonam[c]	>16/4.9	>16/20.8	<.001	5.21 (1.68-15.0)
Cefoxitin[c]	>16/0.9	>16/20.8	<.001	30.0 (9.18-92.4)
Cefepime	16/38.4	4/58.3	.046	2.24 (0.93-5.44)
Ceftazidime	>16/34.5	>16/45.8	.240	1.61 (0.67-3.84)
Ceftriaxone	>32/16.2	32/41.6	<.001	3.7 (1.52-8.89)
Cefuroxime[c]	>16/2.6	>16/25.0	<.001	12.72 (4.41-34.97)
Imipenem	1/71.6	1/66.6	.589	0.79 (0.32-2.02)
Meropenem	2/68.0	4/58.3	.314	0.66 (0.28-1.60)
Ciprofloxacin	>4/32.7	1/50.0	.072	2.06 (0.86-4.90)
Levofloxacin	>4/35.6	≤0.5/50.0	.142	1.81 (0.76-4.31)
Amikacin	32/48.8	8/58.3	.355	1.46 (0.61-3.55)
Gentamicin	>8/37.0	4/50.0	.190	1.70 (0.71-4.05)
Tobramycin	4/53.8	8/45.8	.434	0.73 (0.30-1.73)
Minocycline	≤1/88.4	≤1/87.5	.885	0.91 (0.26-3.89)
Tigecycline[d]	0.5/97.0	0.5/95.8	.729	0.70 (0.10-14.16)
Doxycycline	≤1/74.6	≤1/87.5	.150	2.37 (0.67-10.03)
Tetracycline	8/43.1	≤4/58.3	.133	1.85 (0.77-4.49)
Trimethoprim/sulfamethoxazole	>2/37.9	≤2/54.2	.420	1.39 (0.58-3.31)

a. Category of susceptibility for antimicrobials determined as specified by the M100-S18 CLSI document for *Acinetobacter spp.*
b. *P* value was calculated by χ^2 test; OR and respective 95% CI refer to comparisons of susceptibility rates between polymyxin-resistant *A. baumannii* and polymyxin-susceptible *A. baumannii* for each antimicrobial agent.
c. Category of susceptibility for aztreonam, cefoxitin and cefuroxime determined as specified by the M100-S18 CLSI document for Enterobacteriaceae.
d. Category of susceptibility for tigecycline determined according to the Enterobacteriaceae breakpoints approved by the United States Food and Drug Administration (USA-FDA; breakpoints for susceptibility, ≤2 mg/L; breakpoints for resistance, ≥8 mg/L).

CONCLUSIONS

- It has been postulated that *A. baumannii* showing phenotypic resistance to colistin may possess outer-membrane modifications, consequently increasing permeability for other antimicrobial agents, which may also explain the results obtained in our study.
- Regardless of outer-membrane modifications, they were not favorable for those antimicrobial agents frequently used for treatment of infections caused by MDR *A. baumannii*, such as carbapenems and tobramycin. Furthermore, minocycline and the novel glycylcycline tigecycline, were highly active independent of the polymyxin susceptibility profile.
- It is important to mention that other resistance mechanisms were present among the polymyxin B-resistant isolates, since the extent of the susceptibility increases were modest, except for ampicillin/sulbactam, for which the susceptibility rate increased substantially (45.4% to 70.8%; Table 1).
- This study provides additional insights for combination therapeutic options and for possible novel antimicrobial agents in drug development, and also indicates that local susceptibility testing results will be important to guide treatment.

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