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Characterization of Antibiotic Susceptibility Profiles of Extensively- and Pan-Drug Resistant *Acinetobacter baumannii* Clinical Isolates

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Background

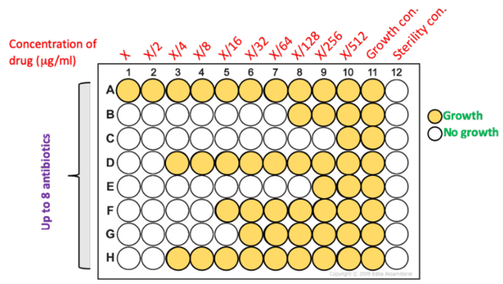
Acinetobacter baumannii is a ubiquitous, Gram-negative bacterium that is an opportunistic human pathogen (1). It is a major cause of hospital-acquired infections, particularly among critically-ill and immunocompromised individuals. Manifestations of *A. baumannii* infections include nosocomial pneumonia, bacteremia, urinary-tract infections, and less commonly, osteomyelitis, endocarditis, meningitis, and necrotizing fasciitis (2, 3).

A. baumannii infections have become increasingly problematic in recent years, as these bacteria rapidly acquire antibiotic resistance, leading to the emergence of multidrug, extensively drug and pan drug-resistant (MDR, XDR, and PDR, respectively) isolates. XDR strains are strains resistant to all antibiotics in a drug class, except for two or fewer classes, while PDR strains are resistant to every available antibiotics (4). The carbapenems, imipenem and meropenem are first-line, standard treatment options for *A. baumannii* infections, however, carbapenem-resistance is on the rise and is now more common (5). Several mechanisms of antibiotic resistance are commonly found among *A. baumannii* isolates, including the production of aminoglycoside-modifying enzymes and beta-lactamases, alteration of penicillin-binding proteins, overexpression of efflux pumps, and reduction in the permeability of the outer membrane (6).

According to the Center for Disease Control and Prevention (CDC), *A. baumannii* is an urgent public health threat due to the difficulty in eradicating them from the environment and the emergence of highly drug-resistant strains (7). In fact, *A. baumannii* has the highest rate of drug resistance of any Gram-negative pathogen that causes nosocomial infections (8,9). Recently, Cooper University Hospital (CUH) experienced a large increase in highly drug-resistant *A. baumannii* infections, which had a mortality rate of 60%. Often times, physicians had to turn to combinations of drugs with no experimental verification or historically shelfed antibiotics, such as the polymyxins, in a desperate attempt to save lives (10). This highlights the critical need for more research to identify new, effective treatment options for these difficult-to-treat infections. Here, we determined the susceptibility of 22 patient isolates from CUH against 22 standard-of-care drugs and three newly released antibiotics (eravacycline, omadacycline and plazomicin). We plan on exploring novel combinations of eravacycline and omadacycline with the standard-of-care drugs, to search for synergistic combinatorial effects using checkerboard assays. This information can be ultimately be used to design new treatment regimens against drug-resistant *A. baumannii* infections.

Materials & Methods

- 22 de-identified *A. baumannii* isolates were collected during routine diagnostic workup at Cooper University Hospital in Camden, NJ, during an increase in MDR infections that occurred from 2018-2019.
- Strains were inoculated into Mueller-Hinton broth (MHB) and grown overnight in a 37°C shaking incubator. Bacterial growth was monitored by measuring the optical density at 600 nm (OD_{600}) when necessary.
- Determination of the minimum inhibitory concentrations (MICs) of 22 antibiotics was performed by broth microdilution, according to standard protocols. A single colony of each strain to be tested was inoculated into 5 ml of MH broth and allowed to grow for 16 hours overnight with aeration at 37°C. The next day, the OD_{600} was determined and cells were diluted into fresh MHB at a starting OD of 0.05. Each drug to be tested was added to the first well of a row in a flat-bottom, 96-well plate. Two-fold serial dilutions were performed and an equal volume of diluted cells added to each well. The plates were incubated overnight in a 37°C incubator, without shaking. The following day, the OD_{600} values were read using a Synergy H1 Microplate reader (Biotek). For each strain, the MICs determinations were made at least two independent times.



Results

CLASS	ANTIBIOTIC	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12	M13	M14	M15	M16	M17	M18	M19	M20	M21	M22
Aminoglycosides	Gentamicin	NS	NS	NS	NS	NS	S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Amikacin	NS	S	NS	NS	NS	NS	S	NS	NS	S	S	NS	S	NS	S	S	NS	S	NS	NS	NS	NS
	Tobramycin	NS	NS	S	NS	NS	S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Netilmicin	NS	NS	NS	NS	NS	S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Plazomicin	NS	NS	S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Tetracyclines	Doxycycline	NS	NS	NS	NS	NS	S	NS	S	NS	S	S	NS	NS	NS	NS	S	NS	S	NS	NS	NS	S
	Tetracycline	NS	NS	NS	NS	NS	NS	NS	NS	NS	S	S	NS	NS	NS	NS	S	NS	S	NS	NS	NS	S
	Tigecycline	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Minocycline	S	S	S	S	NS	S	NS	S	NS	S	S	S	S	S	NS	S	NS	S	NS	NS	NS	S
	Omadacycline	NS	NS	S	S	S	S	S	NS	S	S	S	S	S	S	S	S	NS	S	S	S	S	S
	Eravacycline	S	S	S	S	NS	NS	NS	NS	S	S	S	S	S	S	S	S	NS	S	S	S	S	S
Extended Spectrum Cephalosporins	Ceftazedime	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Ceftriaxone	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Cefotaxime	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Cefepime	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	S	NS	S	NS	NS	NS	NS	NS	NS	NS	NS	NS
Antipseudomonal Carbapenems	Imipenem	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Meropenem	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	S	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Doripenem	NS	NS	NS	NS	NS	NS	NS	NS	NS	S	S	NS	S	NS	NS	NS	NS	NS	NS	NS	NS	NS
Antipseudomonal Fluoroquinolones	Ciprofloxacin	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Levofloxacin	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Penicillin + B-lactamase Inhibitors	Amp/Sul	NS	S	NS	NS	NS	NS	NS	NS	NS	NS	S	NS	S	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Pip/Tazo	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
	Timentin	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Folate Pathway Inhibitors	TMP/SMX	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Antitubercular	Rifampin	S	NS	S	NS	NS	NS	S	S	NS	NS	S	NS	S	NS	NS	S	S	S	S	NS	S	S
Resistance Classification		XDR	XDR	XDR	XDR	PDR	XDR	XDR	XDR	PDR	XDR	XDR	XDR	XDR	XDR	XDR	XDR	PDR	XDR	PDR	PDR	PDR	XDR

Table 1: Antibiotic susceptibility profiles of 22 *A. baumannii* isolates. Strains that are susceptible are denoted as S, and those determined to have intermediate susceptibility or resistance are denoted as NS (non-susceptible). Each isolate is also classified in terms of drug resistance, as either extensively-drug resistant (XDR) or pan-drug resistant (PDR).

CLASS	Antibiotic	Percentage of Non-Susceptible Strains
Aminoglycosides	Gentamicin	95.45%
	Amikacin	59.09%
	Tobramycin	81.82%
	Netilmicin	90.91%
	Plazomicin	95.45%
Tetracyclines	Doxycycline	59.09%
	Tetracycline	72.73%
	Tigecycline	100%
	Minocycline	22.73%
	Omadacycline	18.18%
	Eravacycline	22.73%
Extended Spectrum Cephalosporins	Ceftazedime	95.45%
	Ceftriaxone	95.45%
	Cefotaxime	90.91%
	Cefepime	90.91%
Antipseudomonal Carbapenems	Imipenem	100%
	Meropenem	90.91%
	Doripenem	86.36%
Antipseudomonal Fluoroquinolones	Ciprofloxacin	100%
	Levofloxacin	100%
Penicillin + B-lactamase Inhibitors	Amp/Sul	86.36%
	Pip/Tazo	100%
	Timentin	100%
Folate Pathway Inhibitors	TMP/SMX	100%
Antitubercular	Rifampin	45.45%

Table 2: Percentage of strains found to be non-susceptible to each antibiotic.

Table 1 summarizes the antibiotic susceptibility profiles of each *A. baumannii* isolate, as well as each isolate's drug resistance classification. Strains that had intermediate susceptibility or resistance to a given antibiotic are labeled as non-susceptible (NS). Notably, in this collection of isolates there were no multidrug-resistant strains, which are defined as resistant to at least one drug in less than three classes. Rather, 16 (72.7%) isolates were extensively-drug resistant (XDR) and 6 (27.3%) isolates were pan-drug resistant (PDR). Table 2 provides the percentages of isolates that were found to be non-susceptible to each antibiotic.

The *A. baumannii* isolates displayed varying susceptibility to the aminoglycoside antibiotics tested. Among the 22 isolates, there were high rates of non-susceptibility to the commonly used Gentamicin (95.45%), Tobramycin (81.82%), and Netilmicin (90.91%), and even to the relatively new antibiotic Plazomicin (95.45%). Amikacin was the most effective aminoglycoside tested, but the majority of strains (59.09%) were non-susceptible.

There were also highly varied responses to the tetracycline antibiotics. There was complete non-susceptibility to Tigecycline (100.0%) and relatively high rates of non-susceptibility to Tetracycline (72.73%) and Doxycycline (59.09%). However, there were low non-susceptibility rates in response to Minocycline (22.73%), and the new tetracycline-class drugs Omadacycline (18.18%) and Eravacycline (22.73%). While official breakpoint data is not available from the Clinical Laboratory Standards Institute for the new tetracycline-class drugs, we made these determinations based upon values for other tetracycline class drugs to approximate. Even so, the relatively low MIC value s obtained suggest efficacy.

There were high rates of non-susceptibility to all extended-spectrum cephalosporins tested - Ceftazedime (95.45%), Ceftriaxone (95.45%), Cefotaxime (90.91%), and Cefepime (90.91%), and complete non-susceptibility to both antipseudomonal fluoroquinolones tested - Ciprofloxacin (100.0%) and Levofloxacin (100.0%).

Notably, there were also high rates of non-susceptibility to all carbapenems tested - Meropenem (90.91%), Doripenem (86.36%), and Imipenem (100.0%). High rates of non-susceptibility were also noted in response to the penicillin/β-lactamase inhibitor combinations Ampicillin/Sulbactam (86.36%), Pipercillin/Tazobactam (100.0%), and Timentin (100.0%), as well as the folate pathway inhibitor Trimethoprim/Sulfamethoxazole (100.0%).

Interestingly, there was a comparatively low non-susceptibility rate in response to the anti-tubercular drug Rifampin (45.45%), which is not traditionally used in the treatment of *Acinetobacter* infections.

Discussion

- None of the 22 clinical isolates were found to be MDR, with all samples being highly drug resistant, as either XDR and PDR.
- There were concerning levels of non-susceptibility to antimicrobials of every antibiotic class assessed, especially against those typically reserved for treating MDR species, such as Tigecycline, and the carbapenems.
- Plazomicin, which was recently approved for medical use in the U.S. in 2018, was found to be ineffective against these strains, with 95.45% of strains being non-susceptible. This follows a worrying trend of growing resistance against aminoglycosides in *A. baumannii*, primarily due to expression of various aminoglycoside-modifying enzymes.
- Omadacycline and Eravacycline were both found to be quite effective, with only 22.73% and 27.27% of strains being non-susceptible, respectively. Previous research has shown that both Omadacycline and Eravacycline retain activity against strains expressing both efflux pumps and ribosomal protection mechanisms. Our findings further suggest that Omadacycline and Eravacycline could be implemented as possible standard treatments of drug-resistant *A. baumannii* infections.
- One notable finding from this study is the comparable effectiveness of Rifampin, with 45.5% of strains being non-susceptible. Traditionally used as a antimycobacterial agent for tuberculosis infection, Rifampin has had minimal use in the treatment of *A. baumannii* infections. Our findings suggest that Rifampin may be used as an alternative treatment for *A. baumannii* infections and perhaps further investigation into the effectiveness of other rifamycins and antimycobacterial agents should be performed.
- Moving forward, we plan to evaluate novel combinations of the aforementioned antimicrobial agents, with the goal of identifying synergistic combinations that are effective in treating *A. baumannii*. Furthermore, we plan to evaluate the activity of Cefiderocol, a new siderophore cephalosporin antibiotic that utilizes a novel entry mechanism, whereby the siderophore binds to iron and is actively transported into the bacterial cell. Cefiderocol has been approved for use in treating MDR gram-negative bacteria, and may be useful in treating drug-resistant *A. baumannii*.

Acknowledgements & References

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