



Moving towards a standardized definition of antimicrobial resistance: a comparison of the antimicrobial susceptibility profile of difficult-to-treat resistance (DTR) versus multidrug-resistant (MDR) *Pseudomonas aeruginosa* clinical isolates (CANWARD, 2016-2021)

A. Walkty^{a,b,*}, J.A. Karlowsky^{a,b}, P.R.S. Lagacé-Wiens^{a,b}, M.R. Baxter^a, H.J. Adam^{a,b}, D.C. Bay^a, G.G. Zhanel^a

^a Department of Medical Microbiology and Infectious Diseases, Max Rady College of Medicine, University of Manitoba, Winnipeg, Canada

^b Shared Health, Winnipeg, Canada

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ABSTRACT

Pseudomonas aeruginosa clinical isolates demonstrating difficult-to-treat resistance (DTR) and multidrug-resistant (MDR) phenotypes were evaluated by broth microdilution. Susceptibility was lower for all antimicrobials versus DTR relative to MDR isolates. Ceftazidime-avibactam, ceftolozane-tazobactam, and imipenem-relebactam susceptibility was 35.9%, 64.5%, and 47.0% for DTR isolates and 60.5%, 80.6%, and 71.5% for MDR isolates.

Pseudomonas aeruginosa is an important nosocomial pathogen [1,2]. Some investigators have reported an association between multidrug resistance among *P. aeruginosa* and adverse patient outcomes, including increased mortality [3–5]. However, interpreting the significance of a multidrug-resistant (MDR) phenotype is complicated by various definitions applied in both clinical and surveillance studies [6]. In 2012, Magiorakos *et al.*, proposed that *P. aeruginosa* isolates be considered MDR if they test not susceptible to one or more antimicrobials from at least three of eight antimicrobial classes [6]. While this definition provided a standardized way to categorize isolates as MDR, a major limitation was that all eight antimicrobial classes were given equal weight, even though some classes are much less preferred by clinicians due to toxicity and/or suboptimal efficacy (e.g., aminoglycosides, polymyxins) [7,8]. The MDR definition has also not been modified despite several changes to breakpoints by the Clinical and Laboratory Standards Institute (CLSI) including removal of gentamicin breakpoints, and elimination of the susceptible breakpoint for colistin [9].

Recognizing these limitations, in 2018 Kadri *et al.*, introduced the concept of a “difficult-to-treat” resistance (DTR) phenotype, defined as isolates testing intermediate or resistant to all reported carbapenems, beta-lactams, and fluoroquinolones [10]. The primary purpose of this study is to compare the antimicrobial susceptibility profile of DTR *P. aeruginosa* with MDR *P. aeruginosa*, using clinical isolates obtained

from patients at Canadian hospitals. A secondary objective of this study is to evaluate the impact of a recent change to the CLSI tobramycin breakpoints on the susceptibility of DTR and MDR isolates.

P. aeruginosa clinical isolates were obtained from patients evaluated at hospitals across Canada (January 2016 to December 2021) as part of CANWARD, an ongoing, national, Health Canada partnered antimicrobial resistance surveillance study [1]. Tertiary-care medical centres submitted pathogens from patients attending hospital clinics, emergency rooms, medical and surgical wards, and intensive care units to a coordinating laboratory (Health Sciences Centre, Winnipeg, Canada). All isolates were deemed clinically significant based on local site criteria. Isolates were shipped on Amies semi-solid transport media to the coordinating laboratory (Health Sciences Centre, Winnipeg, Canada), sub-cultured onto appropriate media, and stocked in skim milk at -80°C until MIC testing was performed. Isolate identification was performed by the submitting site and confirmed at the reference site as required (i.e., when morphological characteristics and antimicrobial susceptibility patterns did not fit the reported identification).

Following two subcultures from frozen stock, susceptibility testing was carried out by broth microdilution in accordance with the CLSI guidelines using custom broth microdilution panels [11]. MICs were interpreted according to current (2023) CLSI breakpoints with the exception of tobramycin (interpreted using both the 2022 and 2023

* Corresponding author at: MS673B, Microbiology, Health Sciences Centre, 820 Sherbrook Street, Winnipeg, Manitoba, R3A 1R9, Canada. Phone: (204) 787-1161.
E-mail address: AWalkty@sharedhealthmb.ca (A. Walkty).

Table 1
In vitro activity of common antipseudomonal antimicrobials versus DTR and MDR *P. aeruginosa* clinical isolates.

Antimicrobial	DTR <i>P. aeruginosa</i> (n = 166)				MDR <i>P. aeruginosa</i> (n = 495)			
	MIC ₅₀ (μg/mL)	MIC ₉₀ (μg/mL)	Range (μg/mL)	% Susceptible	MIC ₅₀ (μg/mL)	MIC ₉₀ (μg/mL)	Range (μg/mL)	% Susceptible
Ceftazidime	>32	>32	16 - >32	0	>32	>32	1 - >32	12.7
Cefepime	32	>64	16 - >64	0	16	64	2 - >64	24.4
Ciprofloxacin	4	>16	1 - >16	0	2	16	≤0.06 - >16	28.7
Colistin	1	2	0.12 - 16	-	1	2	≤0.06 - >16	-
Imipenem	32	>32	4 - >32	0	16	32	0.25 - >32	16.6
Meropenem	16	>32	4 - >32	0	8	32	≤0.03 - >32	26.9
Piperacillin-tazobactam	256	512	32 - >512	0	64	512	≤1 - >512	17.4
Tobramycin (2022)	1	32	≤0.5 - >64	77.1	1	32	≤0.5 - >64	80.6
Tobramycin (2023)	1	32	≤0.5 - >64	55.4	1	32	≤0.5 - >64	59.4
Ceftazidime-avibactam *	16	>16	1 - >16	35.9	8	>16	0.12 - >16	60.5
Ceftolozane-tazobactam	4	64	1 - >64	64.5	2	8	≤0.12 - >64	80.6
Imipenem-relebactam	4	8	0.25 - >32	47.0	2	8	0.12 - >32	71.5

DTR isolates: not susceptible (intermediate or resistant) to all of the following antimicrobials: imipenem, meropenem, piperacillin-tazobactam, ceftazidime, cefepime, and ciprofloxacin.

MDR isolates: not susceptible (intermediate or resistant) at least one antimicrobial from three or more of the following categories: antipseudomonal carbapenems (imipenem, meropenem), antipseudomonal cephalosporins (ceftazidime, cefepime), antipseudomonal penicillins (piperacillin-tazobactam), aminoglycosides (tobramycin 2023 breakpoints), fluoroquinolones (ciprofloxacin).

* Susceptibility of ceftazidime-avibactam was only determined for 142 DTR isolates and 428 MDR isolates.

breakpoints) [9,12]. DTR isolates were defined as those testing not susceptible to all of the following antimicrobials: imipenem, meropenem, piperacillin-tazobactam, ceftazidime, cefepime, and ciprofloxacin [10]. MDR isolates were defined as those testing not susceptible to at least one antimicrobial from three or more of the following categories: antipseudomonal carbapenems (imipenem, meropenem), antipseudomonal cephalosporins (ceftazidime, cefepime), antipseudomonal penicillins (piperacillin-tazobactam), aminoglycosides (tobramycin), and fluoroquinolones (ciprofloxacin) [6]. The MDR definition was evaluated with both the 2022 and 2023 CLSI breakpoints for tobramycin. Newer beta-lactam-beta-lactamase inhibitor combinations (ceftazidime-avibactam, ceftolozane-tazobactam, and imipenem/relebactam) were not included in either definition, such that the *in vitro* activity of these agents could be determined versus the isolate subsets they will most likely be used to treat in clinical practice.

Of 1912 *P. aeruginosa* isolates recovered as a part of CANWARD, 166 (8.7%) and 495 (25.9%) demonstrated a DTR and MDR phenotype, respectively. The distribution of DTR *P. aeruginosa* isolates by specimen source was as follows: 88.6% respiratory, 7.2% wound, 3.0% blood, and 1.2% urine. The majority of DTR *P. aeruginosa* isolates (80.7%) were obtained from inpatients (ICU, medical wards, surgical wards), with only 19.3% recovered from outpatients (emergency rooms, clinics). The distribution of MDR *P. aeruginosa* isolates by specimen source was: 84.2% respiratory, 8.7% wound, 4.2% blood, and 2.8% urine. Similar to DTR *P. aeruginosa*, MDR isolates were predominantly recovered from hospital inpatients.

The antimicrobial susceptibility profile of DTR and MDR *P. aeruginosa* isolates is presented in Table 1. The MDR isolates were comprised of 53 distinct phenotypes (Supplemental Table 1). Related to the more heterogeneous definition of resistance for MDR isolates, a small proportion of these remained susceptible to ceftazidime, cefepime, ciprofloxacin, imipenem, meropenem, and piperacillin-tazobactam. In contrast, DTR isolates were not susceptible to any of these antimicrobials. For MDR isolates, susceptibility to tobramycin was 80.6% and 59.4% using the 2022 and 2023 CLSI susceptibility breakpoints, respectively. For DTR isolates, susceptibility to tobramycin was 77.1% and 55.4% applying the 2022 and 2023 breakpoints. The proportion of *P. aeruginosa* isolates meeting the definition of MDR was minimally altered by the recent change in the CLSI susceptibility breakpoint for tobramycin; 24.7% using the 2022 breakpoint versus 25.9% applying the 2023 breakpoint. As aminoglycosides are not included in the DTR definition, the recent change to the tobramycin susceptibility breakpoint had no impact on the number of DTR isolates.

DTR *P. aeruginosa* isolates were less susceptible to newer beta-lactam-beta-lactamase inhibitor combinations than MDR isolates. Among the three newer beta-lactam-beta-lactamase inhibitor combinations evaluated, ceftolozane-tazobactam demonstrated the greatest *in vitro* activity versus both DTR and MDR subsets, followed by imipenem-relebactam, and then ceftazidime-avibactam. The proportion of DTR and MDR isolates testing susceptible to ceftolozane-tazobactam was 64.5% and 80.6%, respectively. The proportion of DTR and MDR isolates testing susceptible to imipenem-relebactam was 47.0% and 71.5%, respectively. Finally, the proportion of DTR and MDR isolates testing susceptible to ceftazidime-avibactam was 35.9% and 60.5% respectively. As a limitation of this analysis, it should be noted that susceptibility to ceftazidime-avibactam was not determined for a small number of DTR and MDR isolates.

Kadri *et al.* provide support for the clinical relevance of a DTR phenotype [10]. These investigators performed a retrospective cohort analysis evaluating treatment outcomes for patients with Gram-negative bacteremia in the setting of resistance to first-line antimicrobials. They demonstrated that a DTR phenotype was associated with decreased patient survival [10]. The association between a DTR phenotype and increased mortality in the setting of bacteremia was also reported by Huh *et al.* in a second retrospective study [13].

In summary, the DTR phenotype defines a more homogeneous group of *P. aeruginosa* clinical isolates with exceptionally limited treatment options. This definition is not impacted by recent changes to aminoglycoside breakpoints made by CLSI, including the elimination of breakpoints for gentamicin and the lowering of breakpoints for tobramycin, in contrast to the definition of MDR. Broad application of the DTR definition among surveillance studies would allow for better comparison of clinically relevant resistance among *P. aeruginosa* in different geographic locations. Further, this definition allows clinicians to more precisely estimate the potential clinical utility of newer beta-lactam-beta-lactamase inhibitor combinations by determining the *in vitro* activity of these antimicrobials against the subset of isolates for which they would most likely be employed.

Author Contribution Statement

AW: writing and editing the manuscript. JAK: reviewing and editing the manuscript. PRSLW: reviewing and editing the manuscript. MRB: data analysis, reviewing and editing the manuscript. HJA: planning the study, reviewing and editing the manuscript. DCB: reviewing and editing the manuscript. GGZ: planning the study, reviewing and editing the

manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

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