



Introduction

Interpreting the significance of a multidrug-resistant (MDR) phenotype is complicated by various definitions applied in both clinical and surveillance studies. Recognizing these limitations, in 2018, Kadri *et al.* introduced the concept of a “difficult-to-treat” (DTR) resistance phenotype, defined as isolates testing intermediate or resistant to all reported β -lactams (including carbapenems) and fluoroquinolones [1]. The purpose of this study was to determine the proportion of *Enterobacterales* and *Pseudomonas aeruginosa* recently recovered from patients at Canadian hospitals that demonstrate a DTR phenotype, and to evaluate the *in vitro* activity of newer β -lactam- β -lactamase inhibitor (BL-BLI) combination agents versus these isolates.

Materials and Methods

Enterobacterales and *P. aeruginosa* clinical isolates were obtained from patients evaluated at hospitals across Canada from January 2016 to December 2023 as part of an ongoing national surveillance study (CANWARD).

Antimicrobial susceptibility testing was carried out using custom broth microdilution panels, with MICs interpreted according to 2024 CLSI M100 breakpoints [2, 3].

DTR isolates were defined as those testing not susceptible to carbapenems (ertapenem, imipenem, meropenem), cephalosporins (ceftazidime, ceftriaxone, cefepime), piperacillin-tazobactam, and ciprofloxacin.

Piperacillin-tazobactam susceptible-dose dependent *Enterobacterales* isolates were considered susceptible and excluded from the DTR isolate subset.

Ceftazidime-avibactam and meropenem-vaborbactam were only tested against 7109 and 1056, respectively, (of 7936) non-DTR *Enterobacterales*. Ceftazidime-avibactam and meropenem-vaborbactam were only tested against 1987 and 443 (of 2228) non-DTR *P. aeruginosa*.

References

1. Kadri SS, Adjemian J, Lai YL, *et al.* Difficult-to-treat resistance in Gram-negative bacteremia at 173 US hospitals: retrospective cohort analysis of prevalence, predictors, and outcome of resistance to all first-line agents. Clin Infect Dis 2018; 67: 1803-14.

2. CLSI. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically - Eleventh Edition: M07. 2018.

3. CLSI. Performance Standards for Antimicrobial Susceptibility Testing – Thirty-fourth Edition: M100. 2024.

Results

1. Of 7952 *Enterobacterales* isolates recovered as a part of CANWARD, only 16 (0.2%) demonstrated DTR.
2. The DTR *Enterobacterales* isolates were recovered from blood (n = 7), respiratory (n = 6), and wound (n = 3) specimens.
3. Of 2414 *P. aeruginosa* isolates recovered as a part of CANWARD, 186 (7.7%) demonstrated DTR.
4. The DTR *P. aeruginosa* isolates were primarily recovered from a respiratory source (89.2%, 166/186).

Table 1. The *in vitro* activity of newer β -lactam- β -lactamase inhibitor combination agents and relevant comparators versus the DTR isolates of *Enterobacterales* and *P. aeruginosa*.

Organism	MIC ₅₀	MIC ₉₀	% Susceptible
Antimicrobial agent (µg/mL) (µg/mL)			
DTR <i>Enterobacterales</i> (n = 16)^a			
Ceftazidime-avibactam	1	>16	81.3
Ceftolozane-tazobactam	64	>64	0
Imipenem-relebactam	1	>32	62.5
Meropenem-vaborbactam	1	>32	68.8
Ceftriaxone	>64	>64	0
Ceftazidime	>32	>32	0
Colistin	0.5	>16	0
Meropenem	8	>32	0
Piperacillin-tazobactam	>512	>512	0
Ciprofloxacin	>16	>16	0
Gentamicin	1	>32	68.8
Tobramycin	16	>64	31.3
TMP-SMX	>8	>8	12.5
DTR <i>P. aeruginosa</i> (n = 186)			
Ceftazidime-avibactam	16	>16	44.6
Ceftolozane-tazobactam	4	64	65.1
Imipenem-relebactam	4	8	46.8
Meropenem-vaborbactam	16	>32	36.6
Ceftazidime	>32	>32	0
Cefepime	32	>64	0
Meropenem	16	>32	0
Piperacillin-tazobactam	256	512	0
Ciprofloxacin	4	>16	0
Colistin	1	2	0
Tobramycin	1	64	53.8

^a 7 *Klebsiella pneumoniae*, 6 *Escherichia coli*, 1 *Enterobacter cloacae*, 1 *Morganella morganii*, 1 *Serratia marcescens*.

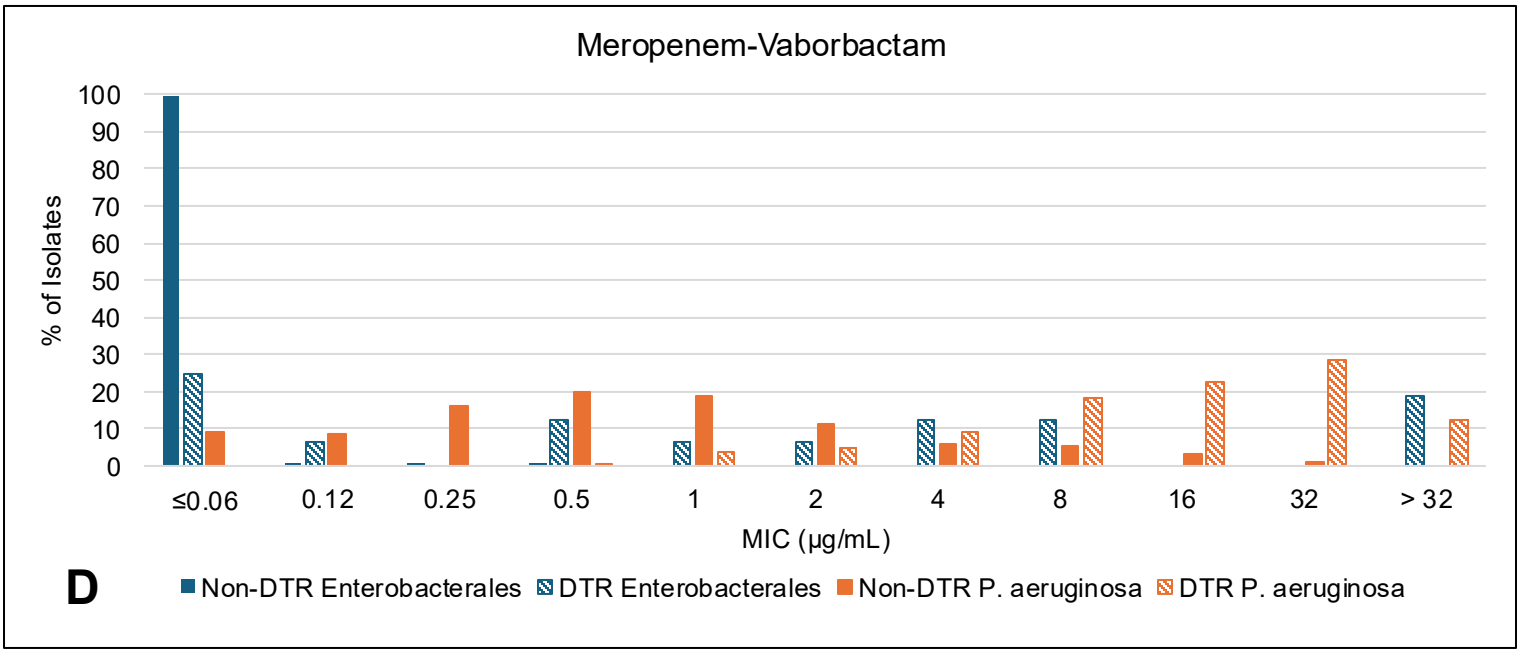
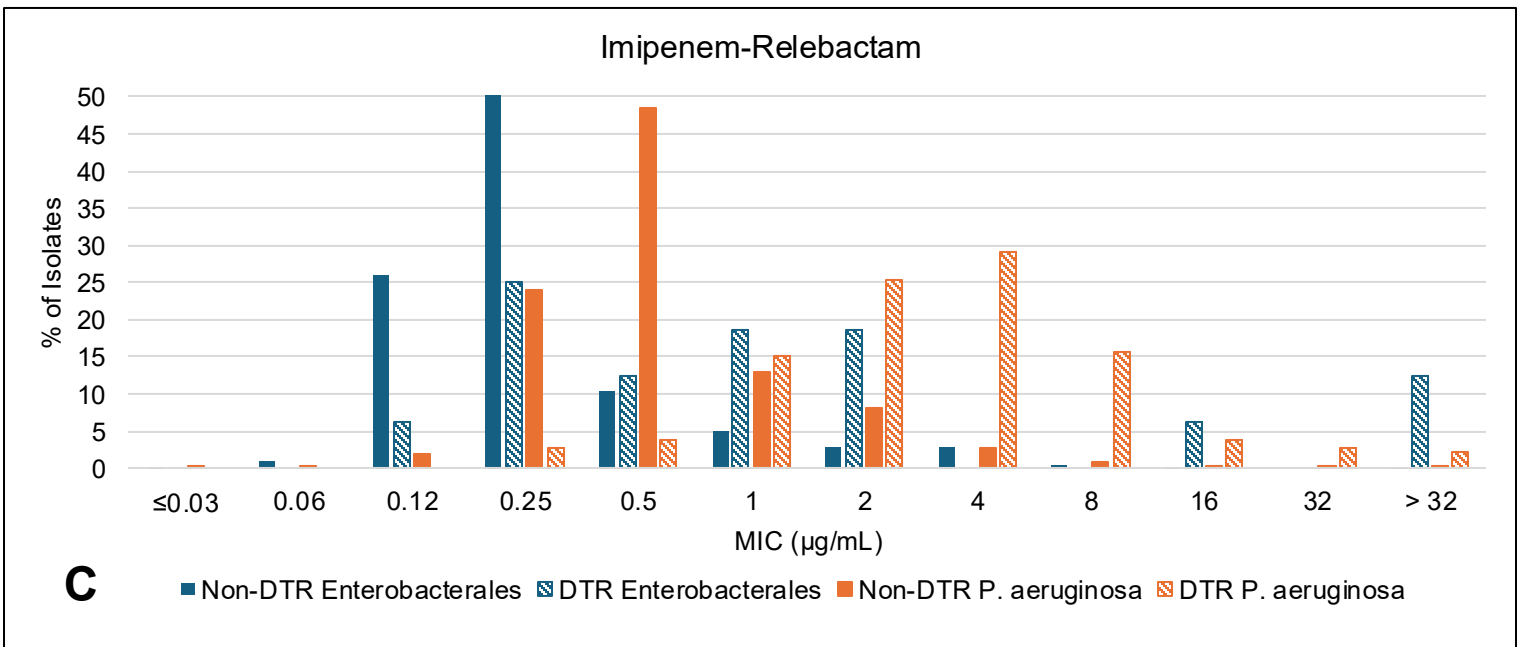
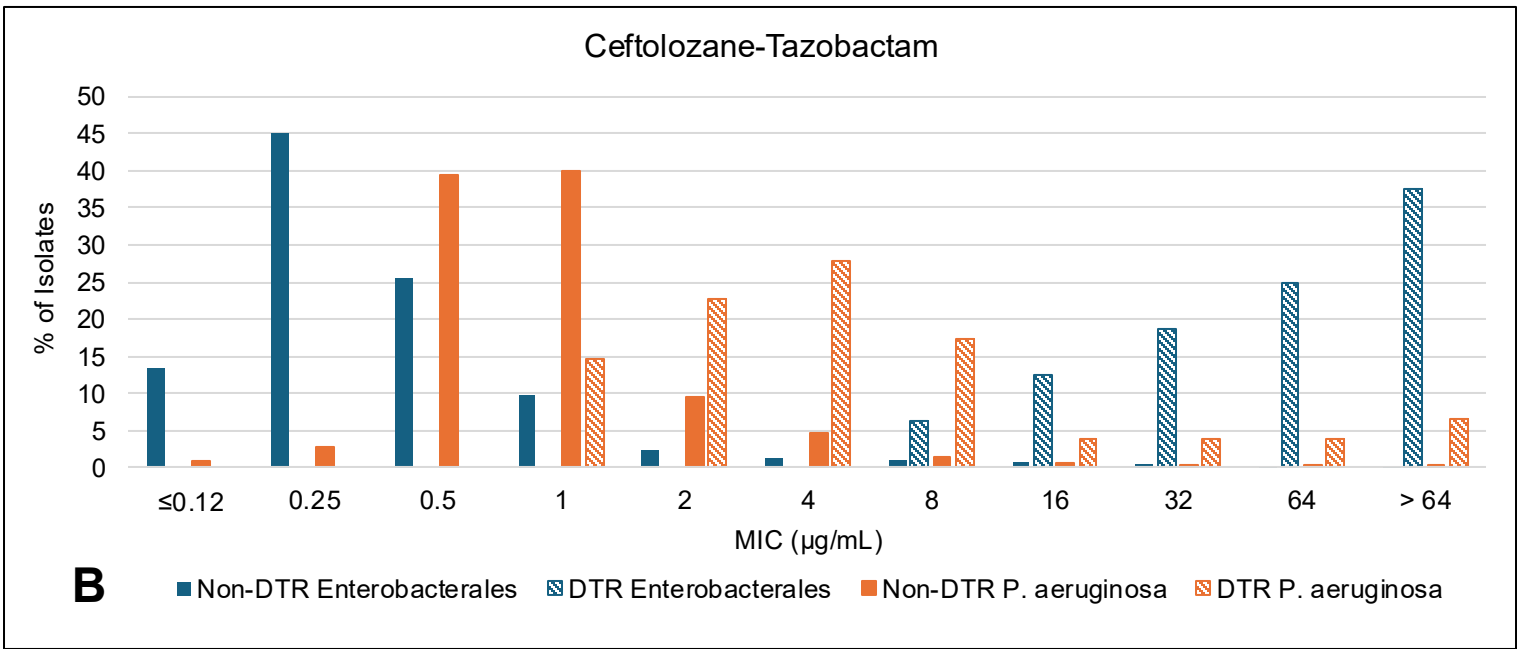
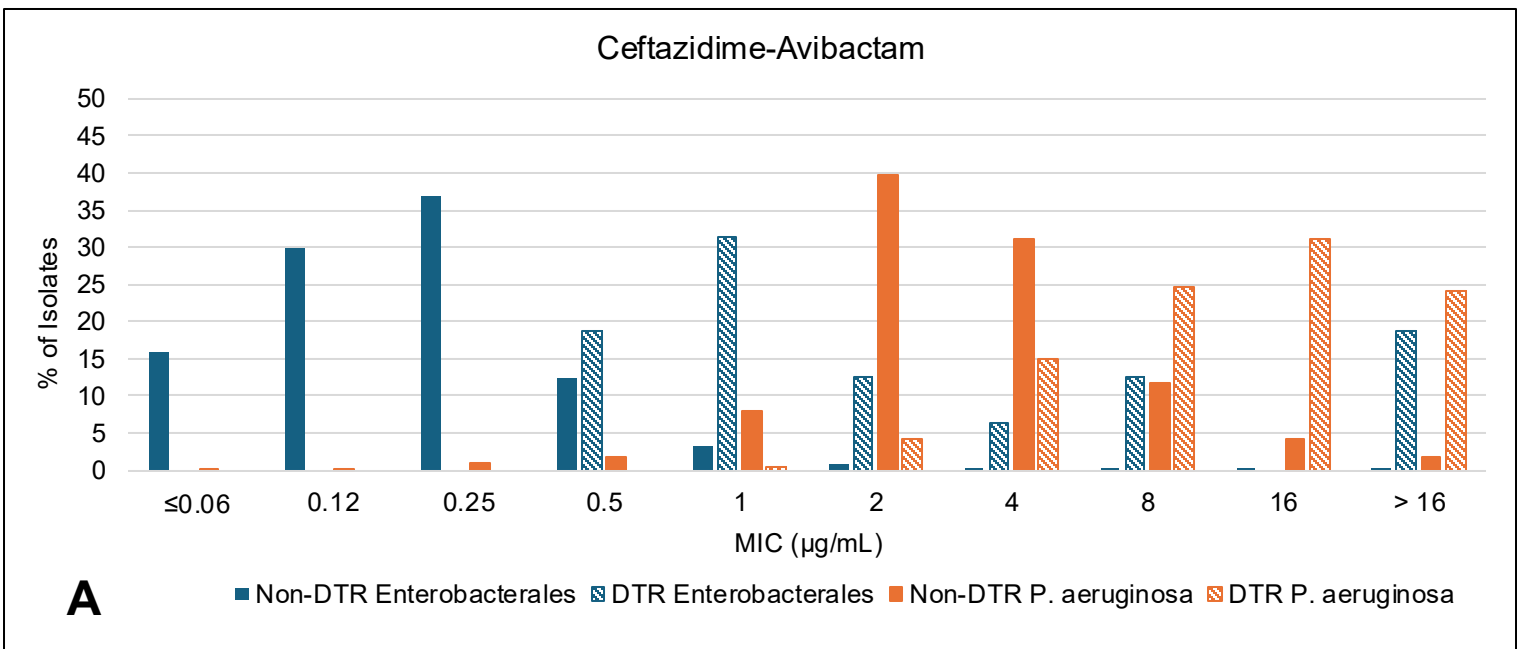


Figure 1. MIC distributions of ceftazidime-avibactam (A), ceftolozane-tazobactam (B), imipenem-relebactam (C), and meropenem-vaborbactam (D) for DTR *Enterobacterales*, non-DTR *Enterobacterales*, DTR *P. aeruginosa*, and non-DTR *P. aeruginosa*.

Conclusions

1. Overall, 0.2% of *Enterobacterales* and 7.7% of *P. aeruginosa* clinical isolates from Canadian patients, collected from 2016 to 2023, displayed a DTR phenotype.
2. Ceftazidime-avibactam and imipenem-relebactam were active against the majority of DTR *Enterobacterales* isolates (81.3% susceptible and 62.5% susceptible, respectively). None of the DTR *Enterobacterales* isolates were susceptible to ceftolozane-tazobactam.
3. Ceftazidime-avibactam and imipenem-relebactam demonstrated bimodal MIC distributions for DTR isolates of *Enterobacterales*. The two *Enterobacterales* isolates with ceftazidime-avibactam and imipenem-relebactam MICs ≥ 16 $\mu\text{g/mL}$ carried NDM-5 (data not shown).
4. Ceftolozane-tazobactam was the most active agent *in vitro* versus DTR *P. aeruginosa* isolates (65.1% susceptible). Less than 50% of DTR *P. aeruginosa* isolates were susceptible to ceftazidime-avibactam and imipenem-relebactam.
5. Clinical isolates of DTR Gram-negative bacilli should be tested for susceptibility to any newer β -lactam- β -lactamase inhibitor combination agent considered for therapy.

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