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Concise Communication

Canadian impact of CLSI aminoglycoside breakpoint changes for selected gram-negative bacteria

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Abstract

We evaluated the impact of recent revisions to the Clinical and Laboratory Standards Institute (CLSI) aminoglycoside breakpoints on susceptibility within Enterobacterales and *Pseudomonas aeruginosa* at a Canadian academic hospital. While the aminoglycoside breakpoint changes minimally affected overall susceptibility, the impact of these changes was notable within beta-lactamase producing Enterobacterales.

Introduction

The Clinical and Laboratory Standards Institute (CLSI) recently revised the long-held aminoglycoside breakpoints for Enterobacterales and *Pseudomonas aeruginosa* in 2023.[1–3] While aminoglycoside monotherapy is not typical nor recommended for definitive therapy of multidrug-resistant (MDR) gram-negative bacilli (GNB), they have importance as a component of empiric regimens.[3] A recent study from the United States (US) demonstrated that while 94.6% of Enterobacterales remained susceptible to amikacin after the recent breakpoint change, isolates expressing an extended spectrum β-lactamase (ESBL) were significantly impacted, with susceptibility declining from 96.9% to 79.7%.[4] Comparably large decreases in amikacin susceptibility were seen among carbapenem-resistant Enterobacterales.[4] Another US institution reported a significant drop in susceptibility to aminoglycosides within GNB, though this was mostly driven by the removal of gentamicin breakpoints and restricting amikacin reporting to urine specimens only for *P. aeruginosa*.[5] The impact of the recent breakpoint changes on Canadian institutions remains unexplored.

Aminoglycoside use has decreased in Canada since the early 2000s[6] in favor of less nephrotoxic agents including third generation cephalosporins and carbapenems, though the increasing incidence of CPE[7] may lead to increased reliance on aminoglycosides. Despite the growing threat of antibiotic resistance, Canada trails other high-income countries in bringing novel antibiotics to market,[8,9] increasing reliance on existing

antibiotic options, including many listed in the current IDSA guidance on the treatment of various resistant GNB.[10] Here we summarize aminoglycoside susceptibility and the impact of recent breakpoint changes for Enterobacterales and *P. aeruginosa* from various specimen sources and resistant subsets of isolates identified at a large Canadian academic teaching hospital.

Methods

Retrospective susceptibility testing data from all clinical samples at a large hospital laboratory in Toronto, Ontario, Canada over a one-year period was collected (January 1, 2022 – Dec 31, 2022). The top ten most common Enterobacterales species (ordered by frequency: *Escherichia coli, Klebsiella pneumoniae, Enterobacter cloacae complex, Proteus mirabilis, Klebsiella oxytoca, Serratia marcescens, Citrobacter koseri, Enterobacter aerogenes, Citrobacter freundii complex*, and *Morganella morganii*) and *P. aeruginosa* were selected for analysis. Respiratory specimens from cystic fibrosis patients and isolates with incomplete susceptibility data for routinely tested antibiotics were excluded. Interpretations of susceptibility data were defined as follows: MIC values reported by the reference laboratory were viewed as the highest quality of evidence, followed by data from a disk diffusion interpretation, and then by MIC values from the automated susceptibility testing system (Vitek II [Biomerieux]). For this study, the highest quality available evidence was used as the final interpretation based on the CLSI M100E32[1] and for reinterpretation with the CLSI M100E33 breakpoints.[2] Only the first isolate of a given species per patient per year was included for analysis. If more than one isolate of a given species was isolated on the same day for a patient, if the susceptibility pattern was distinct between the isolates, both isolates were included.

Table 1. Percent of amikacin, gentamicin, and tobramycin susceptible Enterobacterales isolates based on the 2022 and 2023 breakpoints, as well as the absolute change in susceptibility and the significance of that change ($P \leq 0.05$, McNemar Test). Organisms listed as AmpC, ESBL, AmpC and ESBL, and CPE are classed based on their phenotypic expression or genotypic evidence of a β-lactamase. ESBL; Extended spectrum beta-lactamase Class A. CPE; Carbapenemase-producing Enterobacterales

		Amikacin				Gentamicin				Tobramycin			
Isolate type	TOTAL	2022 (%S)	2023 (%S)	Change in %S	P-value	2022 (%S)	2023 (%S)	Change in %S	P-value	2022 (%S)	2023 (%S)	Change in %S	P-value
All	2713	99.9	99.0	−0.8	<0.001	94.3	94.1	−0.2	0.07	94.1	92.6	−1.6	<0.001
Site													
Urine	1765	99.9	99.2	−0.8	<0.001	93.5	93.2	−0.3	0.07	93.2	91.9	−1.3	<0.001
Respiratory	316	100.0	99.7	−0.3	1.00	98.1	98.1	0.0	1.00	97.5	96.5	−0.9	0.25
Blood	232	100.0	99.6	−0.4	1.00	94.0	94.0	0.0	1.00	94.8	91.4	−3.4	0.01
Resistance													
AmpC	129	99.2	99.2	0.0	1.00	91.5	91.5	0.0	1.00	96.1	93.0	−3.1	0.13
ESBL	218	99.1	93.1	−6.0	<0.001	73.9	73.9	0.0	1.00	64.7	61.5	−3.2	0.02
AmpC & ESBL	13	100.0	84.6	−15.4	0.48	61.5	61.5	0.0	1.00	61.5	61.5	0.0	1.00
CPE	4	50.0	50.0	0.0	1.00	25.0	25.0	0.0	1.00	0.0	0.0	0.0	1.00

Organisms expressing an AmpC or Class A ESBL β-lactamase were identified based on the results of the Kirby–Bauer double-disk diffusion test according to the Institute for Quality Management in Healthcare (IQMH) guidelines. Carbapenemase-producing Enterobacterales (CPE) were identified using either the Rapid Carb screen test (Rosco Diagnostica A/S) with confirmation at the reference Public Health Ontario Laboratory or the NG-Test CARBA 5 (Hardy Diagnostics). The definition of MDR *P. aeruginosa* was based on the IDSA definitions and the available data in our center.[10] MDR was defined as *P. aeruginosa* not susceptible to at least three of the following: piperacillin-tazobactam, ceftazidime, ciprofloxacin, tobramycin, and meropenem based on the 2023 breakpoints. Data was analyzed using Excel version 2310 and R version 4.1.3. Statistical significance (p-value <0.05) was assessed using the McNemar Test for comparing susceptibility using the 2022 and 2023 interpretations.

Results

Susceptibility to aminoglycosides amikacin, gentamicin, and tobramycin are summarized for Enterobacterales (n = 2713) isolates in Table 1 and *P. aeruginosa* isolates (n = 442) in Table 2. Enterobacterales organisms remained highly susceptible to aminoglycosides, with small shifts in susceptibility (−0.8%, −0.2%, and −1.6% to amikacin, gentamicin, and tobramycin respectively). Resistance to gentamicin and tobramycin amongst ESBLs and CPEs was significant before and after the breakpoint changes. Amikacin remained the most active aminoglycoside against ESBLs and CPE (93.1% and 50% of isolates susceptible, respectively), though susceptibility of ESBLs to amikacin decreased (−6%, P < 0.001) due to the breakpoint change.

Non-susceptible organisms included those that were intermediate, resistant, or non-susceptible based on the breakpoint changes, hence the susceptibility of *P. aeruginosa* to gentamicin based on the 2023 breakpoints is 0% for all isolates (Table 2). Tobramycin remained highly active against *P. aeruginosa* isolates in aggregate, though the percentage of susceptible isolates identified in respiratory specimens decreased by 5% after the breakpoint change. MDR *P. aeruginosa* susceptibility to aminoglycosides shifted with the breakpoint change, though this was statistically insignificant.

Discussion

In contrast to recent data from the US,[4] our institution did not observe large decreases in the proportion of Enterobacterales susceptible to aminoglycosides when applying the recent CLSI breakpoint changes. Our data suggests comparatively fewer isolates were close to the breakpoint values. One reason for this observation may be that Canadian hospitals use aminoglycosides infrequently compared to other centers, though we could not identify directly comparable use data between the US and Canada. Although we had low numbers of CPE, our findings corroborate the significant aminoglycoside resistance within CPEs documented by the Canadian Nosocomial Infection Surveillance Program (CNISP).[7] This is concerning in the context of a threefold rise in healthcare-associated CPE colonization in Canada between 2015 and 2019.[7] Of concern, Canada trails in bringing novel antibiotics to market (many which are now preferentially recommended by IDSA guidelines for treating MDR GNB),[10] due to relatively low market-share compared to other countries, and limited incentives to entice companies to undertake the Canadian regulatory approval process.[8,9]

Aminoglycoside susceptibility among *P. aeruginosa* was altered when applying the 2023 breakpoints, though tobramycin remained active against 94.8% of *P. aeruginosa* isolates. Encouragingly, the number of MDR *P. aeruginosa* we identified was small, though susceptibility to aminoglycosides within this subgroup was low. While tobramycin is often used as dual coverage in empiric regimens for patients likely to be harboring resistant strains, our data, though underpowered, suggests resistant subgroups of *P. aeruginosa* isolates are not well covered by this agent. Additional research with a greater sample size of MDR isolates is needed to make definitive conclusions. Our definition of MDR was modified slightly from the IDSA guidelines based on the agents that are routinely tested and reported at Canadian institutions.[10] Of note, aminoglycoside breakpoints are not aligned across breakpoint setting organizations which surely impacts our results and should be considered when comparing data from other regions not using the CLSI breakpoints. USCAST breakpoints are set at lower MICs than CLSI, while EUCAST is similar to CLSI, though divergences in these documents exist.

Table 2. Percent of amikacin, gentamicin, and tobramycin susceptible *P. aeruginosa* isolates based on the 2022 and 2023 breakpoints, as well as the absolute change in susceptibility and the significance of that change ($P \leq 0.05$, McNemar Test). MDR; Multidrug-resistant

Isolate type	TOTAL	Amikacin				Gentamicin				Tobramycin			
		2022 (%S)	2023 (%S)	Change in %S	P-value	2022 (%S)	2023 (%S)	Change in %S	P-value	2022 (%S)	2023 (%S)	Change in %S	P-value
All	442	97.5	26.9*	−70.6*	<0.001*	92.1	0.0*	−92.1*	<0.001*	97.7	94.8	−2.9	<0.001
Site													
Urine	121	98.3	98.3	0.0	1.00	91.7	0.0*	−91.7*	<0.001*	99.2	96.7	−2.5	0.25
Respiratory	160	94.4	0.0*	−94.4*	<0.001*	88.1	0.0*	−88.1*	<0.001*	95.0	90.0	−5.0	0.01
Blood	27	100.0	0.0*	−100.0*	<0.001*	88.9	0.0*	−88.9*	<0.001*	100.0	96.3	−3.7	1.00
Resistance													
MDR	12	75.0	16.7*	−58.3*	0.02*	33.3	0.0*	−33.3*	0.13*	66.7	41.7	−25.0	0.25

*This result reflects the removal of the breakpoint for amikacin for non-urine isolates or the removal of the breakpoint for gentamicin altogether for *P. aeruginosa* in 2023.

Conclusion

Recent aminoglycoside breakpoint changes impacted ESBL-producing Enterobacterales organisms, though these agents remained active against most isolates. Our data, although not robust in CPE or MDR *P. aeruginosa* representation, demonstrates that these subgroups are not well covered by aminoglycosides, supporting the ongoing concern that patients may be at risk of harm in the future if development or commercialization of new antimicrobials stalls. Clinical availability of novel antimicrobials remains problematic in countries where access limitations are observed,[9] which likely influences the high mortality rate associated with CPE infections in Canada.[7] Future research should address susceptibility to novel antimicrobials within resistant GNBs to ensure that the introduction of novel antimicrobials in Canada addresses current gaps in available treatment options.

References

1. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing*. 32nd ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute. 2022.
2. CLSI. *Performance Standards for Antimicrobial Susceptibility Testing*. 33rd ed. CLSI supplement M100. Wayne, PA: Clinical and Laboratory Standards Institute. 2023.
3. Humphries RM. AST News Update June 2023: New! CLSI M100-Ed33: Updated Aminoglycoside Breakpoints for Enterobacterales and Pseudomonas aeruginosa. https://clsi.org/about/blog [online]. Accessed May 20, 2024.
4. Sader HS, Mendes RE, Kimbrough JH, Kantro V, Castanheira M. Impact of the recent clinical and laboratory standards institute breakpoint changes on the antimicrobial spectrum of aminoglycosides and the activity of plazomicin against multidrug-resistant and carbapenem-resistant Enterobacterales from United States medical centers. *Open Forum Infect Dis* 2023;10: ofad058. US: Oxford University Press.
5. Wangchinda W, Aitken SL, Pierce VM, Lephart P, Pogue JM. 356. Impact of CLSI Breakpoint Changes on Unit-Specific Combination Antibiograms for Gram-Negative Respiratory Isolates. *Open Forum Infect Dis* 2023;10: ofad500-427. US: Oxford University Press.
6. Liang JJ, Rudnick W, Mitchell R, Brooks J, Bush K, Conly J, Ellison J, Frenette C, Johnston L, Lavallée C, McGeer A. Antimicrobial use in Canadian acute-care hospitals: Findings from three national point-prevalence surveys between 2002 and 2017. *Infect Control Hosp Epidemiol* 2022;43:1558–64.
7. Public Health Agency of Canada (PHAC). *Canadian Antimicrobial Resistance Surveillance System Report- Update 2021*. Ottawa, ON: Public Health Agency of Canada. 2021.
8. Outterson K, Orubu ESF, Rex J, Årdal C, Zaman MH. Patient access in 14 high-income countries to new antibacterials approved by the US food and drug administration, European medicines agency, Japanese pharmaceuticals and medical devices agency, or health Canada, 2010-2020. *Clin Infect Dis* 2022;74:1183–1190.
9. Council of Canadian Academies (CCA). Overcoming Resistance. Ottawa (ON): Expert Panel on Antimicrobial Availability- CCA. 2023.
10. Tamma PD, Aitken SL, Bonomo RA, Mathers AJ, van Duin D, Clancy CJ. Infectious Diseases Society of America 2023 guidance on the treatment of antimicrobial resistant gram-negative infections. *Clin Infect Dis* 2023; ciad428. https://doi.org/10.1093/cid/ciae403.