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Frequency of 16S ribosomal RNA methyltransferase detection among *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates obtained from patients in Canadian hospitals (CANWARD, 2013–2017)

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ABSTRACT

Pan-aminoglycoside (amikacin, gentamicin, tobramycin, plazomicin)–resistant *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates from patients in Canadian Hospitals (2013–2017) were evaluated by whole genome sequencing for 16S ribosomal RNA methyltransferase genes. The *rmtB* gene was detected in 2 isolates (1 of 3094 *E. coli* [0.03%], 1 of 1039 *K. pneumoniae* [0.1%]).

Plazomicin is a next-generation aminoglycoside derived from sisomicin (Zhanel et al., 2012). It retains *in vitro* activity versus members of the family *Enterobacteriaceae* that produce aminoglycoside modifying enzymes, with the exception of the chromosomal AAC(2′)-I enzyme found in *Providencia stuartii* (Aggen et al., 2010; Cox et al., 2018; Karaiskos et al., 2015; Zhanel et al., 2012). Carbapenem-resistant and extended-spectrum β-lactamase (ESBL)–producing *Enterobacteriaceae* typically remain susceptible to plazomicin *in vitro* (Endimiani et al., 2009; Lopez-Diaz et al., 2017; Martins et al., 2018; Zhang et al., 2017). In a recent small phase 3 open-label study, plazomicin was compared with colistin for the treatment of patients with a confirmed carbapenem-resistant *Enterobacteriaceae* (CRE) bloodstream infection (McKinnell et al., 2017). Twenty-eight day mortality was lower among patients randomized to treatment with plazomicin, supporting a possible role of plazomicin for this indication (McKinnell et al., 2017). Unfortunately, the *in vitro* activity of plazomicin is compromised by acquired 16S ribosomal RNA (rRNA) methyltransferases (Cox et al., 2018; Zhanel et al., 2012). Nine 16S rRNA methyltransferase enzymes have been reported to date (ArmA, RmtA, RmtB, RmtC, RmtD, RmtE, RmtF, RmtG, and RmtH) (Doi et al., 2016). At present, there is a paucity of national surveillance data describing the prevalence of these enzymes among contemporary *Enterobacteriaceae* isolates in many parts of the world. The purpose of this study was to determine the frequency with which 16S rRNA methyltransferase enzymes are detected in clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* obtained from patients in Canadian hospitals between 2013 and 2017 (CANWARD).

From January 2013 to December 2017, inclusive, 12 to 15 sentinel hospitals across Canada submitted clinical isolates from patients attending emergency rooms, medical and surgical wards, hospital clinics, and intensive care units (CANWARD). On an annual basis, each center was asked to submit clinical isolates (consecutive, 1 per patient/infection site) from blood, respiratory, urine, and wound infections. The medical centers submitted clinically significant isolates, as defined by their local site criteria. 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). Isolates were shipped on Amies semi-solid transport media to the coordinating laboratory (Health Sciences Centre, Winnipeg, Canada), subcultured onto appropriate media, and stocked in skim milk at −80 °C until minimum inhibitory concentration (MIC) testing was carried out.

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Table 1
Susceptibility profile of isolates harboring a 16S rRNA methyltransferase gene recovered as part of the CANWARD study, 2013–2017.

	E. coli isolate		*K. pneumoniae* isolate	
Antimicrobial	MIC (mg/L)	Interpretation[a]	MIC (mg/L)	Interpretation[a]
Plazomicin	>64	R	>64	R
Amikacin	>64	R	>64	R
Gentamicin	>32	R	>32	R
Tobramycin	>64	R	>64	R
Cefazolin	16	R	>128	R
Ceftazidime	0.5	S	>32	R
Ceftriaxone	≤0.25	S	>64	R
Ciprofloxacin	>16	R	>16	R
Ertapenem	0.06	S	32	R
Meropenem	≤0.03	S	8	R
Piperacillin–tazobactam	4	S	>512	R
Tigecycline	0.5	S	1	S
Trimethoprim–sulfamethoxazole	>8	R	8	R

[a] Current CLSI breakpoints were used, where available (Clinical and Laboratory Standards Institute, 2018b). FDA-defined breakpoints were used for tigecycline (susceptible, ≤2 mg/L; intermediate, 4 mg/L; resistant, ≥8 mg/L) and plazomicin (susceptible, ≤2 mg/L; intermediate, 4 mg/L; resistant, ≥8 mg/L).

Following 2 subcultures from frozen stock, the *in vitro* activities of plazomicin, amikacin, gentamicin, tobramycin, and relevant nonaminoglycoside comparators were determined by broth microdilution in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (Clinical and Laboratory Standards Institute, 2018a, 2018b). In-house–prepared 96-well broth microdilution panels were used to test all antimicrobial agents. Antimicrobial MICs were interpreted using CLSI breakpoints, where available (Clinical and Laboratory Standards Institute, 2018b). *E. coli* and *K. pneumoniae* isolates that demonstrated pan-aminoglycoside resistance (resistance to amikacin, gentamicin, and tobramycin) including resistance to plazomicin (MIC ≥8 mg/L, United States Food and Drug Administration breakpoint) were submitted to Canada's National Microbiology Laboratory (Winnipeg, Canada) for whole genome sequencing on the NextSeq platform (Illumina, San Diego, CA, USA) to assess the potential mechanism of plazomicin resistance, including the presence of 16S rRNA methyltransferase genes. Reads were assembled into contigs by SPAdes v3.8.0, and the contigs were analyzed by ResFinder on the Center for Genomic epidemiology website (https://cge.cbs.dtu.dk/services/).

In total, 3094 *E. coli* and 1039 *K. pneumoniae* isolates were recovered as part of the CANWARD Study between 2013 and 2017. Only 2 isolates demonstrated pan-aminoglycoside resistance: 1 *E. coli* and 1 *K. pneumoniae*. The susceptibility profile of these isolates is presented in Table 1. Both isolates had a plazomicin MIC of >64 mg/L. All other *E. coli* and *K. pneumoniae* evaluated in this study were susceptible (99.5% and 99.8%, respectively) or intermediately susceptible (0.4% and 0.1%, respectively) to plazomicin. The plazomicin-resistant *E. coli* was obtained from the bloodstream of a patient in the Canadian province of British Columbia, while the plazomicin-resistant *K. pneumoniae* was obtained from the bloodstream of a patient in the Canadian province of Quebec. By whole genome sequencing, both isolates were found to contain the *rmtB* gene. The *K. pneumoniae* isolate was also positive for the NDM-1 and OXA-232 carbapenemase enzymes. The overall frequency of 16S rRNA methyltransferase detection among *E. coli* and *K. pneumoniae* isolates in this dataset was 0.03% and 0.1%, respectively.

Surveillance data on the global distribution of 16S rRNA methyltransferase enzymes remain limited at present (Doi et al., 2016). Prevalence rates of <1% have been reported among *Enterobacteriaceae* from Japan and Argentina (Doi et al., 2016; Tijet et al., 2011; Yamane et al., 2007). Bell et al. found a higher prevalence of 16S rRNA methyltransferases among countries in the Asia-Pacific region that were involved in the SENTRY surveillance study (Bell et al., 2010). Among 4161 *Enterobacteriaceae* recovered between 2007 and 2008, 209 isolates in 5 countries were found to contain at least 1 of these enzymes (Bell et al., 2010). The rate of detection by country was as follows: China 6.9%, India 10.5%, Hong Kong 1.5%, Korea 6.1%, and Taiwan 5.0% (Bell et al., 2010). More recently, Taylor et al. screened 806 *Enterobacteriaceae* isolates with high-level pan-aminoglycoside resistance from the United Kingdom and Ireland collected between 2004 and 2015 (Taylor et al., 2018). Overall, 94.5% of these pan-aminoglycoside resistant isolates were positive for 1 or more 16S rRNA methyltransferase genes (Taylor et al., 2018). It is not possible to determine the prevalence of this resistance mechanism from these data, but they clearly demonstrate that isolates with 16S rRNA methyltransferases are present in these countries. Castanheira et al. screened *Enterobacteriaceae* isolates with high-level plazomicin resistance (MIC ≥128 mg/L) obtained from European and adjacent countries between 2014 and 2015 for the presence of 16S rRNA methyltransferases (Castanheira et al., 2018). Of 4217 isolates evaluated, 60 (1.4%) demonstrated high-level plazomicin resistance, and all of these were found to possess a 16S rRNA methyltransferase enzyme (Castanheira et al., 2018). *Enterobacteriaceae* producing 16S rRNA methyltransferases have been infrequently described in the United States and Canada, in keeping with the data presented here (Denisuik et al., 2015; Fritsche et al., 2008; Peirano et al., 2014). The isolates reported in Canada to date have generally been associated with recent patient travel outside of the country (Peirano et al., 2014).

Of concern, *Enterobacteriaceae* positive for both 16S rRNA methyltransferases and carbapenemase enzymes (often the NDM metallo-β-lactamase) have also been described in several publications. In the recent report by Taylor et al., 93.4% of 16S rRNA methyltransferase–positive *Enterobacteriaceae* isolates also had an acquired carbapenemase, most commonly NDM and/or OXA-48-like (Taylor et al., 2018). The *K. pneumoniae* isolate described here harbored the NDM-1 and OXA-232 enzymes.

There are several limitations to the current study that deserve attention. Pan-aminoglycoside resistance (including plazomicin resistance) was used to screen for the presence of 16S rRNA methyltransferase enzymes rather than performing whole genome sequencing on all isolates. Additionally, limited clinical data were available on the patients from whom the 2 isolates harboring 16S rRNA methyltransferases were obtained. It is quite possible that 1 or both of these isolates were acquired during travel outside of Canada.

In summary, 16S rRNA methyltransferase genes remain uncommon among *E. coli* and *K. pneumoniae* clinical isolates in Canada at present. This is an encouraging finding given the possible role of plazomicin in the treatment of infections due to CRE. Further surveillance data are greatly needed to better define the prevalence of 16S rRNA methyltransferases in Gram-negative bacilli around the world.

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