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Characterization of Meropenem-Resistant and Extensively-Drug Resistant *Pseudomonas aeruginosa* in Canada: CANWARD 2007–2016

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ABSTRACT

Background: Carbapenem-resistant PA is a worldwide problem with increasing reports of carbapenemase producing isolates. These isolates may concomitantly be multi-drug resistant (MDR) and/or XDR. This study characterized MER-resistant and carbapenemase-producing PA from Canadian hospitals.
Methods: From January 2007 to December 2016, tertiary-care hospitals from across Canada submitted PA from patients attending hospitals. Susceptibility testing was performed using CLSI broth microdilution. PCR was used to detect carbapenemases GES, KPC, NDM, IMP, VIM, OXA-48, beta-lactamases SHV, TEM, CTX-M, OXA-1, CMY-2, and the *mcr-1 and -2* genes for colistin resistance. Sequencing identified the specific carbapenemase gene variant. Genetic relatedness was determined with MLST and PFGE using *Spe*I.
Results: 3864 PA were collected with 382 (9.89%) being MER-resistant and/or XDR. These 382 isolates were distributed as follows: 2007 (14.1%, n=54), 2008 (5.2%, n=20), 2009 (8.6%, n=33), 2010 (7.3%, n=28), 2011 (5.8%, n=22), 2012 (6.5%, n=25), 2013 (12.3%, n=47), 2014 (15.5%, n=59), 2015 (15.2%, n=58), 2016 (9.4%, n=36). Patient age ranged from 1-93 yrs (mean age 55.1 yrs) and 245 (64.1%) isolates were from males. Isolates were obtained from respiratory (75.1%; n=287), blood (12.8%; n=49), wound (8.4%; n=32) and urine (3.7%; n=14) specimens. Susceptibilities were: 93.46% colistin; 91.6% ceftolozane/tazobactam, 80.37% amikacin, 73.04% tobramycin, 38.22% piperacillin/tazobactam, 36.39% ceftazidime, 36.65% ciprofloxacin. 27.5% of MER-resistant/XDR isolates were MDR [resistant to 3 different antimicrobial classes] and 24.6% were XDR [resistant to ≥4 different antimicrobial classes]. Among all PA, XDR isolates remained low (1.5 to 3.5%) from 2007-2016. Only 3.4% of isolates harboured a carbapenemase; GES-5 (1.8%, n=7,), VIM-2 (0.5%, n=2), VIM-4 (0.5%, n=2), IMP-18 (0.5%, n=2) and IMP-7 (0.26%, n=1). All GES-5 isolates were genetically related by PFGE and MLST (ST17) and were all from western Canada. Genetic diversity among MER-resistant and XDR PA is reflected in the identification of 206 different sequence types (STs). There were four genetically related clusters with more than 10 isolates per cluster; ST17 (4.3%, n=16), ST235 (3.8%, n=14), ST244 (2.9%, n=11) and ST357 (3.8%, n=14). *mcr* was not found among any colistin resistant PA.
Conclusions: MER-resistant PA have increased over the surveillance period in Canada reaching a peak of 17.3% in 2014. Carbapenemase-producing PA are rare with only 13 (3.4%) identified. Pan drug resistant PA have not yet been identified in Canada. Genetic diversity was independent of MDR or XDR phenotypes.

BACKGROUND

Pseudomonas aeruginosa (PA) is a common nosocomial pathogen. PA is a complex organism that has both intrinsic and acquired mechanisms contributing to carbapenem resistance. Although some carbapenems remain effective in treating PA infections, there has been a rise in carbapenem-resistant PA worldwide (1). Many of these carbapenem-resistant isolates harbour carbapenemases and may concomitantly be multi-drug resistant (MDR) or extensively-drug resistant (XDR) as per recommendations (2). Past studies have shown PA to have the highest rates of MDR among Gram-negative organisms in Canada (3). XDR PA are also on the rise worldwide (4). PA displays a great diversity of STs that often overlap between clinical and environmental sources (5). Internationally, MDR and XDR infections can be associated with three high risk clones ST111, ST175 and ST235 (6). These clones are known to carry many acquired beta-lactamases such as IMP, NDM and VIM (4).

MATERIALS & METHODS

Study Design: From January 2007 to December 2016, tertiary-care medical centres representing 8 of 10 provinces across Canada submitted pathogens from patients attending hospital clinics, emergency rooms, medical and surgical wards, and intensive care units. Each study site was asked to submit clinical isolates (consecutive, one per patient, per infection site) from inpatients and outpatients with respiratory, urine, wound, and bloodstream infections. Meropenem-resistant isolates were screened into the study using the following CLSI guidelines. From 2007-2011 CLSI guidelines for meropenem were ≥16µg/mL. From 2012 to 2016 CLSI guidelines for meropenem were ≥8µg/mL.
Bacterial Strains: Stock cultures were stored at -80°C in Microbank vials (Pro-Lab Diagnostics, Richmond Hill, Ontario, Canada).
Antimicrobial Susceptibility Testing: Antimicrobial susceptibilities were determined via broth microdilution (CLSI).
DNA Methodology: PCR was used to detect carbapenemases GES, KPC, NDM, IMP, VIM, OXA-48, beta-lactamases SHV, TEM, CTX-M, OXA-1, CMY-2, and the *mcr-1 and -2* genes for colistin resistance. Sequencing identified the specific carbapenemase gene variant. Genetic relatedness was determined with MLST (7) and PFGE using *Spe*I.

RESULTS

Figure 1. Incidence of MER-resistant and XDR among all *P. aeruginosa* (n=3864) in Canada from 2007-2016.

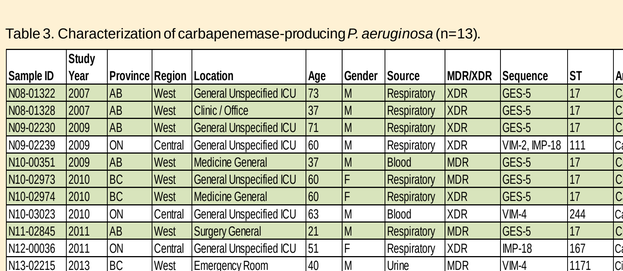

Table 2. Patient demographic information on MER-resistant and/or XDR *P. aeruginosa* from 2007-2016 (n=382).

		2007 n=54 (%)	2008 n=20 (%)	2009 n=33 (%)	2010 n=28 (%)	2011 n=22 (%)	2012 n=25 (%)	2013 n=47 (%)	2014 n=59 (%)	2015 n=58 (%)	2016 n=36 (%)
Gender	Male	28 (51.9)	16 (80.0)	19 (57.6)	15 (53.6)	14 (63.6)	18 (72.0)	33 (70.2)	42 (71.2)	34 (58.6)	26 (72.2)
	Female	26 (48.1)	4 (20.0)	14 (42.4)	13 (46.4)	8 (36.4)	7 (28.0)	14 (29.8)	17(28.8)	24 (41.4)	10 (27.8)
Hospital Wards	Clinics	14 (25.9)	1 (5.0)	6 (18.2)	4 (14.3)	2 (9.1)	5 (20.0)	7 (14.9)	5 (8.5)	2 (3.4)	5 (13.9)
	ER	2 (3.7)	2 (10.0)	0 (0.0)	0 (0.0)	2 (9.1)	0 (0.0)	4 (8.5)	3 (5.1)	1 (1.7)	1 (2.8)
	ICU	21 (38.9)	6 (30.0)	14 (42.4)	12 (42.9)	11 (50.0)	10 (40.0)	18 (38.3)	17 (28.8)	19 (32.8)	13 (36.1)
	Medical	13 (24.1)	6 (30.0)	10 (30.3)	8 (28.6)	4 (18.2)	8 (32.0)	17 (36.2)	33 (55.9)	31 (53.4)	13 (36.1)
	Surgical	4 (7.4)	5 (25.0)	3 (9.1)	5 (7.9)	3 (13.6)	2 (8.0)	1 (2.1)	1 (1.7)	5 (8.6)	4 (11.1)
Speciman Type	Urine	5 (9.3)	2 (10.0)	0 (0.0)	2 (7.1)	0 (0.0)	0 (0.0)	1 (2.1)	1 (1.7)	2 (3.4)	1 (2.8)
	Blood	9 (16.7)	7 (35.0)	4 (12.1)	5 (7.9)	4 (18.2)	4 (16.0)	4 (8.5)	6 (10.1)	5 (8.6)	1 (2.8)
	Wound	7 (13.0)	2 (10.0)	5 (15.1)	3 (10.7)	1 (4.5)	0 (0.0)	2 (4.3)	5 (8.5)	5 (8.6)	2 (5.6)
	Respiratory	33 (61.1)	9 (45.0)	24 (72.7)	18 (64.3)	17 (77.3)	21 (84.0)	40 (85.1)	47 (79.7)	46 (79.3)	32 (88.9)
Region	West	18 (33.3)	9 (45.0)	12 (36.4)	9 (32.1)	3 (13.6)	8 (32.0)	8 (17.0)	8 (13.6)	18 (31.0)	10 (27.8)
	Central	33 (61.1)	11 (55.0)	21 (63.6)	19 (67.9)	17 (77.3)	15 (60.0)	39 (83.0)	49 (83.1)	37 (63.8)	23 (63.9)
	East	3 (5.6)	0 (0.0)	0 (0.0)	0 (0.0)	2 (9.1)	2 (8.0)	0 (0.0)	2 (3.4)	3 (5.2)	3 (8.3)
Age	≤17	3 (5.6)	2 (10.0)	2 (6.1)	1 (3.6)	0 (0.0)	0 (0.0)	2 (4.3)	4 (6.8)	1 (1.7)	4 (11.1)
	18-65	37 (68.5)	10 (50.0)	18 (54.5)	19 (67.9)	13 (59.1)	16 (64.0)	27 (57.4)	31 (52.5)	28 (48.3)	23 (63.9)
	≥66	14 (25.9)	8 (40.0)	13 (39.4)	8 (28.6)	9 (4.09)	9 (36.0)	18 (38.3)	24 (40.7)	29 (50.0)	9 (25.0)

Table 3. Characterization of carbapenemase-producing *P. aeruginosa* (n=13).

Sample ID	Study Year	Province	Region	Location	Age	Gender	Source	MDR/XDR	Sequence	ST	Antibiogram
N08-01322	2007	AB	West	General Unspecified ICU	73	M	Respiratory	XDR	GES-5	17	CazCipMerTob
N08-01328	2007	AB	West	Clinic / Office	37	M	Respiratory	XDR	GES-5	17	CazCipMerTob
N09-02230	2009	AB	West	General Unspecified ICU	71	M	Respiratory	XDR	GES-5	17	CazCipColMerTob
N09-02239	2009	ON	Central	General Unspecified ICU	60	M	Respiratory	XDR	VIM-2, IMP-18	111	Caz, Cip, Mer, Tob
N10-00351	2009	AB	West	Medicine General	37	M	Blood	MDR	GES-5	17	CipMerTob
N10-02973	2010	BC	West	General Unspecified ICU	60	F	Respiratory	MDR	GES-5	17	CipMerTob
N10-02974	2010	BC	West	Medicine General	60	F	Respiratory	XDR	GES-5	17	CazCipMerTob
N10-03023	2010	ON	Central	General Unspecified ICU	63	M	Blood	XDR	VIM-4	244	CazPipTazCipMerTob
N11-02845	2011	AB	West	Surgery General	21	M	Respiratory	MDR	GES-5	17	CipMerTob
N12-00036	2011	ON	Central	General Unspecified ICU	51	F	Respiratory	XDR	IMP-18	167	CazCipMerTob
N13-02215	2013	BC	West	Emergency Room	40	M	Urine	MDR	VIM-4	1171	CipMerTob
N13-02233	2013	SK	West	General Unspecified ICU	27	F	Respiratory	n/a	VIM-2	1996	CipMerTob
N16-03646	2016	ON	Central	General Unspecified ICU	84	M	Respiratory	XDR	IMP-7	2072	CazPipTazCipMerTob

MDR-resistant to 3 different antimicrobial classes. XDR-resistant to 4 or 5 different antimicrobials as per recommendations (2).
Caz-ceftazidime, Cip-ciprofloxacin, Col-colistin, Mer-meropenem, Pip/Taz-piperacillin/tazobactam, Tob-tobramycin.

Table 1a. Antimicrobial profiles of MER-resistant and/or XDR *P. aeruginosa* (n=382).

	Breakpoint Interpretations			MIC (mg/L)		Range of Values
Antimicrobial	%S	%I	%R	MIC_{50}	MIC_{90}	Range
Ceftazidime	36.39	17.80	45.81	16	>32	1 - ≥32
Piperacillin/tazobactam	38.22	28.01	33.77	32	256	≤1 - ≥512
Ciprofloxacin	36.65	14.40	48.95	4	>16	≤0.06 - ≥16
Colistin	93.46	0.00	6.54	1	2	0.12 - ≥16
Amikacin	80.37	7.07	12.57	8	>64	≤1 - ≥64
Meropenem	0.00	2.36	97.64	16	32	8 - ≥64
Tobramycin	73.04	3.66	23.30	1	64	≤0.5 - ≥64
Ceftolozane/tazobactam	91.62	3.4	4.97	1	4	0.25 - ≥64

*%S – percent susceptible; %I – percent intermediate; %R – percent resistant. 2017 CLSI breakpoints (µg/mL): Ceftazidime S ≤8, I 16, R ≥32; Piperacillin/tazobactam S ≤16/4, I 32/4-64/4, R ≥128/4; Ciprofloxacin S ≤1, I 2, R ≥4; Colistin S ≤2, I (n/a), R ≥4; Amikacin S ≤16, I 32, R ≥64; Meropenem S ≤2, I 4, R ≥8; Tobramycin S ≤4, I 8, R ≥ 6; Ceftolozane/tazobactam S ≤4, I 8, R ≥16.

Figure 2. goeBurst diagram showing distribution of all STs subdivided by XDR status. 206 different STs identified. Most common STs found: ST17, ST235, ST244 and ST357.

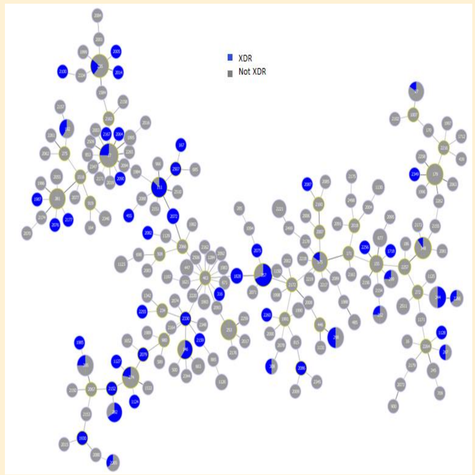

Figure 3. PFGE subtyping with *Spe*I on all GES-5 isolates (n=7). Genetic relatedness seen with MLST (all are ST17), but PFGE also demonstrates high discriminatory power with small band changes over time.

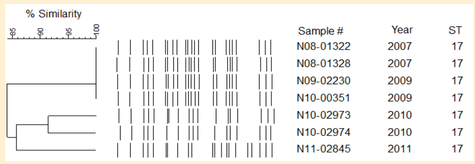

Table 1b. Antimicrobial profiles of XDR *P. aeruginosa* (n=94).

	Breakpoint Interpretations			MIC (mg/L)		Range of Values
Antimicrobial	%S	%I	%R	MIC_{50}	MIC_{90}	Range
Ceftazidime	8.51	4.25	87.23	>32	>32	4 - ≥32
Piperacillin/tazobactam	5.31	1908	76.59	128	512	2 - ≥512
Ciprofloxacin	7.44	4.25	88.29	8	>16	0.12 - ≥16
Colistin	89.36	0.00	10.63	1	4	0.12 - ≥16
Amikacin	56.38	8.51	35.1	16	>64	≤1 - ≥64
Meropenem	0.00	9.57	90.42	16	>32	4 - ≥64
Tobramycin	38.29	5.31	56.38	16	>64	≤0.5 - ≥64
Ceftolozane/tazobactam	78.72	6.38	14.89	2	32	1 - ≥64

CONCLUSIONS

1. Incidence of XDR PA have remained low (1.5 to 3.5%) and constant in CANADA from 2007-2016.

2. MER-resistant PA have increased over the surveillance period, reaching a peak of 17.3% in 2014 and appears to be declining since.

3. Carbapenemase-producing PA are rare with only 3.4% (n=13) identified amongst the MER-resistant and XDR in this study.

4. Pan-drug resistant PA have not yet been identified in Canada, but treatment options are extremely limited.

5. MER-resistant and XDR PA remain susceptible to ceftolozane/tazobactam (91.62% and 78.72%, respectively).

6. XDR isolates do not cluster to specific STs, however they are associated with some high risk clones (ST111, ST175, ST235).

7. High-risk clones (ST111, ST175 and ST235) are seen in limited quantities in central Canada. They do not carry any of the tested carbapenemases as often seen in other parts of the word.

8. All GES-5 positive isolates were genetically related (ST17) and were isolated from 2 hospital sites. This is suggestive of a small outbreak resulting in minor genetic diversity (seen with PFGE) when spread to different sites.

ACKNOWLEDGMENTS

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