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Results from the Canadian Nosocomial Infection Surveillance Program for detection of carbapenemase-producing *Acinetobacter* spp. in Canadian hospitals, 2010–16

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Objectives: Globally there is an increased prevalence of carbapenem-resistant *Acinetobacter* spp. (CRAs) and carbapenemase-producing *Acinetobacter* spp. (CPAs) in the hospital setting. This increase prompted the Canadian Nosocomial Infection Surveillance Program (CNISP) to conduct surveillance of CRA colonizations and infections identified from patients in CNISP-participating hospitals between 2010 and 2016.

Methods: Participating acute care facilities across Canada submitted CRAs from 1 January 2010 to 31 December 2016. Patient data were collected from medical records using a standardized questionnaire. WGS was conducted on all CRAs and data underwent single nucleotide variant analysis, resistance gene detection and MLST.

Results: The 7 year incidence rate of CRA was 0.02 per 10 000 patient days and 0.015 per 1000 admissions, with no significant increase observed over the surveillance period ($P > 0.73$). Ninety-four CRA isolates were collected from 58 hospitals, of which 93 (98.9%) were CPA. Carbapenemase OXA-235 group (48.4%) was the most common due to two separate clusters, followed by the OXA-23 group (41.9%). Patients with a travel history were associated with 38.8% of CRA cases. The all-cause 30 day mortality rate for infected cases was 24.4 per 100 CRA cases. Colistin was the most active antimicrobial agent (95.8% susceptibility).

Conclusions: CRA remains uncommon in Canadian hospitals and the incidence did not increase from 2010 to 2016. Almost half of the cases were from two clusters harbouring OXA-235-group enzymes. Previous medical treatment during travel outside of Canada was common.

Introduction

Acinetobacter baumannii has emerged as an important healthcare-associated pathogen due to its ability to rapidly develop antibiotic resistance, intra- and inter-hospital outbreak spread and international clonal dissemination.[1,2] The acquisition of carbapenem resistance in *Acinetobacter* is most often due to carbapenem-hydrolysing class D β-lactamases (CHDLs) predominantly from the OXA-23, OXA-24/40, OXA-58, OXA-143 and OXA-235 groups.[1–4] This report describes

the incidence, epidemiology and molecular mechanisms of carbapenem-resistant *Acinetobacter* spp. (CRAs) collected over 7 years (2010–16) from a national hospital surveillance network, the Canadian Nosocomial Infection Surveillance Program (CNISP).

Methods

Description of the surveillance study

Surveillance was carried out between 1 January 2010 and 31 December 2016 and included inpatients, outpatients and emergency department patients from Canadian acute care hospitals belonging to CNISP (33 in 2010 increasing to 58 in 2016). The 58 CNISP hospitals were distributed across all 10 Canadian provinces: 10 (17%) in eastern Canada (PEI, Prince Edward Island; NB, New Brunswick; NS, Nova Scotia; and NL, Newfoundland & Labrador), 28 (48%) in central Canada (QC, Quebec; and ON, Ontario) and 20 (35%) in western Canada (MB, Manitoba; SK, Saskatchewan; AB, Alberta; and BC, British Columbia). The first *Acinetobacter* sp. collected from a patient during a single admission was considered eligible for inclusion and sent to the National Microbiology Laboratory (NML) for further analysis. A questionnaire was completed for all patients, which provided detailed patient history regarding travel, underlying medical conditions and patient outcome. Annual incidence rates for CRA from inpatients were calculated per 1000 admissions and 10 000 patient days. Incidence rates were compared using the *z*-test or Fisher's exact test as appropriate. Linear regression was used to identify significant changes in incidence rates over time.

Bacterial identification and antimicrobial susceptibility testing

Bacteria were initially identified at the submitting hospital with confirmation done at the NML using the taxonomic sequence classifier Kraken[5] and using OrthoANI.[6] Antimicrobial susceptibilities were determined using VITEK 2 AST-N219 cards and Etest strips (bioMérieux, St Laurent, Canada) or broth microdilution (colistin) using CLSI or FDA (tigecycline) breakpoints. XDR isolates were defined based on the recently published Canadian recommendations of full resistance to ciprofloxacin, piperacillin/tazobactam, ceftazidime, tobramycin and imipenem or meropenem.[7]

WGS and bioinformatic analyses

All 94 eligible isolates in this study were subjected to WGS on the MiSeq™ platform (Illumina, San Diego, CA, USA). Assembled reads (contigs) were analysed using tools at the Centre for Genomic Epidemiology website (https://cge.cbs.dtu.dk/services/). Single nucleotide variant (SNV) analysis was conducted using the SNVPhyl pipeline.[8] *A. baumannii* TYTH-1 (accession number CP003856) and *A. baumannii* K1-12-008 pseudogenomes were used as reference genomes in the SNV analyses.

GenBank accession numbers

All WGS read data were uploaded to the Sequence Read Archive of NCBI under the BioProject PRJNA390934. The $bla_{OXA-565}$ gene was assigned the accession number KY883665 and the *ampC* genes from *Acinetobacter bereziniae* K1-16-076 and *Acinetobacter soli* K1-13-056 were assigned the accession numbers MG987619 and MG987620, respectively.

Results and discussion

Acinetobacter species and epidemiological data for CRA cases

A total of 94 CRA isolates were collected: 90 *A. baumannii*, 2 *Acinetobacter pittii*, 1 *A. bereziniae* and 1 *A. soli*. The 94 CRAs were isolated in 2010 ($n = 9$), 2011 ($n = 2$), 2012 ($n = 10$), 2013 ($n = 38$), 2014 ($n = 8$), 2015 ($n = 9$) and 2016 ($n = 18$) from either western (22.3%) or central (77.7%) regions. From 2010 to 2016 the overall incidence rates for CRA were 0.02 per 10 000 patient days and 0.015 per 1000 admissions and did not significantly increase over the 7 years of surveillance ($P > 0.73$). Table S1 (available as Supplementary data at *JAC* Online) summarizes the demographic data available for patients. Data on international travel was available for 85 (90.4%) cases, of whom 33 (38.8%) had reported international travel in the past 12 months, with 31 of those having received medical treatment abroad. Thirty day outcome data were available for all 41 infected CRA cases, with 10 deaths reported. All-cause mortality 30 days from the date of positive culture was 24.4%. In all but two deaths the patient had an underlying medical condition.

MLST STs

All *A. baumannii* and *A. pittii* were typed *in silico* from the WGS data using both the Oxford (OX) and Pasteur (PA) MLST schemes. There were 30 different STs using the OX scheme and 17 using the PA scheme. The majority of isolates (79.8%, $n = 75$) belonged to the international clonal group 2 (IC2)[2] with most of those ($n = 70$) being $ST2_{PA}$. The $ST2_{PA}$ isolates represented 12 OX STs, with 71.4% being $ST208_{OX}$. STs are shown in Figure 1.

Antimicrobial susceptibilities and non-β-lactamase resistance genes

A summary of antimicrobial data is found in Table S2 and non-β-lactamase content is shown in Table S3. CRAs were commonly resistant to ciprofloxacin (95.7%), trimethoprim/sulfamethoxazole (89.4%) gentamicin (80.9%) and tobramycin (77.6%). Resistance to tigecycline (5.3%, $n = 5$) and colistin (4.3%, $n = 4$) was rare. Mutations in GyrA (Ser83Leu) and ParC (Ser80Leu) known to be associated with quinolone resistance were detected in 95.6% of ciprofloxacin-resistant isolates.[9,10] Colistin resistance in clinical *Acinetobacter* spp. is most often due to mutations in the *pmrA* or *pmrB* genes or the *lpxA*, *lpxC* or *lpxD* genes.[11–15] All four of the colistin-resistant isolates had amino acid changes in PmrB as compared with the control strains and one isolate, I1-16-001, had an M103K change in LpxC as well (Table S4). The two isolates with the highest MICs (>16 mg/L) had two amino acid changes in PmrB. The mobile colistin resistance genes *mcr-1* to *mcr-5* were not detected. According to recent Canadian guidelines,[10] 78.7% (74/94) of isolates in this study would be classified as XDR.[14]

β-Lactamase gene content

The β-lactamase content (except for intrinsic class C) of all 94 isolates is listed in Table S5 and the number of CHDLs by year is shown in Figure S1. An acquired OXA-type carbapenemase was found in 93 of the isolates with most harbouring an OXA-235-group[4] (48.4%, $n = 45$) or OXA-23-group (41.9%, $n = 39$) enzyme. One *A. baumannii* also harboured NDM-1 and one *A. pittii* also harboured IMP-26. All isolates except the *A. soli* harboured an intrinsic class D gene with ISAba1 located upstream in a single isolate, the one CRA without an acquired CHDL (Figure 2). All *A. baumannii* and *A. pittii* isolates except one harboured an

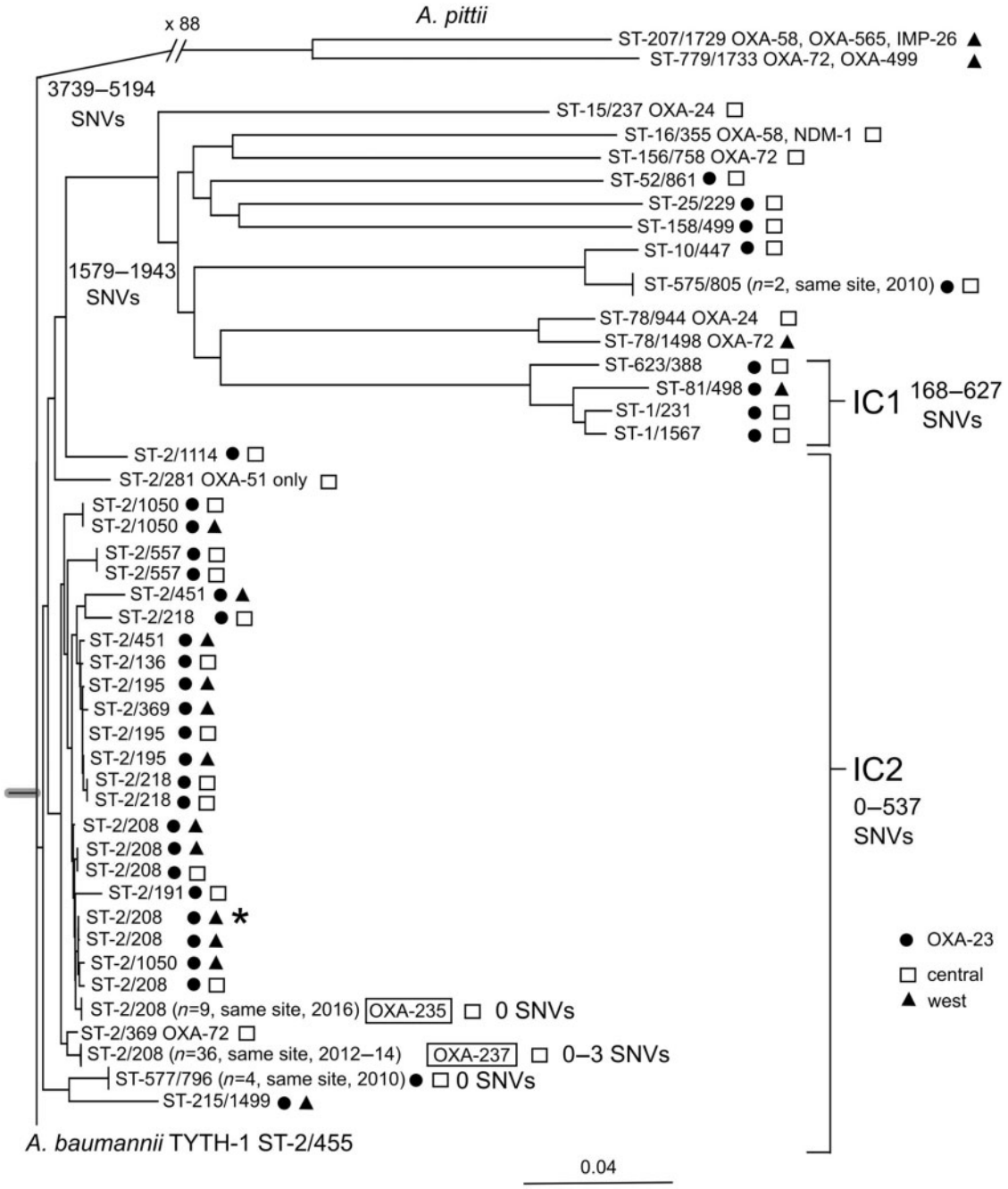

Figure 1. Phylogenetic tree based on SNV differences of the *A. baumannii* and *A. pittii* isolates in this study ($n = 92$). In this analysis 57.2% of the core genome and 12 860 SNV sites were used to determine the phylogeny. Tips are labelled with the ST (PA scheme/OX scheme) and the acquired carbapenemase gene(s) they harbour or a circle indicating OXA-23. The location where the bacterium was isolated is indicated by a square for central Canada or a triangle for western Canada. The gap in the *A. pittii* branch represents 88× reduction of the actual branch length. One OXA-237-harbouring isolate was $ST1024_{OX}$, a single locus variant (*gpi*) of $ST208_{OX}$. The reference genome used in the analysis was *A. baumannii* TYTH-1 (accession number CP003856) and the isolate it was most closely related to is indicated by an asterisk.

intrinsic class C bla_{ADC}-group gene with 88.3% ($n = 83$) of them being preceded by an ISAba1 element. Intrinsic class C *ampC* genes were also found in *A. bereziniae* K1-16-076 (accession number MG987619) and *A. soli* K1-13-056 (accession number MG987620), but they exhibited only a distant relationship (46%–53% identity) to the bla_{ADC} group.

SNV analysis

SNV analysis of the core genome was conducted on all *A. baumannii* and *A. pittii* isolates ($n = 92$) to determine broad phylogenetic relatedness (Figure 1). There were four broad clusters: the *A. pittii* cluster ($n = 2$); a diverse cluster of 15 isolates including the IC1

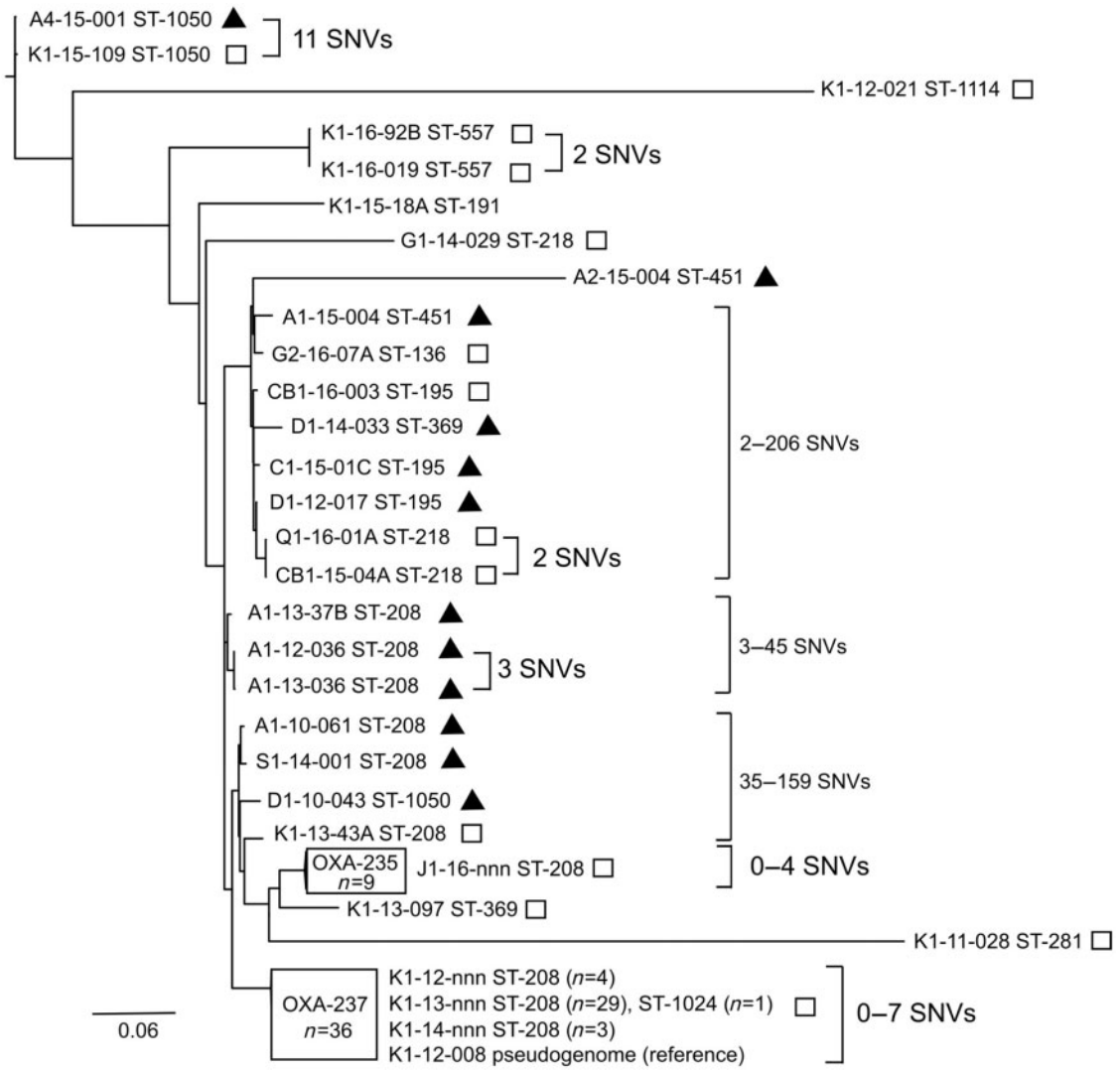

Figure 2. Phylogenetic tree based on SNV differences of the $ST2_{PA}$ *A. baumannii* in this study ($n = 70$). In this analysis 85.5% of the core genome and 6498 SNV sites were used to determine phylogeny. Tips are labelled with the isolate number and the ST_{OX} number. xx(x)-xx-xxx = hospital site-year of isolation (e.g. 16=2016)-individual isolate. The location where the bacterium was isolated is as in Figure 1. *A. baumannii* K1-13-028 did not harbour an acquired CHDL, but had ISAba1 upstream of its intrinsic OXA-82 (OXA-51 group) gene. The reference genome used in the analysis was the *A. baumannii* K1-12-008 pseudogenome.

isolates; the IC2 cluster of 70 isolates mainly consisting of $ST2_{PA}$ isolates; and a cluster of five isolates consisting of four $ST557_{PA}$/796_{OX} and one $ST215_{PA}$/1499_{OX}. To obtain a finer resolution of the $ST2_{PA}$ isolates we conducted SNV analysis of only these 70 isolates (Figure 2). Overall the 70 isolates differ by 0–3234 SNVs in this analysis; however, the majority ($n = 61$) differ by <500 SNVs and could be divided into five subclusters of >2 isolates including the OXA-235 and OXA-237 clusters. All nine OXA-235-harbouring isolates were collected from patients in the ICU of one hospital over a five month period in 2016. All of the isolates were epidemiologically linked to the index case who had recently received medical care in the USA and thus these cases constitute a small outbreak. All 36 OXA-237-harbouring isolates were collected in a different hospital in 2012 ($n = 4$), 2013 ($n = 29$) and 2014 ($n = 3$), with only one case (of 34) with reported international travel. A detailed analysis of possible transmission routes of these isolates in the hospital is the subject of another study (C. Bogaty, L. Mataseje, A. Grey, B. Lefebvre, S. Levesque, M. Mulvey and Y. Longtin, unpublished data). The *A. baumannii* plasmid pORAB01-3 harbouring OXA-237 was previously found in 16 isolates collected from June 2012 to October 2014 in the state of Oregon.[16,17] We did find the pORAB01-3 backbone (~12 kb) in the assemblies of the OXA-237 isolates in this study; however, due to the limitations of short-read sequencing this could not be linked to $bla_{OXA-237}$.

Conclusions

This study indicates that rates of CRA have not significantly increased over 7 years of CNISP surveillance and rates remain low as previously reported.[18,19] A limitation of this study is that

data on previous hospital admission or CRA status were not collected and hence multiple isolates for the same patient may be included in our analysis. Our surveillance data indicate that 38.8% of patients reported travel outside of Canada within the past 12 months and, with the exception of two patients, all received medical treatment while travelling. This suggests that travel and receipt of medical care outside of Canada are important factors in the importation of CPA into Canada though no region of travel predominated. The most prevalent strain type identified in every year of this surveillance period was *A. baumannii* $ST2_{PA}$ harbouring OXA-23 ($n = 38$). This is in agreement with reports of IC2 *A. baumannii* harbouring OXA-23 being the most globally spread CRA.[1,2] In this study 78.7% of isolates were XDR underscoring the prevalence of this feature in healthcare-associated *Acinetobacter* spp. More concerning was the finding that four of the XDR isolates were also resistant to colistin with two of those intermediately resistant to tigecycline, thus further limiting treatment options. The high association with XDR and putative outbreak isolates warrants the continued surveillance of this organism.

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Transparency declarations

None to declare.

Supplementary data

Tables S1 to S5 and Figure S1 are available as Supplementary data at *JAC* Online.

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