Identification of multidrug- and carbapenem-resistant Acinetobacter baumannii in Canada: results from CANWARD 2007

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Objectives: Multidrug-resistant (MDR) Acinetobacter baumannii is a growing concern in many countries. This report describes patient demographics, antimicrobial susceptibilities and molecular characteristics of A. baumannii cases identified through the Canadian Ward Surveillance Study (CANWARD). In addition, clinical cases involving MDR carbapenem-resistant A. baumannii are also detailed in this report.

Methods: From January to December 2007, 12 hospital centres across Canada submitted pathogens from clinics, emergency rooms, intensive care units and medical/surgical wards as part of the CANWARD study. MICs were determined using microbroth dilution (CLSI). PCR and sequence analysis identified OXA genes among carbapenem-resistant isolates. PFGE was used to determine genetic relatedness and compare representatives of the Midlands 2 strain, OXA-23 clone 1 or 2, T strains and isolates collected from military sources.

Results: This study identified A. baumannii in 0.33% (n=26) of infections. The majority of isolates remained susceptible to the antimicrobials tested, however, 7.7% (n=2) displayed an MDR phenotype, including resistance to carbapenems. In one isolate bla_OXA-58 was found to be the likely cause of carbapenem resistance while the other isolate had an insertion sequence element upstream of its intrinsic bla_OXA-51. The clinical data of these two isolates suggest that one is travel-related while the source of the other remains unknown.

Conclusions: A. baumannii infections from Canadian hospitals were relatively low. Carbapenem-resistant MDR A. baumannii were also rare and unrelated to previously observed isolates from military sources. Continued surveillance in Canada is suggested in order to determine if such organisms will become a problem.

Keywords: OXA-type β-lactamases, surveillance, MICs

Introduction

Acinetobacter baumannii is an opportunistic pathogen that often causes nosocomial pneumonia particularly in patients in intensive care units (ICUs) and burn units.¹ In a recent Canadian study, A. baumannii has been ranked as the 20th most common organism identified from ICUs.² Antimicrobial resistance is increasingly being reported in A. baumannii, often leaving carbapenems as the only effective drug to treat severe infections.³ However, carbapenem resistance has been observed on every continent; examples include the UK, Greece, North America and the Asia-Pacific region.³–⁶ Multidrug-resistant (MDR) A. baumannii have been particularly problematic in ICUs where, for example, several clones of carbapenem-resistant A. baumannii...
Multidrug- and carbapenem-resistant *Acinetobacter baumannii* in Canada

have been reported in Greece.³ Outbreaks of MDR *A. baumannii* have been reported in the New York area of the USA over the past 10 years.⁵ MDR (carbapenem-resistant and carbapenem-susceptible) *A. baumannii* have also been reported in Canada. Simor et al.⁷ described an outbreak of MDR *A. baumannii* (carbapenem-susceptible) at a 14-bed burn unit in a Toronto hospital involving 31 patients that was resolved through strict compliance with infection control practices. MDR (carbapenem-resistant) *A. baumannii* originating from injured Canadian military personnel returning from Afghanistan and Iraq have also been described.⁶ In this report, we describe the patient demographics, antimicrobial susceptibilities and molecular characteristics of *A. baumannii* cases identified through the CANWARD national surveillance study in Canada. In addition, the clinical cases involving MDR carbapenem-resistant *A. baumannii* are also detailed in this report.

### Materials and methods

Over a 1 year period from 1 January 2007, 12 tertiary care hospitals across Canada were asked to collect and submit consecutive pathogens (one per patient/infection site) from blood (*n* = 360), respiratory tract (*n* = 200), urine (*n* = 100) and wound/intravenous (*n* = 50) infections.⁹ Pathogens were from patients in hospital clinics, emergency rooms, medical or surgical wards and ICUs. Bacteria isolates were identified at participating clinical laboratories by conventional methods and then shipped to the reference laboratory at the Winnipeg Health Sciences Centre on Amies charcoal swabs. Isolates were sub-cultured onto blood agar and/or chocolate agar and stocked in skimmed milk at −80°C. Molecular characterization was completed at the National Microbiology Laboratory in Winnipeg.

Antimicrobial susceptibilities were determined using microbroth dilution and interpreted following breakpoints described by the CLSI (2008). Antimicrobials tested by this method included amikacin, cefepime, ceftriaxone, ciprofloxacin, gentamicin, levofloxacin, meropenem, piperacillin/tazobactam, trimethoprim/sulfamethoxazole, colistin (polymyxin E) and tigecycline. Breakpoints approved by the Food and Drug Administration (FDA) for Enterobacteriaceae were used to interpret tigecycline MICs (S and Drug Administration (FDA) for Enterobacteriaceae were used to interpret tigecycline MICs (S

### Results and discussion

Of the 7881 pathogens collected in 2007, 26 (0.33%) *A. baumannii* isolates were identified from infections at the 12 Canadian hospitals. Thirty-one percent (*n* = 8) were identified from patients in British Columbia/Alberta, 12% (*n* = 3) from Saskatchewan/Manitoba, 27% (*n* = 7) from Ontario/Quebec and 31% (*n* = 8) from the Maritime Provinces. Most *A. baumannii* were isolated from the ICU (*n* = 8) and the outpatient clinic (*n* = 8) followed by medical (*n* = 4) and surgery (*n* = 1) wards. Specimens were mostly from blood (*n* = 18) followed by the respiratory tract (*n* = 3), urine (*n* = 3) and wounds (*n* = 2). Patient age ranged from 20 to 86 years with the majority (*n* = 17) >50 years of age. Sixty-nine per cent (*n* = 18) of the isolates were from males.

Of the 26 *A. baumannii* isolates, 24 were relatively susceptible to all antimicrobials tested (Table 1). Of these 24 isolates, 21 were pan-susceptible and 3 were resistant to 1 antimicrobial (piperacillin/tazobactam, trimethoprim/sulfamethoxazole and ciprofloxacin, respectively). The remaining two isolates were MDR, which included resistance to the carbapenems.

The first carbapenem-resistant *A. baumannii* (isolate no. 73858) was identified from a 72-year-old Canadian female who was initially admitted to an ICU in a Greek hospital following a motor vehicle accident. During the ICU stay in Greece, the patient displayed no signs of infection but was treated

### Table 1. Antimicrobial resistance of 26 A. baumannii from Canadian hospitals

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt; (mg/L)</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt; (mg/L)</th>
<th>Range (mg/L)</th>
<th>Resistant, % (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin</td>
<td>≤2</td>
<td>2</td>
<td>≤2 to &gt;64</td>
<td>7.7 (2)</td>
</tr>
<tr>
<td>Cefepime</td>
<td>4</td>
<td>16</td>
<td>≤1 to &gt;128</td>
<td>7.2 (2)</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>16</td>
<td>32</td>
<td>≤1 to &gt;64</td>
<td>7.7 (2)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.25</td>
<td>1</td>
<td>≤0.06 to &gt;16</td>
<td>11.5 (3)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>≤0.5</td>
<td>1</td>
<td>≤0.5 to &gt;32</td>
<td>7.2 (2)</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>0.25</td>
<td>0.5</td>
<td>≤0.06–16</td>
<td>7.7 (2)</td>
</tr>
<tr>
<td>Meropenem</td>
<td>0.5</td>
<td>2</td>
<td>≤0.12–32</td>
<td>7.7 (2)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>0.19</td>
<td>0.25</td>
<td>0.032 to &gt;32</td>
<td>7.7 (2)</td>
</tr>
<tr>
<td>Piperacillin/tazobactam</td>
<td>2</td>
<td>32</td>
<td>≤1 to &gt;512</td>
<td>11.5 (3)</td>
</tr>
<tr>
<td>Tigecyclinea&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.5</td>
<td>1</td>
<td>0.12–4</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Trimethoprim/sulfamethoxazole</td>
<td>≤0.12</td>
<td>2</td>
<td>≤0.12 to &gt;8</td>
<td>11.5 (3)</td>
</tr>
<tr>
<td>Colistin (polymyxin E)</td>
<td>1</td>
<td>2</td>
<td>0.5 to &gt;16</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

<sup>a</sup>Breakpoints approved by the FDA for Enterobacteriaceae were used to interpret tigecycline MICs (S ≤2 mg/L; R >8 mg/L).
prophylactically with imipenem, colistin (polymyxin E) and teicoplanin. One month later the patient was transferred to an ICU in Quebec, Canada and MDR A. baumannii was cultured from her blood. This MDR carbapenem-resistant A. baumannii was isolated in April 2007 and was submitted to the CANWARD study for further analysis. It displayed reduced susceptibility to tigecycline (MIC = 4 mg/L) and was resistant to all other antimicrobials tested except for colistin. Catheters were removed 2 days later and new blood cultures were negative. The patient was later discharged to a rehabilitation centre to recover from the injuries.

The second carbapenem-resistant isolate (isolate no. 70582) was identified from a 40-year-old male who was admitted in 2006 for the treatment of recalcitrant decubitus ulcers. The development of the ulcers began in 2002 as a result of a motor vehicle accident that left him a paraplegic. Upon his admission in September 2006, multiple attempts were made to repair the ulcers. Within 2 months of admission a wound swab tested positive for methicillin-resistant Staphylococcus aureus and he was treated with vancomycin. He also developed Clostridium difficile colitis during the hospitalization. Other antimicrobials received over his 4 months in the hospital included trimethoprim/sulfamethoxazole, metronidazole and ciprofloxacin. MDR carbapenem-resistant A. baumannii was first isolated in 2006 and continued to be isolated from swabs and urine cultures until his discharge in January 2007. At this time it was decided that surgical closure of the wounds was no longer an option and the patient was discharged to a wound management care facility. It was the final MDR carbapenem-resistant A. baumannii isolated in January 2007 that was submitted to the CANWARD study for further analysis. It should also be noted that prior to the 2006 admission the patient had not left Canada in 5 years. The isolate submitted to the CANWARD surveillance study was resistant to all antimicrobials tested with the exception of tigecycline and colistin.

All but two DNA fingerprints generated by PFGE from this study were unique when compared with one another and with isolates from the UK (representatives of the Midlands 2 strain, OXA-23 clones 1 or 2, and T strains) and isolates identified from infections from Canadian military personnel returning from Afghanistan (Figure 1). The two related strains showed >90% similarity and were isolated from the same hospital within 1 week of each other.

PCR and sequence analysis confirmed that isolate 73858 harboured the intrinsic blaOXA-51, as well as blaOXA-58. PCR mapping of the blaOXA-58-flanking sequences was used to identify insertion sequence (IS) elements upstream of the gene. We identified ISAba2 within an ISAba3-like element upstream of blaOXA-58. This structure has been previously identified in A. baumannii from outbreaks in France and Spain. Transformation of OXA-58 was attempted in three independent experiments using electrocompetent E. coli (DH10B) and electrocompetent A. baumannii made in-house with no success. If OXA-58 is located on a plasmid it is non-transferable by our methods. In addition, there was no IS element identified upstream of the intrinsic blaOXA-51 in isolate 73858. The second isolate (70582) harboured the intrinsic blaOXA-51. An insertion element ISAba1 was found upstream of this gene and it is thought to provide a promoter, which in turn may contribute to increased expression of blaOXA-51 leading to a carbapenem resistance phenotype. Sequence typing was used to classify the carbapenem-resistant isolates and both belonged to sequence type group 1, corresponding to European clone II. Interestingly, isolates belonging to clonal group 1 have been identified as being a very successful lineage that can persist for long periods of time and have caused outbreaks in hospitals in many countries including the UK, Israel and Greece. In both carbapenem-resistant cases described in this study, no nosocomial transmission was observed.

In conclusion, this is the first study to establish a baseline for A. baumannii susceptibilities in Canada. Unlike reports from some countries, the majority (n = 24) of A. baumannii identified in this Canadian study were susceptible to all antimicrobials tested. However, of the two MDR isolates identified, both were also resistant to carbapenems, seriously limiting therapeutic options. Although one case appears to be travel related, the source of the second case is unknown. As MDR A. baumannii continue to disseminate globally, and isolates continue to be reported from military personnel returning from Afghanistan, continued surveillance is imperative to determine if MDR A. baumannii will become an issue in Canadian hospitals.

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Transparency declarations

None to declare.

References