Hospital-onset Gram-negative Surveillance Program, 2011

Abstract
The Australian Group on Antimicrobial Resistance performs regular period-prevalence studies to monitor changes in antimicrobial resistance in selected enteric Gram-negative pathogens. The 2011 survey focused on hospital-onset infections, examining isolates from all specimens presumed to be causing disease. In 2011, 1,827 Escherichia coli, 537 Klebsiella species and 269 Enterobacter species were tested using a commercial automated method (Vitek 2, BioMérieux) and results were analysed using Clinical and Laboratory Standards Institute breakpoints from January 2012. Of the key resistances, non-susceptibility to the third-generation cephalosporin, ceftriaxone, was found in 9.6% of E. coli and 9.5%–12.1% of Klebsiella spp. Non-susceptibility rates to ciprofloxacin were 10.6% for E. coli, 0.0%–8.3% for Klebsiella spp. and 0.0%–5.0% in Enterobacter spp. Resistance rates to gentamicin were 8.6%, 2.9%–10.9%, and 0.0%–15.6% for the same 3 groups respectively.

Eight strains, 5 Klebsiella spp. and 3 Enterobacter spp. were shown to harbour a carbapenemase (IMP-4). Commun Dis Intell 2014;38 (1):E49–E53.

Keywords: antibiotic resistance; hospital onset; gram-negative; Escherichia coli; Enterobacter; Klebsiella

Introduction
Emerging resistance in common pathogenic members of the family Enterobacteriaceae is a world-wide phenomenon, and presents therapeutic problems for practitioners in both the community and in hospital practice. The Australian Group on Antimicrobial Resistance commenced surveillance of the key Gram-negative pathogens, Escherichia coli and Klebsiella species in 1992. Surveys have been conducted biennially until 2008 when annual surveys commenced alternating between community- and hospital-onset infections (http://www.agargroup.org/surveys). In 2004, another genus of Gram-negative pathogens in which resistance can be of clinical importance, Enterobacter species, was added. E. coli is the most common cause of community-onset urinary tract infection, while Klebsiella species are less common but are known to harbour important resistances. Enterobacter species are less common but prominent in hospital-acquired infections, and of high importance due to intrinsic resistance to first-line antimicrobials.

Taken together, the 3 groups surveyed are considered to be valuable sentinels for multi-resistance and emerging resistance in enteric Gram-negative bacilli.

Resistances of particular interest include resistance to β-lactams due to β-lactamases, especially extended-spectrum β-lactamases, which inactivate the third-generation cephalosporins that are normally considered reserve antimicrobials. Other resistances of interest include resistance to antibiotics commonly used in the hospital setting such as cefazolin; resistance to agents important for serious infections, such as gentamicin; and resistance to reserve agents such as ciprofloxacin and meropenem.

The objectives of the 2011 surveillance program were to:

1. determine the proportion of resistance to the main therapeutic agents in E. coli, Klebsiella species and Enterobacter species in a subset of Australian diagnostic laboratories;
2. examine the extent of co-resistance and multi-resistance in these species; and
3. detect emerging resistance to extended-spectrum cephalosporins and newer last-line agents such as carbapenems.

Methods

Source of isolates
Isolates were collected from patients hospitalised for more than 48 hours. Each institution collected up to 70 E. coli, 20 Klebsiella spp. and 10 Enterobacter spp.

Species identification
Isolates were identified by one of the following methods: Vitek®, Phoenix™ Automated Microbiology System, Microbact; ATB®, or agar replication. In addition, some E. coli isolates were identified using chromogenic agar plus spot indole (DMACA).

Susceptibility testing
Testing was performed by a commercial semi-automated method, Vitek® 2 (BioMérieux), which is calibrated to the ISO reference standard method.
of broth microdilution. Commercially available Vitrek® AST-N149 cards were utilised by all participants throughout the survey period. The most recent Clinical and Laboratory Standards Institute breakpoints from 20121 were employed in the analysis. E. coli ATCC 25922 and E. coli ATCC 35218 were the quality control strains for this survey. For analysis of cefazolin, breakpoints of ≤4 for susceptible and ≥8 for resistant were applied due to the minimum inhibitory concentration (MIC) range available on the Vitrek card, recognising that the January 2012 breakpoint is actually susceptible ≤2 mg/L. Ertapenem MICs were performed using Etest™ strips (BioMérieux). Non-susceptibility, (which includes both intermediate resistant and resistant strains), has been included for some agents because these figures provide information about important emerging acquired resistances.

**Molecular confirmation of resistances**

E. coli and Klebsiella isolates with ceftazidime or ceftriaxone MIC >1 mg/L, or cefoxitin MIC >8 mg/L; Enterobacter spp. with cefepime MIC >1 mg/L; and all isolates with ertapenem MIC >0.5 mg/L or meropenem MIC >0.25 mg/L were referred to a central laboratory for molecular confirmation of resistance.

All isolates were screened for the presence of the bla_TEM, and bla_3GP genes using a real-time polymerase chain reaction (PCR) platform (LC-480) and published primers.2,3 A multiplex real-time TaqMan PCR was used to detect CTX-M-type genes.4 Strains were probed for plasmid-borne AmpC enzymes using the method described by Pérez-Pérez and Hanson,5 and subjected to molecular tests for MBL (bla_VIM, bla IMP, and bla_SPA), bla_KPC, and bla_OXA-48-like genes using real-time PCR.6,7

**Results**

In 2011, 2,633 isolates were examined comprising 1,827 E. coli, 537 Klebsiella spp. and 269 Enterobacter spp. (Table 1). The majority of isolates were from urine, while 5.6% of isolates overall were from blood cultures (comprising 4.8% of E. coli isolates, 7.3% of Klebsiella and 8.2% of Enterobacter species). Other sites of isolation reflect the high incidence of these species in nosocomial and pre– and post-operative surgical infections.

Major resistances and non-susceptibilities are listed in Table 2. Multi-resistance was detected in 12.6% of E. coli isolates, 10.6% of Klebsiella species, and 8.7% of Enterobacter species (Table 3). A more detailed breakdown of resistances and non-susceptibilities by state and territory is provided in the online report from the group (http://www.agargroup.org/surveys). By way of summary, there were no substantial differences across the states and territories in resistance patterns in contrast to what is seen with resistance patterns in Staphylococcus aureus and Enterococcus spp.

**Table 1: Species tested**

<table>
<thead>
<tr>
<th>Group</th>
<th>Species</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>E. coli</td>
<td>1,827</td>
</tr>
<tr>
<td>Klebsiella</td>
<td>K. pneumoniae</td>
<td>396</td>
</tr>
<tr>
<td></td>
<td>K. oxytoca</td>
<td>137</td>
</tr>
<tr>
<td></td>
<td>K. pneumoniae subsp ozaenae</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>Klebsiella not speciated</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>537</td>
</tr>
<tr>
<td>Enterobacter</td>
<td>E. cloacae</td>
<td>180</td>
</tr>
<tr>
<td></td>
<td>E. aerogenes</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>E. asburiae</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>E. gergoviae</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Enterobacter not speciated</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>269</td>
</tr>
</tbody>
</table>

**Escherichia coli**

Moderately high levels of resistance to ampicillin (and therefore amoxycillin) were observed (50.5%), with lower rates for amoxycillin-clavulanate (16.1% intermediate, and 7.7% resistant) (Table 2). Non-susceptibility to third-generation cephalosporins has increased slowly compared with the 2009 survey (ceftiazidime 9.6%, ceftazidime 5.8%, compared with 7.2% and 4.2% respectively in 2009). Most of the strains with extended-spectrum β-lactamase (ESBL) genes harboured genes of the CTX-M type (68%, 128/189). Moderate levels of resistance were detected to cefazolin (22.3%) and trimethoprim (23.4%). Ciprofloxacin non-susceptibility was found in 10.6% of E. coli isolates. Ciprofloxacin resistance was found in 51.1% and gentamicin resistance was found in 42.6% of ESBL-producing strains. Resistance to ticarcillin-clavulanate, cefepime, and gentamicin were below 5%. Two isolates had elevated meropenem MICs (≥0.5 mg/L) but 73 strains (4.0%) had ertapenem MICs above wild-type (>0.06 mg/L), 89% of which contained CTX-M or plasmid-borne AmpC genes. None harboured a known carbapenemase.

**Klebsiella species**

These showed slightly higher levels of resistance to cefazolin and ceftriaxone compared with E. coli, but lower rates of resistance or non-susceptibility to ticarcillin-clavulanate, cefazolin, ceftriaxone, ceftazidime, and gentamicin (Table 2). ESBLs were
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present in 48 of 53 presumptively ESBL-positive isolates of *K. pneumoniae*, 35 of which proved to be of the CTX-M type. Five of 7 *Klebsiella* species (5 *K. pneumoniae* and 1 *K. oxytoca*) with elevated meropenem MICs (≥ 0.5 mg/L) harboured *bla*<sub>IMP-4</sub> while 30 additional strains had elevated ertapenem MICs (>0.06 mg/L), but none of these harboured a known carbapenemase.

Table 2: Non-susceptibility and resistance rates for the main species tested

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Category*</th>
<th><em>E. coli</em> (%)</th>
<th><em>K. pneumoniae</em> (%)</th>
<th><em>K. oxytoca</em> (%)</th>
<th><em>E. cloacae</em> (%)</th>
<th><em>E. aerogenes</em> (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>I</td>
<td>0.9</td>
<td>†</td>
<td>†</td>
<td>†</td>
<td>†</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>R</td>
<td>50.5</td>
<td>†</td>
<td>†</td>
<td>†</td>
<td>†</td>
</tr>
<tr>
<td>Amoxicillin-clavulanate</td>
<td>I</td>
<td>16.1</td>
<td>8.8</td>
<td>4.4</td>
<td>†</td>
<td>†</td>
</tr>
<tr>
<td>Amoxicillin-clavulanate</td>
<td>R</td>
<td>7.7</td>
<td>6.1</td>
<td>10.2</td>
<td>†</td>
<td>†</td>
</tr>
<tr>
<td>Ticarcillin-clavulanate</td>
<td>R</td>
<td>8.0</td>
<td>9.1</td>
<td>11.7</td>
<td>33.9</td>
<td>21.7</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>R</td>
<td>22.3</td>
<td>18.4</td>
<td>68.6</td>
<td>†</td>
<td>†</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>R</td>
<td>4.8</td>
<td>4.3</td>
<td>2.2</td>
<td>†</td>
<td>†</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>NS</td>
<td>9.6</td>
<td>12.1</td>
<td>9.5</td>
<td>43.3</td>
<td>33.7</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>NS</td>
<td>5.8</td>
<td>9.8</td>
<td>3.6</td>
<td>40.6</td>
<td>28.9</td>
</tr>
<tr>
<td>Cefepime</td>
<td>NS</td>
<td>1.8</td>
<td>2.3</td>
<td>0.0</td>
<td>4.4</td>
<td>0.0</td>
</tr>
<tr>
<td>Meropenem</td>
<td>NS</td>
<td>0.1</td>
<td>0.5</td>
<td>0.0</td>
<td>0.6</td>
<td>0.0</td>
</tr>
<tr>
<td>Ertapenem</td>
<td>NS</td>
<td>0.2</td>
<td>1.0</td>
<td>0.0</td>
<td>16.1</td>
<td>4.8</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>NS</td>
<td>10.6</td>
<td>8.3</td>
<td>0.0</td>
<td>5.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>NS</td>
<td>10.2</td>
<td>4.8</td>
<td>0.0</td>
<td>4.4</td>
<td>0.0</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>NS</td>
<td>8.6</td>
<td>10.9</td>
<td>2.9</td>
<td>15.6</td>
<td>0.0</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>R</td>
<td>23.4</td>
<td>18.7</td>
<td>4.4</td>
<td>27.2</td>
<td>2.4</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>NS</td>
<td>5.0</td>
<td>†</td>
<td>†</td>
<td>†</td>
<td>†</td>
</tr>
</tbody>
</table>

*R = resistant, I = intermediate, NS = non-susceptible (intermediate + resistant).
† Considered largely intrinsically resistant due to natural β-lactamases.

Testing for resistance to piperacillin-tazobactam was not available for this survey due to a global recall from BioMérieux.

Table 3: Multiple acquired resistances, by species

<table>
<thead>
<tr>
<th>Species</th>
<th>Total</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>Cumulative</th>
<th>Multi-resistant</th>
<th>Cumulative</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>1,827</td>
<td>45.3</td>
<td>18.6</td>
<td>15.2</td>
<td>8.2</td>
<td>87.4</td>
<td>68</td>
<td>48</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td>28.0</td>
<td>15.8</td>
<td>22</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td><em>Klebsiella spp.</em></td>
<td>537</td>
<td>52.1</td>
<td>29.4</td>
<td>4.1</td>
<td>3.7</td>
<td>89.4</td>
<td>3.7</td>
<td>2.2</td>
</tr>
<tr>
<td>%</td>
<td></td>
<td>50.0</td>
<td>40.0</td>
<td>6.0</td>
<td>4.0</td>
<td>100</td>
<td>16</td>
<td>6</td>
</tr>
<tr>
<td><em>Enterobacter spp.</em></td>
<td>269</td>
<td>39.8</td>
<td>20.8</td>
<td>23.0</td>
<td>6.7</td>
<td>90.3</td>
<td>5.9</td>
<td>2.2</td>
</tr>
</tbody>
</table>

* Antibiotics included: amoxycillin-clavulanate, cefazolin, cefoxitin, ceftriaxone, ceftazidime, cefepime, gentamicin, amikacin, ciprofloxacin, nitrofurantoin, trimethoprin, meropenem.
† Antibiotics excluded: ampicillin (intrinsic resistance), ticarcillin-clavulanate, tobramycin, norfloxacin, nalidixic acid, sulfamethoxazole-trimethoprin (high correlation with antibiotics in the included list).

* Antibiotics included: ceftriaxone, ceftazidime, cefepime, gentamicin, amikacin, ciprofloxacin, nitrofurantoin, trimethoprim, meropenem.
† Antibiotics excluded: ampicillin, amoxycillin-clavulanate, cefazolin, and cefoxitin, (all four due to intrinsic resistance); also excluded were ticarcillin-clavulanate, tobramycin, norfloxacin, nalidixic acid, sulfamethoxazole-trimethoprin (high correlation with antibiotics in the included list).
Enterobacter species

Acquired resistance was common to ticarcillin-clavulanate (29.7%), ceftriaxone (40.1%), ceftazidime (36.4%) and trimethoprim (19.3%) (Table 2). Rates of resistance to cefepime, ciprofloxacin, and gentamicin were all less than 11%. Twenty-seven of 88 strains tested for ESBL based on a suspicious phenotype, harboured ESBL-encoding genes. Five strains had elevated meropenem MICs (≥ 0.5 mg/L) three of which harboured bla\text{IMP-4} while 39% of strains had ertapenem MICs above wild type (>0.125 mg/L), related to the presence of stably-derepressed chromosomal AmpC β-lactamase.

Discussion

Comparing these results with those from the first hospital-onset survey in 2009, there is a small but noticeable increase in resistance or non-susceptibility rates to some reserve antibiotics. For example, rates of resistance in E. coli for ceftriaxone rose from 7.2% to 9.6% and for non-susceptibility to ciprofloxacin rose from 8.1% to 10.6%. Such rises were not observed in Klebsiella or Enterobacter species. Although originally thought to be primarily community-associated, the great bulk of extended-spectrum β-lactamases detected were of the CTX-M type, suggesting that this group has become the dominant form in hospital infections as well. Plasmid-borne AmpC β-lactamases also appear to be increasing substantially, up from 31 strains with genes detected encoding one of these enzymes in 2009, to 51 strains in 2011.

The greatest concern is the emergence of carbapenemases which affect the ‘last-line’ β-lactams such as meropenem. In 2009, we detected 5 strains of Klebsiella with a carbapenemase, all of which were bla\text{IMP-4.} In this 2011 survey, we found 8 strains, 5 Klebsiella spp. and 3 Enterobacter sp., all of which were also bla\text{IMP-4.} This carbapenemase appears to have become endemic in Australia, albeit at a very low level presently. So far our surveys have not detected other carbapenemases, such as KPC-2 and NDM-1, which are known to be prevalent in other countries. However, there are published reports of the detection on these carbapenemases in Australia, all so far imported by overseas visitors or Australian returning from overseas.\textsuperscript{10,11} Surveys such as those conducted by AGAR are critical to determining whether such unwelcome resistances might become established in Australia.

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