Hospital-onset Gram-negative Surveillance Program annual report, 2011

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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 focussed 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-susceptibilty 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 ß-lactams due to ß-lactamases, especially extended-spectrum ß-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 Vitek® AST-N149 cards were utilised by all participants throughout the survey period. The most recent Clinical and Laboratory Standards Institute breakpoints from 2012[1] 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 Vitek 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 intermediately 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_{SHV} 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_{NDM}), 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

Group	Species	Total
E. coli	*E. coli*	1,827
Klebsiella	*K. pneumoniae*	396
	K. oxytoca	137
	K. pneumoniae subsp *ozaenae*	3
	Klebsiella not speciated	1
Total		537
Enterobacter	*E. cloacae*	180
	E. aerogenes	83
	E. asburiae	3
	E. gergoviae	2
	Enterobacter not speciated	1
Total		269

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 (ceftriaxone 9.6%, ceftazidime 5.8%, compared with 7.2% and 4.2% respectively in 2009). Most of the strains with extended-spectrum ß-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

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

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

* 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

Species	Total	Number of acquired resistances												
		Non-multi-resistant					Multi-resistant							
		0	1	2	3	Cumulative %	4	5	6	7	8	9	10	Cumulative %
E. coli	1,827	828	340	278	150		68	48	55	29	26	4	1	
%		45.3	18.6	15.2	8.2	87.4	3.7	2.6	3.0	1.6	1.4	0.2	0.1	12.6
Klebsiella spp.*	537	280	158	22	20		20	12	10	11	3	1		
%		52.1	29.4	4.1	3.7	89.4	3.7	2.2	1.9	2.0	0.6	0.2		10.6
Enterobacter spp.†	269	107	56	62	18		16	6	3	1				
%		39.8	20.8	23.0	6.7	90.3	5.9	2.2	1.1	0.4				9.7

* Antibiotics included: amoxycillin-clavulanate, cefazolin, cefoxitin, ceftriaxone, ceftazidime, cefepime, gentamicin, amikacin, ciprofloxacin, nitrofurantoin, trimethoprim, meropenem.

Antibiotics excluded: ampicillin (intrinsic resistance), ticarcillin-clavulanate, tobramycin, norfloxacin, nalidixic acid, sulfamethoxazole-trimethoprim (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-trimethoprim (high correlation with antibiotics in the included list).

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 $bla_{\text{IMP-4}}$, while 30 additional strains had elevated ertapenem MICs (>0.06 mg/L), but none of these harboured a known carbapenemase.

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_{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* ß-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 ß-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 ß-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' ß-lactams such as meropenem. In 2009, we detected 5 strains of *Klebsiella* with a carbapenemase, all of which were bla_{IMP-4}. In this 2011 survey, we found 8 strains, 5 *Klebsiella* spp. and 3 *Enterobacter* sp., all of which were also bla_{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.[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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