Australian Group on Antimicrobial Resistance Australian Enterobacteriaceae Sepsis Outcome Programme annual report, 2014

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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 2014 survey was the second year to focus on blood stream infections. During 2014, 5,798 Enterobacteriaceae species isolates were tested using commercial automated methods (Vitek 2, BioMérieux; Phoenix, BD) and results were analysed using the Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints (January 2015). Of the key resistances, non-susceptibility to the third-generation cephalosporin, ceftriaxone, was found in 9.0%/9.0% of *Escherichia coli* (CLSI/EUCAST criteria) and 7.8%/7.8% of *Klebsiella pneumoniae,* and 8.0%/8.0% *K. oxytoca*. Non-susceptibility rates to ciprofloxacin were 10.4%/11.6% for *E. coli,* 5.0%/7.7% for *K. pneumoniae,* 0.4%/0.4% for *K. oxytoca,* and 3.5%/6.5% in *Enterobacter cloacae*. Resistance rates to piperacillin-tazobactam were 3.2%/6.8%, 4.8%/7.2%, 11.1%/11.5%, and 19.0%/24.7% for the same 4 species respectively. Fourteen isolates were shown to harbour a carbapenemase gene, 7 bla_{IMP-4}, 3 bla_{KPC-2}, 3 bla_{VIM-1}, 1 bla_{NDM-4}, and 1 $bla_{OXA-181-like}$. *Commun Dis Intell* 2016;40(2):E229–E235.

Keywords: antibiotic resistance; bacteraemia; gram-negative; *Escherichia coli; Enterobacter; Klebsiella*

Introduction

Emerging resistance in common pathogenic members of the 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 (AGAR) 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.[1] 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 in the community, but of high importance due to intrinsic resistance to first-line antimicrobials in the community. Taken together, the three groups of species surveyed are considered to be valuable sentinels for multi-resistance and emerging resistance in enteric Gram-negative bacilli. In 2013 AGAR commenced the Enterobacteriaceae Sepsis Outcome Programme (EnSOP), which focused on the collection of resistance and some demographic data on all isolates prospectively from patients with bacteraemia. The 2014 survey was the second EnSOP survey.

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 are to agents important for treatment of these serious infections, such as gentamicin; and resistance to reserve agents such as ciprofloxacin and meropenem.

The objectives of the 2014 surveillance program were to:

1. monitor resistance in Enterobacteriaceae isolated from blood;
2. examine the extent of co-resistance and multi-resistance; and
3. detect emerging resistance to newer last-line agents such as carbapenems.

Methods

Study design

From 1 January to 31 December 2014, 26 institutions across Australia collected either all or up to 200 isolates from different patient episodes of bacteraemia.

Species identification

Isolates were identified using the routine method for each institution; Vitek®, Phoenix™ Automated Microbiology System, or where available, mass spectrometry (MALDI-TOF).

Susceptibility testing

Testing was performed by 2 commercial semi-automated methods, Vitek 2 (BioMérieux) or Phoenix (BD), which are calibrated to the ISO reference standard method of broth microdilution. Commercially available Vitek AST-N246, or Phoenix NMIC-203 cards were utilised by all participants throughout the survey period. The Clinical and Laboratory Standards Institute (CLSI) M100[2] and European Committee on Antimicrobial Susceptibility Testing (EUCAST) v5.0[3] breakpoints from January 2015 have been employed in the analysis. For analysis of cefazolin, breakpoints of ≤ 4 for susceptible, and ≥ 8 for resistant were applied due to the restricted minimum inhibitory concentration (MIC) range available on the commercial cards, recognising that the January 2015 breakpoint is actually susceptible ≤ 2 mg/L.

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; all isolates with ciprofloxacin MIC > 0.25 mg/L; all isolates with meropenem MIC > 0.25 mg/L; and all isolates with amikacin MIC > 32 mg/L were referred to a central laboratory (SA Pathology) for molecular confirmation of resistance.

All referred 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.[4,5] A multiplex real-time TaqMan PCR was used to detect CTX-M-type genes.[6] Strains were probed for plasmid-borne AmpC enzymes using the method described by Pérez-Pérez and Hanson,[7] 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.[8,9] Known plasmid mediated quinolone resistance mechanisms (Qnr, efflux (*qepA, oqxAB*), and aac(6')-Ib-cr) were examined by PCR on all referred isolates with ciprofloxacin MIC > 0.25 mg/L using published methods.[10,11] All *E. coli* were examined for presence of the O25b-ST131 clone and its H30- and H30-Rx subclones.[12–14]

Results

The species isolated, and the numbers of each are listed in Table 1. Three genera, *Escherichia* spp., *Klebsiella* spp. and *Enterobacter* spp. contributed 87.6% of all isolates. Major resistances and non-susceptibilities for the top 6 ranked species are listed in Table 2. 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. Multiple acquired resistances by species are shown in Table 3. Multi-resistance was detected in 13.4% of *E. coli* isolates, 9.7% of *K. pneumoniae*, and 12.1% of *Ent. cloacae*. 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).

Table 1: Species tested

Species	Total	%
Escherichia coli	3,493	60.2
Klebsiella pneumoniae	877	15.1
Enterobacter cloacae	343	5.9
Klebsiella oxytoca	226	3.9
Proteus mirabilis	187	3.2
Serratia marcescens	136	2.3
Enterobacter aerogenes	105	1.8
Salmonella species (non Typhi)	94	1.6
Morganella morganii	57	1.0
Citrobacter freundii	53	0.9
Citrobacter koseri	50	0.9
Salmonella Typhi/Paratyphi	26	0.4
Enterobacter asburiae	16	0.3
Raoultella ornithinolytica	15	0.3
Pantoea species	12	0.2
Pantoea agglomerans	12	0.2
Enterobacter species	11	0.2
Providencia stuartii	10	0.2
Other species (n=27)	75	1.3
Total	5,798	

Escherichia coli

Moderately high levels of resistance to ampicillin (and therefore amoxycillin) were maintained (50.1%/51.9%, CLSI/EUCAST criteria), with lower rates for amoxycillin-clavulanate (12.7%/-intermediate, 8.2%/20.9% resistant). Non-susceptibility to third-generation cephalosporins was low (ceftriaxone 9.0%/9.0%, ceftazidime

Table 2: Non-susceptibility and resistance rates for the top 6 ranked species tested

Antimicrobial	Category*	*Escherichia coli* (%)		*Klebsiella pneumoniae* (%)		*Klebsiella oxytoca* (%)		*Enterobacter cloacae* (%)		*Proteus mirabilis* (%)		*Serratia marcescens* (%)	
		CLSI	EUCAST	CLSI	EUCAST	CLSI	EUCAST	CLSI	EUCAST	CLSI	EUCAST	CLSI	EUCAST
Ampicillin	I	1.8	–	†	†	†	†	†	†	0.5	–	†	†
	R	50.1	51.9	†	†	†	†	†	†	16.8	17.3	†	†
Amoxycillinclavulanate‡	I	12.7	–	5.1	–	4.4	–	†	†	8.7	–	†	†
	R	8.2	–	5.3	–	8.8	–	†	†	1.6	–	†	†
Ticarcillin-clavulanate	R	9.4	19.3	7.2	11.4	10.4	12.2	24.4	29.7	1.1	1.7	0.0	2.2
Piperacillintazobactam	R	3.2	6.8	4.8	7.2	11.1	11.5	19.0	24.7	1.1	1.6	0.0	0.0
Cefazolin	R	20.5	/	13.0	/	66.0	/	†	†	26.5	/	†	†
Cefoxitin	R	3.8	/	6.2	/	0.9	/	†	†	0.0	/	†	†
Ceftriaxone	NS	9.0	9.0	7.8	7.8	8.0	8.0	27.6	27.6	0.5	0.5	2.9	2.9
Ceftazidime	NS	4.4	8.0	6.1	8.0	0.4	0.4	24.6	27.0	0.0	0.0	2.2	2.2
Cefepime	NS	3.3	6.4	3.7	6.1	0.0	0.0	4.1	14.4	1.1	1.1	1.5	2.2
Meropenem	NS	0.1	0.1	1.1	1.0	0.0	0.0	2.9	2.3	0.5	0.5	0.7	0.7
Ciprofloxacin	NS	10.4	11.6	5.0	7.6	0.4	0.4	3.5	6.5	2.7	3.2	1.5	3.7
Norfloxacin	NS	10.4	18.2	4.5	13.7	0.0	1.8	3.2	13.5	3.2	5.4	0.7	3.7
Gentamicin	NS	7.5	8.0	5.5	6.1	1.3	1.8	6.7	7.6	1.6	2.2	1.5	1.5
Trimethoprim	R	29.2	29.4	15.5	16.6	4.0	4.4	19.1	19.1	21.7	22.3	1.5	2.2
Nitrofurantoin	NS	6.6	1.6	88.8	/	41.6	/	72.6	/	†	†	†	†

* R = resistant, I = intermediate, NS = non-susceptible (intermediate + resistant), using criteria as published by the Clinical and Laboratory Standards Institute (CLSI) [2014] and European Committee on Antimicrobial Susceptibility Testing (EUCAST) [2014].

† Considered largely intrinsically resistant due to natural β-lactamases; – no intermediate category; / no breakpoints defined

‡ For EUCAST interpretation, the clavulanate is fixed at 2 mg/L, rather than a 2:1 ratio used in CLSI guidelines. As all cards used have a 2:1 ratio of clavulanate no EUCAST category has been applied.

Table 3: Multiple acquired resistances, by species

Species	Total	Number of acquired resistances (CLSI breakpoints)															
		Non-multi-resistant					Multi-resistant										
		0	1	2	3	Cumulative %	4	5	6	7	8	9	10	11	12	13	Cumulative %
Escherichia coli	2,958	1,324	506	505	226		133	109	71	44	22	11	6	0	0	0	
	%	44.8	17.1	17.1	7.6	86.6	4.5	3.7	2.4	1.5	0.7	0.4	0.2	0.0	0.0	0.0	13.4
*Klebsiella pneumoniae**	746	417	192	51	14		17	15	11	6	6	5	4	2	6		
	%	55.9	25.7	6.8	1.9	90.3	2.3	2.0	1.5	0.8	0.8	0.7	0.5	0.3	0.8		9.7
Enterococcus cloacae†	330	163	66	24	37		20	12	5	1	2						
	%	49.4	20.0	7.3	11.2	87.9	6.1	3.6	1.5	0.3	0.6						12.1
Proteus mirabilis	148	3	87	35	13		7	2	0	0	0	0	1				
	%	2.0	58.8	23.6	8.8	93.2	4.7	1.4	0.0	0.0	0.0	0.0	0.7				6.8
Serratia marcescens†	98	1	94	0	1		2										
	%	1.0	95.9	0.0	1.0	98.0	2.0										2.0
*Klebsiella oxytoca**	197	66	98	14	10		7	2									
	%	33.5	49.7	7.1	5.1	95.4	3.6	1.0									4.6
Enterococcus aerogenes†	104	36	31	12	18		5	1	1								
	%	34.6	29.8	11.5	17.3	93.3	4.8	1.0	1.0								6.7
Salmonella spp. (non-Typhi)	71	57	8	4	2												
	%	80.3	11.3	5.6	2.8	100.0											0.0

* Antibiotics included: amoxycillin-clavulanate, piperacillin-tazobactam, 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: piperacillin-tazobactam, 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).

4.4%/8.0%). Moderate levels of resistance were detected to cefazolin (20.5%/-) and trimethoprim (29.2%/29.4%). Ciprofloxacin non-susceptibility was found in 10.4%/11.6% of *E. coli* isolates. Resistance to ticarcillin-clavulanate (9.4%/19.3%), gentamicin (7.3%/7.5%), piperacillin-tazobactam (3.2%/6.8%), and cefepime (1.6%/2.9%) were low. Nine isolates had elevated meropenem MICs (≥ 0.5 mg/L). For the extended-spectrum ß-lactamase (ESBL)-producing strains, ciprofloxacin and gentamicin resistance was found in 51.6%/51.6% and 33.8%/34.1% respectively.

In line with international trends among community strains of *E. coli*, most of the strains with ESBL genes harboured genes of the CTX-M type (222/272 = 82%). Over 60% of *E. coli* with CTX-M group 1 types were found to belong to sequence type 131 (O25b-ST131). ST131 accounted for 68% of *E. coli* ESBL phenotypes that were ciprofloxacin resistant (MIC > 1 mg/L), and only 6% of ciprofloxacin susceptible ESBL phenotypes. Ninety-one per cent and 41% of O25b-ST131 were associated with the H30 and *H30-Rx* subclones, respectively, with their reported association with more antibiotic resistances and greater virulence potential.[13]

Klebsiella pneumoniae

K. pneumoniae showed slightly higher levels of resistance to piperacillin-tazobactam and ceftazidime compared with *E. coli*, but lower rates of resistance to amoxycillin-clavulanate, ticarcillin-clavulanate, cefazolin, ceftriaxone ciprofloxacin, gentamicin, and trimethoprim. Thirteen *K. pneumoniae* isolates had elevated meropenem MICs. ESBLs were present in 61 of 69 (88%) presumptively ESBL-positive isolates of *K. pneumoniae,* 47 (77%) of which proved to be of the CTX-M type.

Enterobacter species

Acquired resistance was common to ticarcillin-clavulanate (24.4%/29.7% and 26.7%/40.6%), piperacillin-tazobactam (19.0%/24.7% and 23.1%/31.7%), ceftriaxone (27.3%/27.3% and 37.5%/37.5%), ceftazidime (24.3%/24.6% and 29.8%/33.7%) and trimethoprim (19.1%/19.1% and 1.9%/1.9%) for *Ent. cloacae* and *Ent. aerogenes,* respectively. Cefepime, ciprofloxacin, and gentamicin resistance were all less than 10%. Seventeen of 45 *Ent. cloacae* tested for ESBL based on a suspicious phenotype, harboured ESBL-encoding genes. Eighteen *Ent. cloacae* strains had elevated meropenem MICs.

Carbapenemase resistance

Overall, 14 isolates (14 patients) in 9 institutions from 5 states or territories were found to harbour a carbapenemase gene. Bla_{IMP-4} was detected in *E. cloacae* (5) and *K. pneumoniae* (2); bla_{KPC-2} was detected in 3 *K. pneumoniae* isolates from 1 institution; bla_{VIM-1} was detected in 2 *K. pneumoniae; *bla_{NDM-4} in 1 *E. coli,* and $bla_{OXA-181-like}$ in 1 *K. pneumoniae.*

Discussion

AGAR has been tracking resistance in sentinel enteric Gram-negative bacteria since 1992. From 2008, surveillance was segregated into hospital- versus community-onset infections. The last year of hospital-onset only surveillance was 2011.[15] In 2013, the first survey of antimicrobial resistance among Enterobacteriaceae isolates from bacteraemic patients throughout Australia was conducted using an approach similar to that conducted by the European EARS-Net program. The 2014 survey was the second survey conducted of antimicrobial resistance among Enterobacteriaceae isolates from bacteraemic patients throughout Australia.

CTX-M-producing *E. coli* and *Klebsiella* species and gentamicin- and ciprofloxacin-resistant *E. coli* continued to be a problem in patients with bacteraemia. Of concern is the high proportion of *E. coli* that belong to the ST131 *H30-Rx* subclone, and its reported association with more antibiotic resistance and greater virulence potential.[13] Carbapenem resistance attributable to acquired carbapenemases are still uncommon in patients with bacteraemia in Australia, although 5 different types (IMP, KPC, VIM, NDM and OXA-181-like) were detected from 9 of the participating institutions. Compared with many other countries in our region, resistance rates in Australian Gram-negative bacteria are still relatively low,[16] but similar to those observed in 2014 in many Western European countries.[17]

Multi-resistance is being increasingly observed, especially in *E. coli* and *E. cloacae,* both of which have multi-resistance rates (as defined by AGAR) above 10%. This is likely to drive more broad-spectrum antibiotic use, and increase the resistance selection pressure for important reserve classes, especially the carbapenemases.

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