Australian Group on Antimicrobial Resistance Enterobacteriaceae Sepsis Outcome Programme (EnSOP) Annual Report, 2013

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Abstract

The Australian Group on Antimicrobial Resistance (AGAR) performs regular period-prevalence studies to monitor changes in antimicrobial resistance in selected enteric Gram-negative pathogens. The 2013 survey focussed for the first time on blood stream infections. Four thousand nine hundred and fifty-eight *Enterobacteriaceae* species were tested using commercial automated methods (Vitek® 2, BioMérieux; Phoenix™, BD) and results were analysed using Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints (January 2014). Of the key resistances, non-susceptibility to the third-generation cephalosporin, ceftriaxone, was found in 7.5%/7.5% of *Escherichia coli* (CLSI/EUCAST criteria); 6.3%/6.3% of *Klebsiella pneumoniae*, and 7.4%/7.4% of *K. oxytoca*. Non-susceptibility rates to ciprofloxacin were 10.3%/11.3% for *E. coli*, 4.6%/7.5% for *K. pneumoniae,* 0.6%/0.6% for *K. oxytoca,* and 3.6%/6.1% in *Enterobacter cloacae*. Resistance rates to piperacillin-tazobactam were 3.1%/6.2%, 4.2%/7.0%, 11.9%/12.6%, and 17.3%/22.2% for the same four species respectively. Fourteen isolates were shown to harbour a carbapenemase gene, nine *bla*$_{IMP}$, three *bla*$_{KPC}$, and two *bla*$_{NDM}$.

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 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 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.

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 2013 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 1st January to 31st December 2013, 25 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 two 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, Vitek AST-N247, Phoenix NMIC/ID-80 or Phoenix NMIC-203 cards were utilized by all participants throughout the survey period. The CLSI M100[1] and EUCAST v4.0[2] breakpoints from January 2014 have been employed in the analysis. For analysis of cefazolin, breakpoints of ≤4 for susceptible, ≥8 for resistant were applied due to the restricted minimum inhibitory concentration (MIC) range available on the commercial cards, recognising that the January 2014 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; and all isolates with meropenem MIC >0.25 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.[3,4] A multiplex real-time TaqMan PCR was used to detect CTX-M-type genes.[5] Strains were probed for plasmid-borne AmpC enzymes using the method described by Pérez-Pérez and Hanson,[6.] 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.[7,8] Known plasmid mediated quinolone resistance (PMQR) 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.[9,10] All *E. coli* were examined for presence of the O25b-ST131 clone and its *H30*- and *H30*-Rx subclones.[11-13]

Results

A total of 4,958 Enterobacteriaceae species were tested, the species isolated and the numbers of each are listed in Table 1. Three genera, *E. coli*, *Klebsiella* spp. and *Enterobacte*r spp. contributed 86.3% of all isolates. Major resistances and non-susceptibilities for the top six ranked species are listed in Table 2. Non-susceptibility (which includes 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 11.7% of *E. coli* isolates, 7.0% of *K. pneumoniae*, and 12.6% of *Enterobacter 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	2958	59.7%
Klebsiella pneumoniae	727	14.7%
Enterobacter cloacae	311	6.3%
Proteus mirabilis	184	3.7%
Klebsiella oxytoca	163	3.3%
Serratia marcescens	156	3.1%
Enterobacter aerogenes	98	2.0%
Salmonella species (non Typhi)	78	1.6%
Morganella morganii	54	1.1%
Citrobacter koseri	51	1.0%
Citrobacter freundii	38	0.8%
Salmonella Typhi/Paratyphi	23	0.5%
Pantoea agglomerans	13	0.3%
Raoultella ornithinolytica	11	0.2%
Enterobacter asburiae	11	0.2%
Other species (n=31)	82	1.7%

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

		E. coli (%)		*K. pneumoniae* (%)		*K. oxytoca* (%)		*E. cloacae* (%)		*P. mirabilis* (%)		*S. marcescens* (%)	
Antimicrobial	*Category**	CLSI	EUCAST	CLSI	EUCAST	CLSI	EUCAST	CLSI	EUCAST	CLSI	EUCAST	CLSI	EUCAST
Ampicillin	I	2.0	-	†	†	†	†	†	†	2.8	-	†	†
	R	50.2	52.2	†	†	†	†	†	†	17.0	19.8	†	†
Amoxycillin-clavulanate	I	12.7	-	5.5	-	4.3	-	†	†	5.5	-	†	†
	R	8.8	21.5	6.0	11.5	8.7	13.0	†	†	5.0	10.5	†	†
Ticarcillin-clavulanate	R	8.1	18.3	5.9	9.6	10.6	12.5	23.3	27.5	0.6	1.7	1.9	5.1
Piperacillin-tazobactam	R	3.1	6.2	4.2	7.0	11.9	12.6	17.3	22.2	0.6	1.1	0.0	2.1
Cefazolin	R	19.1	/	10.0	/	62.1	/	†	†	24.2	/	†	†
Cefoxitin	R	2.9	/	4.2	/	0.0	/	†	†	1.1	/	†	†
Ceftriaxone	NS	7.5	7.5	6.3	6.3	7.4	7.4	26.8	26.8	1.6	1.6	5.1	5.1
Ceftazidime	NS	4.1	7.0	4.9	6.6	1.3	1.9	23.3	26.9	0.5	1.1	0.6	1.9
Cefepime	NS	3.5	6.0	2.8	5.0	0.6	0.6	4.5	12.0	0.5	1.1	0.6	1.3
Meropenem	NS	0.1	0.1	0.7	0.5	0.0	0.0	4.2	3.9	0.0	0.0	1.3	1.3
Ciprofloxacin	NS	10.3	11.3	4.6	7.5	0.6	0.6	3.6	6.1	2.2	3.8	1.3	2.6
Norfloxacin	NS	10.0	17.0	4.0	13.0	0.7	1.4	2.8	13.8	1.7	4.7	0.7	5.6
Gentamicin	NS	7.9	8.4	3.9	4.2	0.6	0.6	9.4	9.7	3.8	7.1	1.3	1.9
Trimethoprim	R	26.9	28.7	14.1	15.9	3.2	3.8	19.7	21.4	20.8	21.4	1.3	1.3
Nitrofurantoin	NS	6.1	1.3	81.6	36.7	41.0	2.5	73.4	20.1	†	†	†	†

* R = resistant, I = intermediate, NS = non-susceptible (intermediate + resistant), using criteria as published by the CLSI [2014] and EUCAST [2014].

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

Table 3: Multiple acquired resistances, by species

		Number of acquired resistances (CLSI breakpoints)														
		Non-multi-resistant					Multi-resistant									
Species	Total	**0**	**1**	**2**	**3**	**Cumulative%**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Cumulative%**
E. coli	2,434	1031	490	431	197		101	54	65	37	19	7	2			
	%	42.4	20.1	17.7	8.1	88.3%	4.1	2.2	2.7	1.5	0.8	0.3	0.1			11.7%
K. pneumoniae.*	598	328	163	53	12		7	13	8	5	2	2	1	2	2	
		54.8	27.3	8.9	2.0	93.0%	1.2	2.2	1.3	0.8	0.3	0.3	0.2	0.3	0.3	7.0%
E. cloacae †	301	162	55	13	33		16	13	4	2	3					
		53.8	18.3	4.3	11.0	87.4%	5.3	4.3	1.3	0.7	1.0					12.6%
P. mirabilis	151	8	78	41	12		9	1	0	0	1	0	1			
		5.3	51.7	27.2	7.9	92.1%	6.0	0.7	0.0	0.0	0.7	0.0	0.7			7.9%
S. marcescens †	142	1	134	5	1		0	0	1							
		0.7	94.4	3.5	0.7	99.3%	0.0	0.0	0.7							0.7%
K. oxytoca.*	139	47	70	10	5		7									
		33.8	50.4	7.2	3.6	95.0%	5.0									5.0%
E. aerogenes †	94	30	36	4	14		10									
		31.9	38.3	4.3	14.9	89.4%	10.6									10.6%
Salmonella spp. (non Typhi)	65	53	8	2	1		1									
		81.5	12.3	3.1	1.5	98.5%	1.5									1.5%

* 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).

Escherichia coli

Moderately high levels of resistance to ampicillin (and therefore amoxycillin) were observed (50.2%/52.2%, CLSI/EUCAST criteria), with lower rates for amoxycillin-clavulanate (12.7%/-intermediate, 8.8%/21.5% resistant). Non-susceptibility to third-generation cephalosporins was low ceftriaxone 7.5%/7.5%, ceftazidime 4.1%/7.0%). Moderate levels of resistance were detected to cefazolin (19.1%/-) and trimethoprim (26.9%/28.7%). Ciprofloxacin non-susceptibility was found in 10.3%/11.3% of *E. coli* isolates. Resistance to ticarcillin-clavulanate (8.1%/18.3%), gentamicin (7.7%/7.9%), piperacillin-tazobactam (3.1%/6.2%), cefepime (1.9%/2.8%) were low. Four isolates had elevated meropenem MICs (≥ 0.5 mg/L). For the ESBL-producing strains. ciprofloxacin and gentamicin resistance was found in 57.3%/59.0% and 41.0%/41.4% respectively.

In line with international trends among community strains of *E. coli*, most of the strains with extended-spectrum β-lactamase (ESBL) genes harboured genes of the CTX-M type (171/229 = 75%). Over half of the *E. coli* with CTX-M group 1 types were found to belong to sequence type 131 (O25b-ST131). ST131 accounted for 66% of *E. coli* ESBL phenotypes that were ciprofloxacin resistant (MIC >1 mg/L), and only 2% of ciprofloxacin susceptible ESBL phenotypes. Ninety-eight percent and 57% 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.[12].

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. Four *K. pneumoniae* isolates had elevated meropenem MICs (see below). ESBLs were present in 38 of 45 (84%) presumptively ESBL-positive isolates of *K. pneumoniae*, 31 of which proved to be of the CTX-M type.

***Enterobacter* species**

Acquired resistance was common to ticarcillin-clavulanate (23.3%/27.5% and 27.8%/32.0%), piperacillin-tazobactam (17.3%/22.2% and 20.6%/28.9%), ceftriaxone (26.5%/26.5% and 28.9%/28.9%), ceftazidime (22.7%/23.3% and 28.9%/28.9%) and trimethoprim (19.7/21.4% and 3.2%/3.2%) for *E. cloacae* and *E. aerogenes*, respectively. Cefepime, ciprofloxacin, and gentamicin

resistance were all less than 10%. Fifteen of 33 *E. cloacae* tested for extended-spectrum β-lactamases based on a suspicious phenotype, harboured ESBL-encoding genes. Thirteen *E. cloacae* strains had elevated meropenem MICs.

Carbapenemase resistance

Overall, fourteen isolates from 12 patients were found to harbour a carbapenemase gene. bla_{IMP} was detected in nine strains (*E. cloacae* (4), *Citrobacter* spp. (2) *E. coli* (1), *S. marcescens* (1), *K. pneumoniae* (1); bla_{KPC} was detected in three *K. pneumoniae* isolates (one patient with multiple admission); and bla_{NDM} in one patient with two bacteraemic episodes.

Discussion

The Australian Group on Antimicrobial Resistance 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.[14] This is the first comprehensive survey of antimicrobial resistance among Enterobacteriaceae isolates from bacteraemic patients through Australia, using an approach similar to that conducted by the European EARS-Net program (http://www.ecdc.europa.eu/en/healthtopics/antimicrobial_resistance/database/Pages/database.aspx).

CTX-M-producing *E. coli* and *Klebsiella* species and gentamicin- and ciprofloxacin-resistant *E. coli* are well established among bacteraemic patients. 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.[12] Carbapenem resistance attributable to acquired carbapenemases are still rare in patients with bacteraemia in Australia, although three different types (IMP, KPC, and NDM) were detected from seven of the participating institutions. Compared with many other countries in our region, resistance rates in Australian Gram-negative bacteria are still relatively low[15], but similar to those observed in 2012 in many Western European countries (http://ecdc.europa.eu/en/publications/Publications/antimicrobial-resistance-surveillance-europe-2012.pdf).

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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