Australian Government
Department of Health

COMMUNICABLE DISEASES INTELLIGENCE

2020 Volume 44

https://doi.org/10.33321/cdi.2020.44.79

Australian Group on Antimicrobial Resistance (AGAR) Australian Gram-negative Sepsis Outcome Programme (GNSOP) Annual Report 2018

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Communicable Diseases Intelligence
ISSN: 2209-6051 Online

This journal is indexed by Index Medicus and Medline.

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Communicable Diseases Intelligence is produced by:
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Annual report

Australian Group on Antimicrobial Resistance (AGAR) Australian Gram-negative Sepsis Outcome Programme (GNSOP) Annual Report 2018

Jan M Bell, Thomas Gottlieb, Denise A Daley, Geoffrey W Coombs

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 2018 survey was the sixth year to focus on bloodstream infections, and included Enterobacterales, *Pseudomonas aeruginosa* and *Acinetobacter* species.

Eight thousand three hundred and fifty isolates, comprising Enterobacterales (7,512, 90.0%), *P. aeruginosa* (743, 8.9%) and *Acinetobacter* species (95, 1.1%), were tested using commercial automated methods. The results were analysed using Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints (January 2019). Of the key resistances, resistance to the third-generation cephalosporin, ceftriaxone, was found in 13.4%/13.4% of *Escherichia coli* (CLSI/EUCAST criteria), and 9.4%/9.4% of *Klebsiella pneumoniae*. Resistance rates to ciprofloxacin were 15.2%/15.2% for *E. coli*, 11.3%/11.3% for *K. pneumoniae*, 7.4%/7.4% for *Enterobacter cloacae c*omplex, and 3.6%/7.7% for *P. aeruginosa*. Resistance rates to piperacillin-tazobactam were 3.0%/6.0%, 4.3%/7.9%, 18.2%/22.0%, and 5.1%/11.1% for the same five species respectively. Thirty-one isolates from 27 patients were shown to harbour a carbapenemase gene: 14 $bla_{\text{IMP-4}}$ (11 patients), including one with $bla_{\text{IMP-4}}$+$bla_{\text{OXA-23}}$, four bla_{KPC} (three patients), three $bla_{\text{OXA-48}}$, three bla_{NDM}, three bla_{GES}. two $bla_{\text{OXA-181}}$, and two $bla_{\text{OXA-23}}$.

Keywords: Australian Group on Antimicrobial Resistance (AGAR); antibiotic resistance; bacteraemia; gram-negative; *Escherichia coli*; Enterobacter; Klebsiella

Introduction

Emerging resistance in common pathogenic members of the Enterobacterales is a world-wide phenomenon and presents therapeutic problems for practitioners, both in 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 (http://www.agargroup.org.au/agar-surveys). In 2004, another genus of gram-negative pathogens in which resistance can be of clinical importance, *Enterobacter*, was added. *E. coli* is the most common cause of community-onset urinary tract infection; *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 com-

menced 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. In 2015, *Pseudomonas aeruginosa* and *Acinetobacter* species were added, and the program has subsequently been identified as the Gram-negative Sepsis Outcome Program (GNSOP).

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, meropenem and colistin.

The objectives of the 2018 surveillance program were to:

- Monitor resistance in Enterobacterales, *P. aeruginosa* and *Acinetobacter* species isolated from blood cultures taken from patients presenting to the hospital or already in hospital;
- Examine the extent of co-resistance and multidrug resistance in the major species;
- Detect emerging resistance to newer last-line agents such as carbapenems and colistin; and
- Examine the molecular basis of resistance to third-generation cephalosporins, quinolones and carbapenems.

Methods

Study design

From 1 January to 31 December 2018, a total of 36 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 systems, or where available matrix assisted laser desorption/ionisation – time of flight (MALDI-ToF) mass spectrometry.

Susceptibility testing

Testing was performed by two commercial semi-automated methods, Vitek 2 (BioMérieux, France) or Phoenix (Becton Dickinson, USA), which are calibrated to the ISO reference standard method of broth microdilution. Commercially available Vitek AST-N246 and AST-N247, or Phoenix NMIC-404 and NMIC-422 cards were utilised by all participants throughout the survey period. The CLSI M100[1] and EUCAST v9.0[2] breakpoints from January 2019 have been employed in the analysis. For analysis of cefazolin, breakpoints of ≤ 4 mg/L for susceptible, ≥ 8 mg/L for resistant were applied due to the restricted minimum inhibitory concentration (MIC) range available on the commercial cards, recognising that the January 2019 breakpoint is actually susceptible ≤ 2 mg/L.

Multidrug resistance

The definitions defined by Magiorakos et al.[3] were applied in this survey, where multidrug resistance was defined as resistance to one or more agent in three or more antimicrobial categories. For each species, antimicrobials were excluded from the count if they were affected by natural resistance mechanisms.

Confirmation of resistances

E. coli, *Klebsiella* spp., *Proteus* spp. and *Salmonella* spp. with ceftazidime or ceftriaxone MIC > 1 mg/L, or cefoxitin MIC > 8 mg/L; any other Enterobacterales with cefepime MIC > 1 mg/L; all isolates with ciprofloxacin MIC > 0.25 mg/L; all isolates with meropenem MIC > 0.25 mg/L; all isolates with amikacin MIC > 32 mg/L, and all isolates with colistin MIC > 2 mg/L were referred to a central laboratory (University of Adelaide) for confirmation of resistance.

All referred isolates were screened using real-time polymerase chain reaction (PCR) platform (LC-480) and published primers for the presence of bla_{TEM} and bla^{SHV}, CTX-M-type genes (groups 1, 2, 9, 8/25), plasmid-borne AmpC (bla_{CIT}, bla_{DHA}, bla_{EBC}, bla_{ACC}, bla_{FOX}, bla_{MOX}), and carbapenemases genes (bla_{IMP}, bla_{NDM}, bla_{KPC}, bla_{OXA-48}-like, bla_{VIM}, bla_{GES}, bla_{SME}, bla_{IMI}).[4–6]

The RT-PCR technique was also used to detect plasmid-mediated quinolone resistance mechanisms (*qnr*, efflux [*qepA*, *oqxAB*] and *aac (6')-Ib-cr*), aminoglycoside ribosomal methyltransferases (*armA*, *rmtB*, *rmtC*, *rmtF*), and mobile colistin resistance genes (*mcr-1*, *mcr-2*, *mcr-3*).[7–12] All referred *E. coli* were examined for membership of the O25b-ST131 clone.[13] All isolates with demonstrated carbapenemase activity and any amikacin resistant isolates were also screened for OXA-23-like, -24, and -58 carbapenemases.[14]

All isolates with carbapenemase activity were subjected to whole genome sequencing using the Illumina NextSeq 500 platform. Data were analysed using a modification of the Nullarbor bioinformatic pipeline.[15] The pipeline was used to identify the multi-locus sequence type and the resistome.

Results

The species isolated, and the numbers of each, are listed in Table 1. Enterobacterales accounted for 90.0%, followed by *P. aeruginosa* (8.9%) and *Acinetobacter* species (1.1%). Of the Enterobacterales, three genera—*Escherichia* (61.0%), *Klebsiella* (20.4%) and *Enterobacter* (5.6%)—contributed 87.0% of all isolates. Major resistances and non-susceptibilities for the top six ranked species are listed in Table 2. Non-susceptibility (which includes both intermediately resistant and resistant isolates) 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 26.9% of *E. coli* isolates, 12.2% of *K. pneumoniae*, and 8.2% of *E. cloacae* complex. A more detailed breakdown of resistances and non-susceptibilities by state and territory is provided in the online AGAR report.

Escherichia coli

Moderately high levels of resistance to ampicillin (and therefore amoxicillin) were maintained (54.7%/56.8%, CLSI/EUCAST criteria), with lower rates for amoxicillin-clavulanic acid (13.6%/– intermediate, 8.8%/– resistant). Non-susceptibility to third generation cephalosporins was low (ceftriaxone 13.5%/13.5%, ceftazidime 6.6%/12.7%).

Table 1. Number and proportion of species isolated, blood cultures, 2018

Species	Percentage (n)
Escherichia coli	54.8 (4,577)
Klebsiella pneumoniae	13.3 (1,107)
Pseudomonas aeruginosa	8.9 (743)
Enterobacter cloacae complex	5.0 (420)
Proteus mirabilis	3.1 (261)
Klebsiella oxytoca	2.8 (230)
Serratia marcescens	2.4 (199)
Klebsiella aerogenes	1.5 (125)
Salmonella species (non-typhoidal)	1.3 (107)
Citrobacter freundii complex	1.1 (91)
Morganella morganii	1.0 (81)
Klebsiella variicola	0.8 (65)
Acinetobacter baumannii complex	0.8 (63)
Citrobacter koseri	0.8 (63)
Salmonella species (typhoidal)	0.6 (46)
Raoultella ornithinolytica	0.3 (22)
Providencia rettgeri	0.2 (17)
Acinetobacter species	0.2 (13)
Raoultella planticola	0.1 (11)
Hafnia alvei	0.1 (10)
Pantoea species	0.1 (10)
Proteus vulgaris	0.1 (10)
Other species (total n = 27)	0.9 (79)
Total	8,350

Table 2. Non-susceptibility and resistance rates for the top six ranked species tested, 2018

		E. coli (%)		*K. pneumoniae* (%)		*P. aeruginosa* (%)		*E. cloacae complex* (%)		*P. mirabilis* (%)		*K. oxytoca* (%)	
Antimicrobial	**Category[a]**	**CLSI**	**EUCAST**	**CLSI**	**EUCAST**	**CLSI**	**EUCAST**	**CLSI**	**EUCAST**	**CLSI**	**EUCAST**	**CLSI**	**EUCAST**
Ampicillin	I	2.1	–	*b*	*b*	na	na	*b*	*b*	*1.2*	–	*b*	*b*
	R	54.7	56.8	*b*	*b*	na	na	*b*	*b*	17.1	18.2	*b*	*b*
Amoxicillin-clavulanic acid (2:1)[c]	I	13.6	na	6.3	–	na	na	*b*	*b*	5.8	–	3.5	–
	R	8.8	–	5.5	–	na	na	*b*	*b*	2.7	–	9.2	–
Piperacillin-tazobactam	R	3.0	6.0	4.3	7.9	5.1	11.1	18.2	22.0	0.0	0.8	9.6	10.5
Cefazolin	R	24.8	24.8	14.5	14.5	na	na	*b*	*b*	18.7	18.7	62.1	62.1
Cefoxitin	R	4.1	/	5.6	/	na	na	*b*	*b*	0.8	/	0.4	/
Ceftriaxone	NS	13.5	13.5	9.6	9.6	na	na	25.6	25.6	2.0	2.0	8.7	8.7
Ceftazidime	NS	6.6	12.7	7.4	10.4	8.1	8.1	22.2	24.6	1.6	2.0	0.4	0.8
Cefepime	NS	4.6	10.5	4.5	8.0	5.6	5.6	8.6	13.4	0.8	1.6	0.0	0.4
Meropenem	NS	0.1	0.1	1.0	1.0	7.7	7.7	3.1	2.7	0.4	0.0	0.0	0.0
Ciprofloxacin	NS	19.1	19.1	12.7	12.7	7.7	7.7	8.4	8.4	2.7	2.7	0.8	0.8
Gentamicin	R	8.2	8.4	4.3	4.4	0.7	2.2	6.0	6.9	1.2	1.9	0.0	0.4
Trimethoprim–sulfamethoxazole	R	32.1	32.0	20.5	19.6	na	na	17.2	17.2	14.3	14.3	3.0	3.0
Nitrofurantoin	R	0.9	0.0	35.8	/	na	na	17.3	/	*b*	*b*	1.8	/

a R = resistant, I = intermediate, NS = non-susceptible (intermediate + resistant), using criteria as published by the CLSI [2019] and EUCAST [2019].
b Considered largely intrinsically resistant due to natural β-lactamases; – no intermediate category; / no breakpoints defined; na = not applicable (testing not recommended)
c For EUCAST interpretation, the clavulanate is fixed at 2 mg/L, rather than a 2:1 ratio used in CLSI guidelines. As all susceptibility test cards used have a 2:1 ratio of clavulanate no EUCAST category has been applied.

Table 3. Multiple acquired resistances by species, 2018

	Number of acquired resistances (EUCAST breakpoints)[a]													
		Non-multi-resistant				Multi-resistant								
Species	**Total**	**0**	**1**	**2**	**Cumulative %**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**Cumulative %**
Escherichia coli	4,508	1,746	763	786		321	315	309	159	69	27	10	3	
	%	38.7	16.9	17.5	73.1	7.1	7.0	6.9	3.5	1.5	0.6	0.2	0.1	26.9
Klebsiella pneumoniae[b]	1,088	768	137	50		27	21	36	20	16	6	7	na	
	%	70.6	12.6	4.6	87.8	2.5	1.9	3.3	1.8	1.5	0.6	0.6		12.2
Enterobacter cloacae complex[c]	402	251	52	66		8	9	11	5	na	na	na	na	
	%	62.4	12.9	16.4	91.8	2.2	2.0	2.7	1.2					8.2
Proteus mirabilis	257	179	32	23		17	2	0	2	2	0	0	0	
	%	69.6	12.5	8.9	91.1	6.6	0.8	0.0	0.8	0.8	0.0	0.0	0.0	8.9
Klebsiella oxytoca[b]	226	82	115	6		5	16	2	0	0	0	0	na	
	%	36.3	50.9	2.7	89.8	2.2	7.1	0.9	0.0	0.0	0.0	0.0		10.2
Salmonella species (non-typhoidal)[d]	100	86	11	0		1	1	1	0	0	0	0	na	
	%	86.0	11.0	0.0	97.0	1.0	1.0	1.0	0.0	0.0	0.0	0.0		3.0
Serratia marcescens[e]	165	45	104	11		3	2	0	0	0	na	na	na	
	%	27.3	63.0	6.7	97.0	1.8	1.2	0.0	0.0	0.0				3.0
Klebsiella aerogenes[c]	123	76	10	27		6	3	1	0	na	na	na	na	
	%	61.8	8.1	22.0	91.9	4.9	2.4	0.8	0.0					8.1

a Antimicrobial categories (agents) included: aminoglycosides (gentamicin or tobramycin or amikacin), antipseudomonal penicillins + β-lactamase inhibitor (piperacillin–tazobactam), carbapenems (meropenem), non-extended cephalosporins (cefazolin), extended-spectrum cephalosporins (ceftriaxone or ceftazidime or cefepime), cephamycins (cefoxitin), fluoroquinolones (ciprofloxacin), folate pathway inhibitors (trimethoprim–sulfamethoxazole), penicillins (ampicillin), and penicillins + β-lactamase inhibitor (amoxicillin-clavulanic acid, CLSI), na = not applicable

b Antimicrobial categories excluded: penicillins

c Antimicrobial categories excluded: penicillins, non-extended cephalosporins, cephamycins, penicillins + β-lactamase inhibitor

d Antimicrobial categories excluded: aminoglycosides

e Antimicrobial categories excluded: penicillins, non-extended cephalosporins, penicillins + β-lactamase inhibitor

Moderate levels of resistance were detected to cefazolin (24.8%/24.8%) and trimethoprim–sulfamethoxazole (32.1%/32.0%). Ciprofloxacin non-susceptibility was found in 19.2%/19.2% of *E. coli* isolates. Resistance to gentamicin (8.2%/8.4%), piperacillin-tazobactam (3.0%/6.0%) and cefepime (2.9%/3.7%) was low. Nine isolates (0.2%) had elevated meropenem MICs (≥ 0.5 mg/L). For the strains with extended-spectrum β-lactamase (ESBL) phenotype, ciprofloxacin and gentamicin resistance was found in 58.4%/58.4% and 30.4%/30.7% respectively.

Most of the *E. coli* strains with ESBL genes harboured genes of the CTX-M type (525/609 = 86%). Fifty-one percent of *E. coli* with CTX-M group 1 types were found to belong to sequence type 131 (O25b-ST131). ST131 accounted for 63% of *E. coli* ESBL phenotypes that were ciprofloxacin resistant (MIC > 1 mg/L), and only 4% of ciprofloxacin-susceptible ESBL phenotypes.

Klebsiella pneumoniae

K. pneumoniae showed slightly higher levels of resistance to ceftazidime and piperacillin-tazobactam than did *E. coli*, but lower rates of resistance to amoxicillin-clavulanic acid, cefazolin, ceftriaxone, ciprofloxacin, gentamicin, and trimethoprim-sulfamethoxazole. Fourteen (1.3%) *K. pneumoniae* isolates had elevated meropenem MICs (see below). ESBLs were present in 108 of 120 (90%) presumptively ESBL-positive isolates of *K. pneumoniae*, 89 (82%) of which were confirmed to be of the CTX-M type.

Enterobacter cloacae complex

Acquired resistance was common to piperacillin-tazobactam (18.5%/22.0%) ceftriaxone (25.1%/25.1%), ceftazidime (21.7%/22.2%) and trimethoprim–sulfamethoxazole (17.2%/17.2%) among *E. cloacae* complex isolates. Cefepime, ciprofloxacin and gentamicin resistance were all less than 10%. Twenty-seven *E. cloacae* complex strains (6.5%) had elevated meropenem MICs.

Carbapenemase resistance

Overall, 31 isolates (27 patients) in sixteen institutions from six states/territories were found to harbour a carbapenemase gene. $bla_{\text{IMP-4}}$ was detected in 14 isolates from 11 patients: *E. cloacae* (eight from five patients), *K. pneumoniae* (two), *K. aerogenes* (one), *K. variicola* (one), *C. freundii* (one), and one *A. radioresistens* which also harboured $bla_{\text{OXA-23}}$. $bla_{\text{OXA-48}}$ was detected in two *E. coli* and one *K. pneumoniae*; $bla_{\text{OXA-181}}$ was detected in two *K. pneumoniae*. $bla_{\text{NDM-5}}$ was detected in two *E. coli* and $bla_{\text{NDM-4}}$ in one *K. pneumoniae*; $bla_{\text{KPC-2}}$ was detected in two *K. pneumoniae* and $bla_{\text{KPC-3}}$ in two *K. pneumoniae* from one patient. $bla_{\text{GES-5}}$ was detected in three *P. aeruginosa*; and $bla_{\text{OXA-23}}$ was detected in one *A. baumannii* and one *A. pittii*.

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.[16] In 2013, the first survey of antimicrobial resistance among Enterobacterales isolates from bacteraemic patients through Australia was conducted using an approach similar to that conducted by the European EARS-Net program. 2018 was the sixth survey of antimicrobial resistance among Enterobacterales, and the fourth for *P. aeruginosa* and *Acinetobacter* spp. from bacteraemic patients through 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 O25b-ST131 clone. Carbapenem resistance attributable to acquired carbapenemases is still uncommon in patients with bacteraemia in Australia, although six different types (IMP, KPC, NDM, OXA-48-like, OXA-23, and GES) were detected from sixteen of the participating institutions. Compared with many other countries in our region, resistance

rates in Australian gram-negative bacteria are still relatively low,[17] but similar to those observed in 2018 in many Western European countries. [18]

Multi-resistance is being increasingly observed, especially in *E. coli*, with multi-resistance rates above 25%. This is likely to drive more broad-spectrum antibiotic use, and increase the resistance selection pressure for important reserve classes, especially the carbapenemases.

Acknowledgments

This study was funded by a grant from the Australian Commission on Safety and Quality in Health care.

AGAR acknowledges the Antimicrobial Resistance Laboratory, Centre for Infectious Diseases and Microbiology, Westmead Institute for Medical Research, for performing whole genome sequencing on carbapenemase-producing isolates.

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Peter Collignon and Susan Bradbury, Canberra Hospital

New South Wales

Thomas Gottlieb and Steven Siarakis, Concord Hospital

Rodney Givney and Kimberly Ross, John Hunter Hospital

James Branley and Linda Douglass, Nepean Hospital

Peter Huntington, Royal North Shore Hospital

Sebastiaan van Hal and Alicia Beukers, Royal Prince Alfred Hospital

Jock Harkness and David Lorenz, St Vincent's Hospital Sydney

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Marcel Leroi and Elizabeth Grabsch, Austin Health

Tony Korman and Despina Kotsanas, Monash Health, Monash Medical Centre and Monash Children's Hospital

Andrew Daley and Gena Gonis, Royal Women's and Children's Hospital

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