

Australian Group on Antimicrobial Resistance (AGAR) Australian Enterobacteriaceae
Sepsis Outcome Programme (EnSOP) Annual Report 2014

Author Details

¹Ms Jan M Bell, ¹Prof John D Turnidge, ^{3,4}A/Prof Geoffrey W Coombs, ⁵Ms Denise A Daley, ⁶A /Prof Thomas Gottlieb, ⁷Dr Jenny Robson, ⁸Ms Narelle George,

¹Departments of Pathology, Paediatrics and Molecular and Biomedical Sciences,
University of Adelaide, Adelaide, South Australia, Australia

²Microbiology and Infectious Diseases Directorate, SA Pathology, Adelaide

³Australian Collaborating Centre for *Enterococcus* and *Staphylococcus Species*
(**ACCESS**) Typing and Research, School of Veterinary and Life Sciences, Murdoch
University, Murdoch, Western Australia, Australia

⁴Department of Microbiology and Infectious Diseases, PathWest Laboratory
Medicine-WA, Fiona Stanley Hospital, Murdoch, Western Australia, Australia

³Australian Group on Antimicrobial Resistance, Fiona Stanley Hospital, Murdoch,
Western Australia, Australia

⁶Microbiology Department, Austin Health, Heidelberg, Victoria, Australia

⁶Concord Hospital, Concord, New South Wales

⁷Sullivan Nicolaides Pathology, , Queensland

⁸Microbiology, Pathology Queensland, Royal Brisbane and Women's Hospital,
Herston, Queensland

Corresponding Author

Prof John D Turnidge

Departments of Pathology, Paediatrics and Molecular and Biomedical Sciences,
University of Adelaide, Adelaide, South Australia, Australia

Telephone: +61 417 811 552

Email: john.turnidge@adelaide.edu.au

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 2014 survey was the second year to focus on blood stream infections. Five thousand seven hundred and ninety-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 2015). Of the key resistances, non-susceptibility to the third-generation cephalosporin, ceftriaxone, was found in 9.0%/9.0% of *E. 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 four species respectively. Fifteen isolates were shown to harbour a carbapenemase gene, seven *bla*_{IMP}, three *bla*_{KPC}, three *bla*_{VIM}, and one *bla*_{NDM}, and one *bla*_{OXA-181-like}.

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. The 2014 survey was the second EnSOP survey.

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 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 1st January to 31st 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 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, or Phoenix NMIC-203 cards were utilized by all participants throughout the survey period. The CLSI M100¹ and EUCAST v5.0² breakpoints from January 2015 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 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.^{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

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 six 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 *Escherichia coli* isolates, 9.7% of *Klebsiella pneumoniae*, and 12.1% of *Enterobacter cloacae*. A more detailed breakdown of resistances and non-susceptibilities by state and territory is provided in the [online report](http://www.agargroup.org/surveys) from the group (<http://www.agargroup.org/surveys>).

Table 1: Species tested

Species	Total	
<i>Escherichia coli</i>	3493	60.2%
<i>Klebsiella pneumoniae</i>	877	15.1%
<i>Enterobacter cloacae</i>	343	5.9%
<i>Klebsiella oxytoca</i>	226	3.9%
<i>Proteus mirabilis</i>	187	3.2%
<i>Serratia marcescens</i>	136	2.3%
<i>Enterobacter aerogenes</i>	105	1.8%
<i>Salmonella</i> species (non Typhi)	94	1.6%
<i>Morganella morganii</i>	57	1.0%
<i>Citrobacter freundii</i>	53	0.9%
<i>Citrobacter koseri</i>	50	0.9%
<i>Salmonella</i> Typhi/Paratyphi	26	0.4%
<i>Enterobacter asburiae</i>	16	0.3%
<i>Raoultella ornithinolytica</i>	15	0.3%
<i>Pantoea</i> species	12	0.2%
<i>Pantoea agglomerans</i>	12	0.2%
<i>Enterobacter</i> species	11	0.2%
<i>Providencia stuartii</i>	10	0.2%
Other species (n=27)	75	1.3%
Total	5798	

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

Antimicrobial	Category*	<i>E. coli</i> (%)		<i>K. pneumoniae</i> (%)		<i>K. oxytoca</i> (%)		<i>E. cloacae</i> (%)		<i>P. mirabilis</i> (%)		<i>S. marcescens</i> (%)	
		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	†	†
Amoxicillin-clavulanate ‡	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
Piperacillin-tazobactam	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 CLSI [2014] and 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	Number of acquired resistances (CLSI breakpoints)																	
	Total	Non-multi-resistant					Cumulative%	Multi-resistant										Cumulative%
		0	1	2	3	4		5	6	7	8	9	10	11	12	13		
<i>E. coli</i>	2,958	1324	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%	
<i>K. pneumoniae</i> .*	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%	
<i>E. cloacae</i> †	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%	
<i>P. mirabilis</i>	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%	
<i>S. marcescens</i> †	98	1	94	0	1		2											
		1.0	95.9	0.0	1.0	98.0%	2.0										2.0%	
<i>K. oxytoca</i> .*	197	66	98	14	10		7	2										
		33.5	49.7	7.1	5.1	95.4%	3.6	1.0									4.6%	
<i>E. aerogenes</i> †	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%	
<i>Salmonella</i> spp. (non Typhi)	71	57	8	4	2													
		80.3	11.3	5.6	2.8	100.0%											0.0%	

* Antibiotics included: amoxicillin-clavulanate, piperacillin-tazobactam, cefazolin, 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, amoxicillin-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 amoxicillin) were maintained (50.1%/51.9%, CLSI/EUCAST criteria), with lower rates for amoxicillin-clavulanate (12.7%/- intermediate, 8.2%/20.9% resistant). Non-susceptibility to third-generation cephalosporins was low ceftriaxone 9.0%/9.0%, ceftazidime 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%), cefepime (1.6%/2.9%) were low. Nine isolates had elevated meropenem MICs (≥ 0.5 mg/L). For the 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 extended-spectrum β -lactamase (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 percent 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.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 amoxicillin-

clavulanate, ticarcillin-clavulanate, cefazolin, ceftriaxone ciprofloxacin, gentamicin, and trimethoprim. Thirteen *K. pneumoniae* isolates had elevated meropenem MICs (see below). 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 *E. cloacae* and *E. aerogenes*, respectively. Cefepime, ciprofloxacin, and gentamicin resistance were all less than 10%. Seventeen of 45 *E. cloacae* tested for extended-spectrum β -lactamases based on a suspicious phenotype, harboured ESBL-encoding genes. Eighteen *E. cloacae* strains had elevated meropenem MICs.

Carbapenemase resistance

Overall, fifteen isolates (15 patients) in nine institutions from five states/territories were found to harbour a carbapenemase gene. *bla*_{IMP-4} was detected in *E. cloacae* (5) and *K. pneumoniae* (2); *bla*_{KPC} was detected in three *K. pneumoniae* isolates from one institution; *bla*_{VIM} was detected in *K. pneumoniae* (2) and *S. marcescens* (1); *bla*_{NDM} in one *E. coli*; and *bla*_{OXA-181-like} in one *K. pneumoniae*.

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 In 2013, the first survey of antimicrobial resistance among Enterobacteriaceae isolates from bacteraemic patients through

Australia was conducted using an approach similar to that conducted by the European EARS-Net program. 2014 was the second survey of antimicrobial resistance among Enterobacteriaceae isolates from bacteraemic patients through Australia was conducted in 2014.

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.¹² Carbapenem resistance attributable to acquired carbapenemases are still uncommon in patients with bacteraemia in Australia, although five different types (IMP, KPC, VIM, NDM and OXA-181-like) were detected from nine of the participating institutions. Compared with many other countries in our region, resistance rates in Australian Gram-negative bacteria are still relatively low¹⁵, but similar to those observed in 2014 in many Western European countries (<http://ecdc.europa.eu/en/publications/Publications/antimicrobial-resistance-annual-epidemiological-report.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.

Agar participants

Australian Capital Territory

Peter Collignon and Susan Bradbury, The Canberra Hospital

New South Wales

Thomas Gottlieb and Graham Robertson, Concord Hospital

John Ferguson and Jack (Ian) Winney, John Hunter Hospital

James Branley and Donna Barbaro, Nepean Hospital

George Kotsiou and Peter Huntington, Royal North Shore Hospital

David Mitchell and Lee Thomas, Westmead Hospital

Northern Territory

Rob Baird and Jann Hennessy, Royal Darwin Hospital

Queensland

Enzo Binotto and Bronwyn Thomsett, Pathology Queensland Cairns Base Hospital

Graeme Nimmo and Narelle George, Pathology Queensland Central Laboratory

Petra Derrington and Sharon Dal-Cin, Pathology Queensland Gold Coast Hospital

Robert Horvath, Pathology Queensland Prince Charles Hospital

Naomi Runnegar and Joel Douglas, Pathology Queensland Princess Alexandra Hospital

Jenny Robson and Georgia Peachey, Sullivan Nicolaides Pathology

South Australia

Kelly Papanoum and Nicholas Wells, SA Pathology (Flinders Medical Centre)

Morgyn Warner and Kija Smith, SA Pathology (Royal Adelaide Hospital)

John Turnidge and Jan Bell, SA Pathology (Women's and Children's Hospital)

Tasmania

Louise Cooley and Rob Peterson, Royal Hobart Hospital

Victoria

Denis Spelman, Amanda Dennison and Christopher Lee, Alfred Hospital

Benjamin Howden and Peter Ward, Austin Hospital

Tony Korman and Despina Kotsanas, Southern Health, Monash Medical Centre

Andrew Daley and Gena Gonis, Royal Women's Hospital

Mary Jo Waters and Linda Joyce, St Vincent's Hospital

Western Australia

David McGeachie and Rebecca Wake, PathWest Laboratory Medicine - WA,
Fremantle Hospital

Ronan Murray and Barbara Henderson, PathWest Laboratory Medicine – WA,
Queen Elizabeth II Hospital

Owen Robinson, Denise Daley and Geoffrey Coombs, PathWest Laboratory
Medicine – WA, Royal Perth Hospital

Sudha Pottumarthy-Boddu and Fay Kappler, St John of God Pathology

References

1. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Twenty-Fifth Informational Supplement M100–S25. Villanova, PA, USA 2015.
2. European Committee on Antimicrobial Susceptibility Testing (2014). Breakpoint tables for interpretation of MICs and zone diameters. Version 5.0, January 2015. Available at http://www.eucast.org/clinical_breakpoints/. Accessed January 1, 2015.
3. Hanson ND, Thomson KS, Moland ES, Sanders CC, Berthold G, Penn RG. Molecular characterization of a multiply resistant *Klebsiella pneumoniae* encoding ESBLs and a plasmid-mediated AmpC. *J Antimicrob Chemother* 1999;44(3):377–380.

4. Chia JH, Chu C, Su LH, Chiu CH, Kuo AJ, Sun CF, et al. Development of a multiplex PCR and SHV melting-curve mutation detection system for detection of some SHV and CTX-M β -lactamases of *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter cloacae* in Taiwan. *J Clin Microbiol* 2005;43(9):4486–4491.
5. Birkett CI, Ludlam HA, Woodford N, Brown DFJ, Brown NM, Roberts MTM, et al. Real-time TaqMan PCR for rapid detection and typing of genes encoding CTX-M extended-spectrum β -lactamases. *J Med Microbiol* 2007;56(Pt 1):52–55.
6. Perez-Perez FJ, Hanson ND. Detection of plasmid-mediated AmpC β -lactamase genes in clinical isolates by using multiplex PCR. *J Clin Microbiol* 2002;40(6):2153–2162.
7. Poirel L, Héritier C, Tolün V, Nordmann P. Emergence of oxacillinase-mediated resistance to imipenem in *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 2004;48(1):15–22.
8. Mendes RE, Kiyota KA, Monteiro J, Castanheira M, Andrade SS, Gales AC, et al. Rapid detection and identification of metallo- β -lactamase-encoding genes by multiplex real-time PCR assay and melt curve analysis. *J Clin Microbiol* 2007;45(2):544–547.
9. Cattoir V, Poirel L, Rotimi V, Soussy C-J, Nordmann P. Multiplex PCR for detection of plasmid-mediated quinolone resistance qnr genes in ESBL-producing enterobacterial isolates. *J Antimicrob Chemother* 2007;60:394–397.
10. Ciesielczuk H, Hornsey M, Choi V, Woodford N, Wareham DW. Development and evaluation of a multiplex PCR for eight plasmid-mediated quinolone-resistance determinants. *J Med Microbiol* 2013;62:1823–1827.
11. Dhanjii H, Doumith M, Clermont O, Denamur E, Hope R, Livermore DM, et al. Real-time PCR for detection of the O25b-ST131 clone of *Escherichia coli* and its CTX-M-15-like extended-spectrum β -lactamases. *J Antimicrob Agents Chemother* 2010;36:355–358.
12. Banerjee R, Robicsek A, Kuskowski MA, Porter S, Johnston BD, Sokurenko E, et al. Molecular Epidemiology of *Escherichia coli* Sequence Type 131 and Its H30 and H30-Rx Subclones among Extended-Spectrum- β -Lactamase-Positive and -Negative *E. coli* Clinical Isolates from the Chicago Region, 2007 to 2010. *Antimicrob Agents Chemother* 2013;57(12):6385–6388.
13. Colpan A, Johnston B, Porter S, Clabots C, Anway R, Thao L, et al. *Escherichia coli* Sequence Type 131 (ST131) Subclone H30 as an Emergent Multidrug-Resistant Pathogen Among US Veterans. *Clin Infect Dis* 2013;57(9):1256–65.
14. Turnidge J, Gottlieb T, Mitchell D, Pearson J, Bell J, for the Australian Group for Antimicrobial Resistance. Gram-negative Survey 2011 Antimicrobial Susceptibility Report. 2011 Adelaide. Available from: <http://www.agargroup.org/files/AGAR%20GNB08%20Report%20FINAL.pdf>
15. Sheng WH, Badal RE, Hsueh PR; SMART Program. Distribution of extended-spectrum β -lactamases, AmpC β -lactamases, and carbapenemases among Enterobacteriaceae isolates causing intra-abdominal infections in the Asia-Pacific region: results of the study for Monitoring Antimicrobial Resistance Trends (SMART). *Antimicrob Agents Chemother* 2013;57(7):2981–2988.