Community-onset Gram-negative Surveillance Program annual report, 2012

John D Turnidge, Thomas Gottlieb, David H Mitchell, Geoffrey W Coombs, Denise A Daley, Jan M Bell for the Australian Group on Antimicrobial Resistance

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 2012 survey focussed on community-onset infections, examining isolates from urinary tract infections from patients presenting to outpatient clinics, emergency departments or to community practitioners. In 2012, 2,025 *Escherichia coli,* 538 *Klebsiella* species and 239 *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-susceptibility to the third-generation cephalosporin, ceftriaxone, was found in 4.2% of *E. coli* and 4.6%–6.9% of *Klebsiella* spp. Non-susceptibility rates to ciprofloxacin were 6.9% for *E. coli,* 0.0%–3.5% for *Klebsiella* spp. and 0.8%–1.9% in *Enterobacter* spp, and resistance rates to piperacillin-tazobactam were 1.7%, 0.7%–9.2%, and 8.8%–11.4% for the same 3 groups respectively. Only 1 *Enterobacter cloacae* was shown to harbour a carbapenemase (IMP-4). *Commun Dis Intell* 2014;38(1):E54–E58.

Keywords: antibiotic resistance; community 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 in the community, but of high importance due to intrinsic resistance to first-line antimicrobials in the community. Taken together, the 3 groups of species 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 community such as trimethoprim; resistance to agents important for serious infections, such as gentamicin; and resistance to reserve agents such as ciprofloxacin and meropenem.

The objectives of the 2012 surveillance program were to:

1. determine proportions of resistance to the main therapeutic agents in *Escherichia 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 newer last-line agents such as carbapenems. Isolates from the urinary tract were selected for this program.

Methods

Source of isolates

Isolates were collected from non-hospitalised patients with urinary tract infections, including those presenting to emergency departments, outpatient departments or to community practitioners. Each institution collected up to 70 *E. coli,* 20 *Klebsiella* spp. and 10 *Enterobacter* spp. isolates. Urinary tract isolates were selected because of their high frequency and high rates of exposure to antimicrobial agents in the community.

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-N246 cards were utilised by all participants throughout the survey period. The most recent Clinical and Laboratory Standards Institute breakpoints from 2013[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 2013 breakpoint is actually susceptible ≤2 mg/L. 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 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 2012, 2,802 isolates were examined, comprising 2,025 *E. coli,* 538 *Klebsiella* spp. and 239 *Enterobacter* spp. (Table 1). Major resistances and non-susceptibilities are listed in Table 2. Multi-resistance was detected in 7.6% of *E. coli* isolates, 5.1% of *Klebsiella* spp. and 5.4% of *Enterobacter* spp. (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*	2,025
Klebsiella	*K. pneumoniae*	434
	K. oxytoca	101
	K. pneumoniae subsp *ozaenae*	3
Total		538
Enterobacter	*E. cloacae*	128
	E. aerogenes	107
	E. asburiae	2
	E. gergoviae	1
	Enterobacter not speciated	1
Total		239

Escherichia coli

Moderately high levels of resistance to ampicillin (and therefore amoxycillin) were observed (44.3%), with lower rates for amoxycillin-clavulanate (11.3% intermediate, 5.3% resistant) (Table 2). Non-susceptibility to third-generation cephalosporins was low but appears to be increasing slowly compared with the 2010 survey (ceftriaxone 4.2%, ceftazidime 2.2%). In line with international trends amongst community strains of *E. coli*, most of the strains with extended-spectrum ß-lactamase (ESBL) genes harboured genes of the CTX-M type (75%, 68/91). Moderate levels of resistance were detected to cefazolin (14.3%) and trimethoprim (22.7%). Ciprofloxacin non-susceptibility was found in 6.9% of *E. coli* isolates. Ciprofloxacin resistance was found in 51.8% and gentamicin resistance was found in 30.1% of ESBL-producing strains. Resistance to ticarcillin-clavulanate, piperacillin-tazobactam, cefepime, and gentamicin were below 5%. No isolates had elevated meropenem MICs.

Klebsiella species

These isolates showed slightly higher levels of resistance to cefazolin, ceftriaxone and piperacillin-tazobactam compared with *E. coli*, but lower rates of resistance to amoxycillin-clavulanate, ticarcillin-clavulanate, ciprofloxacin, gentamicin, and trimethoprim (Table 2). ESBLs were present in 17 of 21 presumptively ESBL-positive isolates of *K. pneumoniae*, 14 of which proved to be of the CTX-M type. No *Klebsiella* species had elevated meropenem MICs.

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	1.9	†	†	†	†
Ampicillin	R	44.3	†	†	†	†
Amoxycillinclavulanate	I	11.3	2.8	1.0	†	†
Amoxycillinclavulanate	R	5.3	2.1	9.9	†	†
Ticarcillin-clavulanate	R	5.7	1.8	12.5	16.8	19.8
Piperacillintazobactam	R	1.7	0.7	9.2	8.8	11.4
Cefazolin	R	14.3	6.9	75.8	†	†
Cefoxitin	R	1.5	1.4	0.0	†	†
Ceftriaxone	NS	4.2	4.6	6.9	27.3	21.5
Ceftazidime	NS	2.2	3.0	0.0	19.5	18.7
Cefepime	NS	0.7	0.5	0.0	0.8	0.0
Meropenem	NS	0.0	0.0	0.0	1.6	0.0
Ciprofloxacin	NS	6.9	3.5	0.0	0.8	1.9
Norfloxacin	NS	6.8	2.3	0.0	0.0	1.9
Gentamicin	NS	4.5	3.0	0.0	5.5	0.0
Trimethoprim	R	22.7	9.9	3.0	17.2	1.9
Nitrofurantoin	NS	5.4	†	†	†	†

* R = resistant, I = intermediate, NS = non-susceptible (intermediate + resistant).

† Considered largely intrinsically resistant due to natural β-lactamases.

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	11	Cumulative %
E. coli	1,871	940	368	304	117		62	33	23	16	4	3		1	
%		50.2	19.7	16.2	6.3	92.4	3.3	1.8	1.2	0.9	0.2	0.2		0.1	7.6
Klebsiella spp.*	508	303	150	21	8		12	4	5	3	2				
%		59.6	29.5	4.1	1.6	94.9	2.4	0.8	1.0	0.6	0.4				5.1
Enterobacter spp.†	224	122	51	19	20		7	4		1					
%		54.5	22.8	85.0	8.9	94.6	3.1	1.8		0.4					5.4

* 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).

Enterobacter species

Acquired resistance was common to ticarcillin-clavulanate (17.8%), piperacillin-tazobactam (9.8%), ceftriaxone (24.3%), ceftazidime (18.8%) and trimethoprim (10.0%) (Table 2). Rates of resistance to cefepime, ciprofloxacin, and gentamicin were all less than 5%. Three of 4 strains tested for ESBL based on a suspicious phenotype, harboured ESBL-encoding genes. Two strains had elevated meropenem MICs (≥ 0.5 mg/L) one of which harboured $bla_{\text{IMP-4}}$.

Discussion

The Australian Group on Antimicrobial Resistance has been tracking resistance in sentinel enteric Gram-negative bacteria since 1992. Until 2008, surveillance was segregated into hospital– versus community-onset infections. The first year of community-onset only surveillance was 2008.[8] Comparing results from that year with 2012, there has been a noticeable increase in resistance rates to some important and reserve antibiotics. For example, rates of resistance in *E. coli* for ceftriaxone rose from 2.1% to 4.2% and for non-susceptibility to ciprofloxacin rose from 4.2% to 6.9%. Intermediate percentages were observed in 2010, confirming the definite upward trend.

Overall though, there are worrying trends in the emergence of CTX-M-producing *E. coli* and *Klebsiella* species and gentamicin– and ciprofloxacin-resistant *E. coli* now presenting in or from the community. Other resistance patterns appear stable. Carbapenem resistance attributable to acquired carbapenemases are still rare in community onset infections in Australia. Compared with many other countries in our region, resistance rates in Australian Gram-negative bacteria are still relatively low.[9]

Agar participants

Australian Capital Territory

Peter Collignon and Susan Bradbury, The Canberra Hospital

New South Wales

Thomas Gottlieb and Graham Robertson, Concord Hospital

Miriam Paul and Richard Jones, Douglass Hanly Moir Pathology

James Branley and Donna Barbaro, Nepean Hospital

George Kotsiou and Peter Huntington, Royal North Shore Hospital

Sebastian van Hal and Bradley Watson, Royal Prince Alfred Hospital

Iain Gosbell and Annabelle LeCordier, South West Area Pathology Service

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

Chris Coulter and Tobin Hillier, 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 Fleur Manno, SA Pathology, Royal Adelaide Hospital

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

Tasmania

Kathy Wilcox, Launceston General Hospital

Louise Cooley and Rob Peterson, Royal Hobart Hospital

Victoria

Denis Spelman and Michael Huysmans, Alfred Hospital

Benjamin Howden and Peter Ward, Austin Hospital

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

Suzanne Garland and Gena Gonis, Royal Women's Hospital

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

Western Australia

David McGechie and Rebecca Wake, PathWest Laboratory Medicine, WA, Fremantle Hospital

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

Keryn Christiansen and Geoffrey Coombs, PathWest Laboratory Medicine, WA Royal Perth Hospital

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

Author details

John D Turnidge[1,2]
Thomas Gottlieb[3]
David H Mitchell[4]
Geoffrey W Coombs[5,6]
Denise A Daley[6]
Jan M Bell[1]

1. Microbiology and Infectious Diseases, SA Pathology, Women's and Children's Hospital, North Adelaide, South Australia
2. Departments of Pathology, Paediatrics and Molecular Biosciences, University of Adelaide, South Australia
3. Department of Microbiology and Infectious Diseases, Concord, Concord, New South Wales
4. Centre for Infectious Diseases and Microbiology, Westmead Hospital, Westmead, New South Wales
5. Australian Collaborating Centre for Enterococcus and Staphylococcus Species (ACCESS) Typing and Research, School of Biomedical Sciences, Curtin University, Perth, Western Australia
6. Department of Microbiology and Infectious Diseases, PathWest Laboratory Medicine-WA, Royal Perth Hospital, Perth, Western Australia

Corresponding author: Professor John Turnidge, Microbiology and Infectious Diseases, SA Pathology, Women's and Children's Hospital, 72 King William Road, NORTH ADELAIDE SA. Telephone: +61 8 8161 6873 Email: john.turnidge@health.sa.gov.au

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