Australian Group on Antimicrobial Resistance Community-onset Gram-negative Surveillance Program annual report, 2010

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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 2010 survey focussed on community-onset infections, examining isolates from urinary tract infections from patients presenting to outpatient clinics, emergency departments or to community practitioners. Two thousand and ninety-two *Escherichia coli,* 578 *Klebsiella* species and 268 *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 3.2% of *E. coli* and 3.2%–4.0% of *Klebsiella* spp. Non-susceptibility rates to ciprofloxacin were 5.4% for *E. coli*, 1.0%–2.3% for *Klebsiella* spp., and 2.5%–6.6% in *Enterobacter* spp, and resistance rates to piperacillin-tazobactam were 2.8%, 3.2%–6.9%, and 16.8%–18.0% for the same 3 groups respectively. Only 3 strains, 2 *Klebsiella* spp. and 1 *Enterobacter* spp, were shown to harbour a carbapenemase (IMP-4). *Commun Dis Intell* 2013;37(3):E219–E223.

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

Introduction

Emerging resistance in common pathogenic members of the Enterobacteriaceae is a worldwide 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 2010 surveillance program were to:

1. determine the proportion 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. 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-N149 cards were utilised by all participants throughout the survey period. The most recent Clinical and Laboratory Standards Institute breakpoints from 2012[1] have been 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, ≥8 for resistant were applied due to the minimum inhibitory concentration (MIC) range available on the Vitek card, recognising that the January 2012 breakpoint is actually susceptible ≤2 mg/L. Ertapenem MICs were performed using Etest™ strips (BioMérieux).

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 ertapenem MIC >0.5 mg/L or 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

The species isolated, and the numbers of each are listed in Table 1. Major resistances and non-susceptibilities 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 7.3% of *E. coli* isolates, 4.3% of *Klebsiella* species, and 8.6% of *Enterobacter* species. A more detailed breakdown of resistances and non-susceptibilites 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*	2,092
Klebsiella	*K. pneumoniae*	475
	K. oxytoca	101
	K. pneumoniae subsp *ozaenae*	2
	Total	578
Enterobacter	*E. cloacae*	137
	E. aerogenes	122
	E. asburiae	7
	E. sakazakii	1
	Enterobacter not speciated	1
	Total	268

Escherichia coli

Moderately high levels of resistance to ampicillin (and therefore amoxycillin) were observed (43.4%), with lower rates for amoxycillin-clavulanate (14.8% intermediate, 6.2% resistant). Non-susceptibility to third-generation cephalosporins is low but appears to be increasing slowly compared with the 2008 survey (ceftriaxone 3.2%, ceftazidime 1.9%). In line with international trends among community strains of *E. coli*, most of the strains with extended-spectrum ß-lactamase (ESBL) genes harboured genes of the CTX-M type (51/65 = 78%). Moderate levels of resistance were detected to cefazolin (15.2%) and trimethoprim (21.2%). Ciprofloxacin non-susceptibility was found in 5.4% of *E. coli* isolates. Ciprofloxacin resistance was found in 60.3% and gentamicin resistance was found in 49.2% of ESBL-producing strains. Resistance to ticarcillin-clavulanate, piperacillin-tazobactam, cefepime, and gentamicin were below 5%. No isolates had elevated meropenem MICs (≥0.5 mg/L) but 28 (1.3%) strains had ertapenem MICs above wild-type (>0.06 mg/L), 85% of which contained CTX-M or plasmid-borne AmpC genes.

Klebsiella species

These showed slightly higher levels of resistance to cefazolin, ceftriaxone and piperacillin-tazobactam

compared with *E. coli*, but lower rates of resistance to amoxycillin-clavulanate, ticarcicllin-clavulanate, ciprofloxacin, gentamicin, and trimethoprim. ESBLs were present in all 17 presumptively ESBL-positive isolates of *K. pneumoniae*, 12 of which proved to be of the CTX-M type. Two of 3 strains of *K. pneumoniae* with elevated meropenem MICs (≥ 0.5 mg/L) harboured bla_{IMP-4}, while 13 additional strains had elevated ertapenem MICs (> 0.06 mg/L), but none of these harboured a known carbapenemase.

Enterobacter species

Acquired resistance was common to ticarcillin-clavulanate (19.8%), piperacillin-tazobactam (17.2%), ceftriaxone (24.6%), ceftazidime (20.9%) and trimethoprim (12.3%). Rates of resistance to cefepime, ciprofloxacin, and gentamicin were all less than 5%. Five of 12 strains tested for extended-spectrum ß-lactamases based on a suspicious phenotype, harboured ESBL-encoding genes. Three strains had elevated meropenem MICs (≥ 0.5 mg/L) one of which harboured bla_{IMP-4}, while 37% of strains had ertapenem MICs above wild-type (> 0.125 mg/L), which appeared to bear some relationship to stably-derepressed chromosomal AmpC ß-lactamase.

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 2010, the next community-onset surveillance year, shows a small but noticeable increase in resistance rates to some reserve antibiotics. For example, rates of resistance in *E. coli* for ceftriaxone rose from 2.1% to 3.2% and for non-susceptibility to ciprofloxacin rose from 4.2% to 5.4%. Further surveys will establish whether this is a genuine trend or simply a sampling issue.

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

Table 2: Non-susceptibility and resistance rates for the main species tested

Antimicrobial	Category*	*E. coli* (%)	*K. pneumonia* (%)	*K. oxytoca* (%)	*E. cloacae* (%)	*E. aerogenes* (%)
Ampicillin	I	1.3	†	†	†	†
Ampicillin	R	43.4	†	†	†	†
Amoxycillin-clavulanate	I	14.8	2.3	4.0	†	†
Amoxycillin-clavulanate	R	6.2	2.3	5.0	†	†
Ticarcillin-clavulanate	R	4.5	2.3	3.0	22.6	17.2
Piperacillin-tazobactam	R	2.8	3.2	6.9	16.8	18.0
Cefazolin	R	15.2	6.7	68.3	†	†
Cefoxitin	R	1.8	2.7	2.0	†	†
Ceftriaxone	NS	3.2	3.4	4.0	27.7	22.1
Ceftazidime	NS	1.9	1.9	0.0	22.6	19.7
Cefepime	NS	0.7	0.0	0.0	1.5	0.0
Meropenem	NS	0.0	0.2	0.0	0.0	0.0
Ertapenem	NS	0.1	0.6	0.0	16.2	5.8
Ciprofloxacin	NS	5.4	2.3	1.0	6.6	2.5
Norfloxacin	NS	5.2	2.1	1.0	3.6	2.5
Gentamicin	NS	4.2	2.3	0.0	4.4	0.8
Trimethoprim	R	21.2	10.5	5.9	16.1	7.4
Nitrofurantoin	NS	0.5	†	†	†	†

* 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	Non-multi-resistant					Multi-resistant					Cumulative (%)
		Number of acquired resistances										
		0	1	2	3	Cumulative (%)	4	5	6	7	8	9	10	
E. coli	2,092	1,073	407	357	105		66	32	26	13	8	3	2	
%		51.3	19.5	17.1	5.0	92.8	3.2	1.5	1.2	0.6	0.4	0.1	0.1	7.2
Klebsiella spp.*	578	292	209	37	15		13	6	2	3		1		
%		50.5	36.2	6.4	2.6	95.7	2.2	1.0	0.3	0.5		0.2		4.3
Enterobacter spp.†	268	108	92	20	25		14	7	2					
		40.3	34.3	7.5	9.3	91.4	5.2	2.6	0.7					8.6

* 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).

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