ESBL producing Enterobacteriaceae in Australia Results of the AGAR Studies (2004-2008; preliminary 2009)

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Background

Results

There is little published data on the prevalence of antimicrobial resistance among clinical isolates of Gram-negative bacteria in Australia. The Australian Group on Antimicrobial Resistance (AGAR) commenced multi-centre surveillance of Escherichia coli and Klebsiella spp. in 1992. Surveys have been conducted biennially since then. In 2000, Enterobacter spp. was included; and in 2006 Acinetobacter (non A. Iwoffii). For the 2008 study, E. coli, Klebsiella spp. and Enterobacter spp. from outpatients with urinary tract infections were examined. For the 2009 study, isolates from any site were collected from hospital inpatients. The objectives of these surveys were to determine the proportion of resistance to the main therapeutic agents; the extent of co-resistance and multi-resistance; and to detect emerging resistance to newer last-line agents such as carbapenems and more recently tigecycline. Particular emphasis was put on the detection of extended-spectrum β-lactamases (TEM, SHV, CTX-M), plasmid-borne AmpC, and carbapenemases. Isolates from the 2004, 2006 and 2008 surveys with these phenotypes were examined by molecular techniques for the presence of known resistance genes.

Methods

Isolates

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Clinically significant, non-duplicate isolates were collected from patients as indicated in Table 1.

Susceptibility testing

MICs were determined using custom made broth microdilution panels (MicroScan® Dade Behring in 2004; Vitek AST-N044 BioMerieux in 2006, Vitek AST-N083 BioMerieux in 2008 and 2009). All panels were inoculated according to the manufacturers instructions. MICs were interpreted according to CLSI guidelines (CLSI (2010), M100s20).

ESBL Phenotype

E. coli or Klebsiella spp. with ESBL phenotype (ceftriaxone and/or ceftazidime MIC >1mg/L) or possible plasmidborne AmpC β-lactamases (cefoxitin MIC >8 mg/L) and Enterobacter species with cefepime MIC >0.5mg/L (>1 mg/L in 2008) were referred to a central laboratory for molecular characterisation of their resistance genes.

Molecular Methods

All referred isolates were screened for the presence of the bla_{TEM} and bla_{SHV} genes using previously reported oligonucleotide primers. A multiplex real-time TaqMan PCR was used to detect CTX-M-type genes (Birkett et al. J Med Micro (2007) 56; 52). Strains were probed for plasmid-borne AmpC enzymes using the method described by Pérez-Pérez et al. (J Clim Microbiol(2002) 402(153-2162)

O25b-ST131 clones and Phylogenetic groups

Allele-specific PCR for the *pabB* gene was used to screen strains of *E. coli* belonging to ST131 and potentially pathogenic *E. coli* groups were determined using the methods described by Clermont et.al. (JAC (2009) 64:274; Appl. Environ. Microbiol. (2000) 66:4555).

Table 1. Study Design (Isolates per site)

	2004	2006	2008	2009	
Patients	all	all	community	hospital	
Source	restricted	restricted	UTI	all	
E. coli	25	25	70	70	
Klebsiella spp.	25	25	20	20	
Enterobacter spp.	50	25	10	10	

- Since 2004 over 3,476 *E. coli*, 1,910 *Klebsiella* spp. and 2,233 *Enterobacter* spp. have been examined from over 30 participating laboratories throughout Australia.
- The incidence of ESBL phenotypes remained low (see Table 2), however significant regional variation was evident (0% to 29%).
- Of those isolates referred for molecular characterisation, 91% of *E. coli*, and 98% of *K. pneumoniae* with an ESBL phenotype were confirmed to contain an ESBL.
- CTX-M enzymes were detected in many institutions throughout the period. Among *E. coli* strains, CTX-M-1 and CTX-M-9 groups were in equal proportion; however, in *K. pneumoniae*, CTX-M-1 groups dominated.
- The number of strains with plasmid-borne AmpC βlactamases was low. Only CMY-2 (n=8) and CMY-7 (n=1) were detected among *E. coli*; and EBC (n=1) and DHA (n=1) in *K. pneumoniae*.
- Only 21% of cefoxitin-resistant *E. coli* confirmed as having plasmid-borne AmpC.
 - ESBLs were more common among Enterobacter

Table 2. Details of ESBLs received for the AGAR studies 2004-2008, with 2009 preliminary data

	Escherichia coli			Klebsiella pneumoniae			Enterobacter cloacae				
	2004	2006	2008ª	2009 ^b	2004	2006	2008	2009	2004	2006	2008
Number of isolates	597	782	2097	907	418	520	474	222	780	508	161
Number of institutions	25	31	31	15	25	31	31	15	25	31	30
ESBL	17	24	49	73	28	29	17	27	185	65	9
phenotype (%)	(2.8)	(3.1)	(2.3)	(8.0)	(6.7)	(5.6)	(3.6)	(12.2)	(23.7)	(12.8)	(5.6)
Any ESBL	E/0	12/14	12/14	10/50	10/10	17/10	12/12	22/25	E2/00	10/56	2/6
(No. received)	5/6	12/14	43/44	40/00	19/19	17/10	13/13	22/25	52/99	19/50	2/0
CTX-M-type	3	6	36	31	2	8	10	9	4	1	0
(% of ESBL)	(38)	(43)	(82)	(53)	(11)	(44)	(77)	(36)	(4)	(2)	(0)
Any AmpC°	3	3	3	8	0	2	0	4	8	0	1
O25b-ST131	1	2	13	7							

^a Isolates from outpatients with urinary tract infections ^b plasmid-borne AmpC

preliminary data only

Table 3. ESBL Genotype profiles (2008-2009)

	2008		2009 prelim		
ESBL profile ^a	ECOL⁵	KPNE	ECOL	KPNE	
Tem - CTX -	21		13		
CTX -	15		17		
- ShvCTX -		8	1	5	
Tem	4		6		
Tem ampC	2		2		
TemShv		2	1	2	
TemShvCTX -		2		4	
	1		10	3	
ampC	1		5		
- Shv		1	1	5	
TemShv - ampC			4	4	
	44	13	57	23	

* Tem, bla_{TEM} detected; Shv, bla_{BFV} detected; CTX, bla_{CTKM} detected; ampC, plasmid-borne AmpC detected ^b ECOL, *E. coli*, KPNE, *K. pneumoniae*

CONCLUSIONS

- Detection of ESBLs among Enterobacteriaceae often can be difficult in a clinical diagnostic laboratory, especially when often more than one molecular class is found.
- The highly virulent *E. coli* O25b-ST131 clone which often produces CTX-M-type enzymes is well established in the Australian community
- Ongoing surveillance is necessary to determine the prevalent resistance gene types and monitor any emergence of new types.

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spp., and confirmed in 48% (2004) and 33% (2006)

- of isolates referred, with cefepime MIC >1 mg/L. Over 80% of all ESBL positive *E. coli* and *K. pneumoniae* from outpatients with UTI (2008 study) had CTX-M-types detected (Table 3). Preliminary data from 2009 (inpatients) indicates that only half of the ESBL positive strains contain CTX-M-types.
- Multiple enzymes were common in Klebsiella spp. and Enterobacter spp. (73%); and E. coli (51%).
- One K. pneumoniae wound isolate collected in 2006 contained bla_{TEM}, bla_{SHV}, bla_{CTX-M-9}, and DHA.
- In the 2008 outpatient study over 33% of all CTX-M containing *E. coli* were determined to belong to the O25b-ST131 clone. All these strains belong to the highly resistant and virulent *E. coli* phylogenetic group B2.
- Preliminary data from the 2009 study (isolates from inpatients only) reveals a significant increase in the number of ESBLs detected among *E. coli* and *K. pneumoniae*.