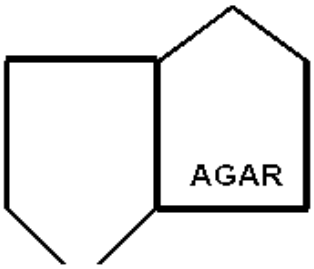

The Australian Group on Antimicrobial Resistance

2011 Gram-negative Survey

Ertapenem Susceptibility Report

Prepared by

Geoffrey Coombs
PathWest Laboratory Medicine WA, Royal Perth Hospital
Perth

Julie Pearson
PathWest Laboratory Medicine WA, Royal Perth Hospital
Perth

Jan Bell
SA Pathology - Women's and Children's Hospital
Adelaide

Professor John Turnidge
SA Pathology - Women's and Children's Hospital
Adelaide

On behalf of the Australian Group for Antimicrobial Resistance (AGAR)

Funded by
Merck Sharp & Dohme (Australia)

JUNE 2012

1 TABLE OF CONTENTS

1 Table of Contents 2
2 Executive Summary 3
3 Background 4
3.1 Objectives of the Program 4
3.2 Importance of Species Surveyed 4
4 Study Design 4
4.1 Methods 5
4.1.1 Species Identification 5
4.2 Susceptibility Testing 5
4.2.1 Minimum Inhibitory Concentration 5
4.3 Molecular Characterisation 5
4.4 Quality Control 5
5 Source of Isolates 6
6 Results 7
6.1 Ertapenem MIC 7
6.2 Comparative Activity 7
6.3 Resistance Mechanisms 8
6.3.1 *Escherichia coli* 8
6.3.2 *Klebsiella* species 8
6.3.3 *Enterobacter* species 8
7 References 9
8 Acknowledgements 9
9 Appendix. Ertapenem MIC Distributions 10

2 EXECUTIVE SUMMARY

The 2011 MSD Ertapenem Susceptibility Study was performed by the Australian Group for Antimicrobial Resistance (AGAR) and forms part of the 2011 AGAR Gram-negative survey.

Twenty-nine institutions from each state and mainland territories of Australia participated. A total of 2,633 isolates (1,827 *E. coli*, 537 *Klebsiella* species and 269 *Enterobacter* species) from inpatients. were included in this survey. Ertapenem minimum inhibitory concentrations (MICs) were determined using Etest® strips (bioMérieux). All isolates with ertapenem MIC >0.25 mg/L were screened for the presence of β-lactamase and carbapenemase genes

This study demonstrates that ertapenem resistance in Enterobacteriaceae in Australian inpatients is still infrequent. Carbapenemases were detected in eight isolates (all harboring *bla*IMP-4). Three of the eight isolates (*K. pneumoniae* (n=2) and *K. oxytoca* (n=1) tested as susceptible to ertapenem. A small percentage of isolates *E. coli* and *Klebsiella* spp. were non-susceptible presumably due to the combination of porin loss associated with extended-spectrum β-lactamases (particularly CTX-M-15 types) and/or plasmid borne AmpC β-lactamases. No VIM, NDM, KPC, or OXA-48-like carbapenemases were detected.

A report of the 2011 AGAR Gram-negative inpatient survey Susceptibility Surveillance Programme is available on the AGAR website (www.antimicrobial-resistance.com).

3 BACKGROUND

3.1 OBJECTIVES OF THE PROGRAM

The objectives of this study were:

1 Determine the proportion of ertapenem resistance in *E. coli*, *Klebsiella* species and *Enterobacter* species isolated from hospital-acquired urinary tract infections.

2 Compare the ertapenem susceptibility of these isolates to a panel of commonly used antibiotics.

3 Investigate the ertapenem resistance mechanism.

3.2 IMPORTANCE OF SPECIES SURVEYED

All species surveyed are members of the family Enterobacteriaceae. This family contains the most important Gram-negative pathogens in a wide range of common conditions in both the community and in hospitals. The three groups surveyed are considered to be valuable sentinels for multi-resistance and emerging resistance.

Escherichia coli is the commonest cause of upper and lower urinary tract infection, and is prominent in a number of other conditions including intra-abdominal sepsis, post-operative wound infections and neonatal sepsis, cholangitis and septicaemia in the profoundly neutropenic patient. It is one of the commonest isolates in the routine microbiology laboratory. In recent years, *E. coli* harboring a CTX-M type extended-spectrum-β-lactamase (ESBL) have been reported in the community in several countries.

Klebsiella species are associated with similar conditions to those of *E. coli* but occur less frequently. They are more likely than *E. coli* to acquire and transmit resistance determinants. This genus is usually intrinsically resistant to aminopenicillins through the possession of one of a small number of natural β-lactamases.

Enterobacter species are predominantly hospital-acquired pathogens, and they were included in this hospital study because as they act as a reservoir of β-lactamases. They are intrinsically resistant to aminopenicillins, first and second generation cephalosporins including cefamycins. Hence, they are naturally multi-resistant. They acquire resistance to important Gram-negative agents relatively easily.

4 STUDY DESIGN

Twenty-nine institutions from each state and mainland territory of Australia participated in the 2011 survey. Each institution collected up to 70 *E. coli*, 20 *Klebsiella* species, 10 *Enterobacter* species from different patients hospitalised for more than 48 hours. The regional distribution of the isolates tested is shown in Table 1.

Table 1. Isolates Tested

Region	Number of Institutions	*E. coli*	*Enterobacter* species	*Klebsiella* species	Total
New South Wales (NSW) Australian Capital Territory (ACT)	8	538	71	145	754
Northern Territory (NT) Queensland (QLD)	7	467	69	139	675
South Australia (SA)	3	163	30	50	243
Victoria (VIC) Tasmania (TAS)	7	381	60	123	564
Western Australia (WA)	4	278	39	80	397
Total	**29**	**1,827**	**269**	**537**	**2,633**

4.1 METHODS

4.1.1 SPECIES IDENTIFICATION

All species were identified by one of the following methods according to each laboratory's standard procedure. Methods included API20E, Vitek®, MicroScan®, Phoenix™, Agar replication, chromogenic agar plus indole. Species included in this study are listed in Table 2.

Table 2. Species included

Group	Organism		Total
E. coli	*E. coli*		1,827
Klebsiella	*K. pneumoniae*		396
	K. oxytoca		137
	K. pneumoniae subsp *ozaenae*		3
	Klebsiella not speciated.		1
		Total	537
Enterobacter	*E. cloacae*		180
	E. aerogenes		83
	E. asburiae		3
	E. gergoviae		2
	Enterobacter not speciated.		1
		Total	269

4.2 SUSCEPTIBILITY TESTING

4.2.1 MINIMUM INHIBITORY CONCENTRATION

Ertapenem minimum inhibitory concentration (MIC) was determined by using Etest® strips (bioMérieux) as recommended by the manufacturer. All other antimicrobials were tested using the Vitek®2 (bioMérieux) AST-N149 card. The ertapenem interpretative breakpoints are the same for both CLSI and EUCAST methods.

4.3 MOLECULAR CHARACTERISATION

Isolates with either ertapenem or meropenem MIC >0.25mg/L were screened for the presence of carbapenemases (VIM, IMP, NDM, KPC, OXA-48-like enzymes) using previously published primers and methodology. Isolates were also tested for the presence of ESBLs and plasmid-borne AmpC.

4.4 QUALITY CONTROL

E. coli ATCC 25922 and *E. coli* ATCC 35218 were used for quality control.

5 SOURCE OF ISOLATES

The majority of isolates were from urine. 5.6% of isolates overall were from blood cultures; comprising 4.8% of *E. coli* isolates, 7.3% of *Klebsiella* and 8.2% of *Enterobacter* species. Other sites of isolation reflect the high incidence of these species in nosocomial and pre- and post-operative surgical infections.

Table 3. Source of Isolates

Source	*E. coli*		*Enterobacter*		*Klebsiella*		Total	
Urine	1448	79.3%	118	43.9%	317	59.0%	1883	71.5%
Respiratory	92	5.0%	66	24.5%	91	16.9%	249	9.5%
Blood	87	4.8%	22	8.2%	39	7.3%	148	5.6%
Skin & soft tissue	81	4.4%	26	9.7%	40	7.4%	147	5.6%
Other	50	2.7%	16	5.9%	22	4.1%	88	3.3%
Intra-abdominal	47	2.6%	8	3.0%	18	3.4%	73	2.8%
Bone & Joint	10	0.5%	6	2.2%	3	0.6%	19	0.7%
Intravascular line	4	0.2%	4	1.5%	6	1.1%	14	0.5%
Sterile site	8	0.4%	3	1.1%	1	0.2%	12	0.5%
Total	**1827**		**269**		**537**		**2633**	

6 RESULTS

6.1 ERTAPENEM MIC

Summary MIC data for ertapenem tested against the most prevelant species isolated is shown in the the Table 4. The full MIC distribution for these organisms is presented in the Appendix.

Table 4. Ertapenem MICs

Genus	MIC Range (mg/L)	MIC_{50}	MIC_{90}	Percentage at MIC: ≤0.5 mg/L (Susceptible)	I mg/L (Intermediate)	≥2 mg/L (Resistant)
***Escherichia coli* (n=1,827)**	≤0.002-8	0.008	0.023	99.8	0.1	0.1
***Klebsiella pneumoniae* (n=396)**	≤0.002-16	0.012	0.047	99.0	0.2	0.8
***Klebsiella oxytoca* (n=137)**	0.006-0.25	0.008	0.032	100		
***Enterobacter cloacae* (n=180)**	≤0.002-3	0.064	1.0	83.9	11.1	5.0
***Enterobacter aerogenes* (n=83)**	0.003-4	0.064	0.38	95.2	2.4	2.4

6.2 COMPARATIVE ACTIVITY

Table 5. Comparative Susceptibility of Ertapenem

Antimicrobial Agent	*E. coli*	*K. pneumoniae*	*K. oxytoca*	*E. cloacae*	*E. aerogenes*
Ampicillin	48.6%	3.0%	2.9%	11.8%	28.6%
Amoxycillin-clavulanate	76.2%	85.1%	85.4%	5.6%	3.6%
Ticarcillin-clavulanate	82.5%	85.0%	86.4%	56.7%	68.8%
Cefazolin	77.7%	81.5%	31.4%	3.4%	15.9%
Ceftriaxone	90.4%	87.9%	90.5%	56.7%	66.3%
Ceftazidime	94.2%	90.2%	96.4%	59.4%	71.1%
Cefepime	98.2%	97.7%	100.0%	95.6%	100.0%
Gentamicin	91.4%	89.1%	97.1%	84.4%	100.0%
Trimethoprim	76.6%	81.3%	95.6%	72.8%	97.6%
Ciprofloxacin	89.4%	91.7%	100.0%	95.0%	100.0%
Meropenem	99.9%	99.5%	100.0%	99.4%	100.0%
Ertapenem	99.8%	99.0%	100.0%	83.9%	95.2%

6.3 RESISTANCE MECHANISMS

No carbapenemase genes (VIM, IMP, NDM, KPC, OXA-48-like enzymes) were detected in any species.

6.3.1 *Escherichia coli*

Of all the *E. coli* isolates (n=10) with ertapenem MIC >0.25 mg/L, all except one strain contained either a confirmed ESBL (CTX-M-15 like) and/ or a plasmid-borne AmpC (CIT).

Isolate#	Ert	Carbapenamase	ESBL	AmpC	Region
2011-2327	0.38	-	CTXM	-	VIC/TAS
2011-1081	0.38	-	CTXM	-	QLD/NT
2011-0417	0.38	-	CTXM	-	ACT/NSW
2011-2112	0.38	-	CTXM	-	VIC/TAS
2011-0658	0.38	-	CTXM	CIT	ACT/NSW
2011-1301	0.5	-	-	CIT	QLD/NT
2011-0847	0.5	-	-	CIT	ACT/NSW
2011-3147	1	-	-	CIT	VIC/TAS
2011-2978	1	-	-	CIT	QLD/NT
2011-2842	8	-	-	-	QLD/NT

6.3.2 *Klebsiella* species

bla $_{IMP-4}$ was detected in three of eight *K. pneumoniae* isolates with ertapenem MIC >0.25 mg/L, and two additional isolates with ertapenem MIC 0.25 mg/L. ESBL (CTX-M-15-like and/or TEM-types) were detected in half of the isolates, and a further two strains contained a plasmid-borne AmpC (DHA).

Isolate#	Ert	Carbapenamase	ESBL	AmpC	Region
2011-0535	0.25	IMP-4	Tem	-	ACT/NSW
2011-0544	0.25	IMP-4	Tem	-	ACT/NSW
2011-2305	0.38	-	TemCTX	-	VIC/TAS
2011-1049	0.38	-	-	DHA	QLD/NT
2011-1974	0.5	-	-	-	VIC/TAS
2011-0274	0.5	IMP-4	TemCTX	-	ACT/NSW
2011-0282	1	IMP-4	TemCTX	-	ACT/NSW
2011-0873	1.5	IMP-4	TEM	-	ACT/NSW
2011-2722	2	-	-	DHA	WA
2011-0485	16	-	CTXM	-	ACT/NSW

6.3.3 *Enterobacter* species

There were 10 *E. cloacae* isolates with ertapenem MIC >1 mg/L of which three contained *bla* $_{IMP-4}$.

7 REFERENCES

1 Clinical and Laboratory Standards Institute (2012). Performance standards for antimicrobial susceptibility testing; twenty-second Informational Supplement. M100-S22. CLSI, Villanova, PA, USA.

2 European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters, Version 2.0, January 2012 (http://www.eucast.org/clinical_breakpoints/).

8 ACKNOWLEDGEMENTS

Alfred Hospital, VIC	Denis Spelman and Michael Huysmans
Austin Health	Ben Howden and Peter Ward
Concord Hospital, NSW	Tom Gottlieb and Graham Robertson
Douglass Hanly Moir Pathology, NSW	Miriam Paul and Richard Jones
Launceston General Hospital, TAS	Mhisti Rele and Kathy Wilcox
Southern Health, (Monash Medical Centre)	Tony Korman and Despina Kotsanas
Nepean Hospital, NSW	James Branley and Donna Barbaro
PathWest Laboratory Medicine-WA, Fremantle Hospital, WA	David McGechie and Rebecca Wake
PathWest Laboratory Medicine-WA, QEII Medical Centre, WA	Ronan Murray and Barbara Henderson
PathWest Laboratory Medicine-WA, Royal Perth Hospital, WA	Keryn Christiansen and Geoffrey Coombs
Pathology Queensland, Cairns Base Hospital, QLD	Enzo Binotto and Bronwyn Thomsett
Pathology Queensland, Gold Coast Hospital, QLD	Petra Derrington and Dale Thorley
Pathology Queensland, Princess Alexandra Hospital, QLD	Joan Faoagali and Joel Douglas
Pathology Queensland, Prince Charles Hospital, QLD	Chris Coulter and Sonali Coulter
Pathology Queensland, Central Laboratoryl, QLD	Graeme Nimmo and Narelle George
Royal Children's Hospital, VIC	Suzanne Garland and Gena Gonis
Royal Darwin Hospital, NT	Rob Baird and Jann Hennessy
Royal Hobart Hospital, TAS	Alistair McGregor and Rob Peterson
Royal North Shore Hospital, NSW	George Kotsiou and Peter Huntington
Royal Prince Alfred Hospital, NSW	Colin MacLeod and Bradley Watson
SA Pathology (Flinders Medical Centre)	Kelly Papanaoum and Hendrik Pruul
SA Pathology (Royal Adelaide Hospital)	Morgyn Warner and Rachael Pratt
SA Pathology (Women's and Children's Hospital)	John Turnidge and Jan Bell
Sydney South West Pathology Service, NSW	Iain Gosbell and Annabelle LeCordier
St John of God Pathology, WA	Victoria D'Abrera
St Vincent's Hospital, VIC	Mary Jo Waters and Linda Joyce
Sullivan Nicolaides Pathology, QLD	Jenny Robson and Georgia Peachey
The Canberra Hospital, ACT	Peter Collignon and Susan Bradbury
Westmead Hospital, NSW	David Mitchell and Lee Thomas

9 APPENDIX. ERTAPENEM MIC DISTRIBUTIONS

Species [b]	Minimum inhibitory concentration (mg/L) [a]																										Total
	0.002	0.003	0.004	0.006	0.008	0.012	0.016	0.023	0.032	0.047	0.064	0.094	0.125	0.19	0.25	0.38	0.5	0.75	1	1.5	2	3	4	8	12	16	
ECOL	12	8	129	541	569	233	140	42	46	12	22	13	17	14	19	5	2		2					1			1827
KPNE	2		3	43	127	57	67	19	25	15	8	6	6	5	5	2	2		1	1	1					1	396
KOXY				34	60	15	10	2	9			1	5		1												137
KPNO					2	1																					3
KLEB						1																					1
ECLO	2				7	5	19	16	18	11	15	8	9	9	12	10	10	6	14	3		3	3				180
EAER		1	1	1	6	5		7	7	9	6	4	6	7	10	6	3		2		1	1					83
EASB					1				1											1							3
ENTR										1					1												2
EGER																						1					1

[a] Vertical lines indicate the CLSI/EUCAST susceptible (blue) and resistant (red) breakpoints

[b] ECOL = *E. coli*, KPNE = *K. pneumoniae*, KOXY = *K. oxytoca*, ECLO = *E. cloacae*, EAER = *E. aerogenes*, EASB = *E. asburiae*, KPNO = *K pneumoniae* subsp. *ozaenae*, ENTR = *Enterobacter* not speciated, EGER = *E. gergoviae*, KLEB = *Klebsiella* not speciated