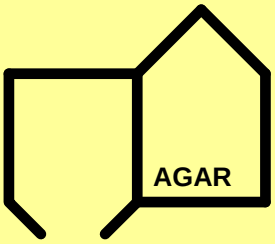

The Australian Group on Antimicrobial Resistance
http://antimicrobial-resistance.com

2010 Ertapenem Susceptibility Report (AGAR Community Enterobacteriaceae Susceptibility Surveillance Programme)

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

The 2010 MSD Ertapenem Susceptibility Study was performed by the Australian Group for Antimicrobial Resistance (AGAR) and forms part of the 2010 AGAR Community Enterobacteriaceae Susceptibility Surveillance Programme.

Thirty institutions from each state and mainland territories of Australia participated in the programme. A total of 2,931 isolates (2,090 *E. coli*, 576 *Klebsiella* species and 265 *Enterobacter* species) from outpatients with urinary tract infections were included in the Ertapenem study. Ertapenem minimum inhibitory concentrations (MICs) were determined using Etest® strips (bioMerieux). The recently approved CLSI[1] ertapenem interpretive breakpoints were applied (susceptible ≤0.5 mg/L). All isolates with ertapenem MIC >0.25 mg/L were screened for the presence of carbapenemases (VIM, IMP, NDM, KPC, OXA-48-like enzymes).

Ertapenem MIC Distributions:

E coli:	Range 0.002 to 1.0 mg/L (MIC_{50} 0.008 mg/L, MIC_{90} 0.016 mg/L).
	99.9% of isolates were ertapenem susceptible.
Klebsiella pneumoniae:	Range 0.002 to 32 mg/L (MIC_{50} 0.012 mg/L, MIC_{90} 0.047 mg/L).
	99.3% of isolates were ertapenem susceptible. Two *K. pneumoniae* strains contained bla_{IMP}.
Klebsiella oxytoca:	Range 0.004 to 0.25 mg/L (MIC_{50} 0.008 mg/L, MIC_{90} 0.016 mg/L). All isolates were susceptible to ertapenem.
Enterobacter cloacae:	Range 0.004 to 3 mg/L (MIC_{50} 0.125 mg/L, MIC_{90} 1.0 mg/L).
	83.8% of isolates were ertapenem susceptible. One isolate with ertapenem MIC = 2mg/L contained bla_{IMP}.
Enterobacter aerogenes:	Range 0.008 to 3 mg/L (MIC_{50} 0.064 mg/L, MIC_{90} 0.38 mg/L).
	93.9% of isolates were ertapenem susceptible.

This study demonstrates that carbapenem resistance in Enterobacteriaceae urinary tract infections in Australian outpatients is not frequently seen. Carbapenemases were detected in three isolates (all harboring bla_{IMP}). This suggests the low-level ertapenem resistance seen among Australian Enterobacteriaceae may primarily be due to a combination of impermeability, arising via porin loss, or the presence of potent ß-lactamases, such as CTX-M ESBLs in *Klebsiella pneumoniae* and *E. coli* and AmpC in the *Enterobacter*. Efflux changes may also be involved.

New Delhi Metallo-β-lactamase (NDM), *Klebsiella pneumoniae* carbapenemase (KPC), or OXA-48-like carbapenemases were not detected.

A report of the 2010 AGAR Community Enterobacteriaceae Susceptibility Surveillance Programme will be available on the AGAR website (www.antimicrobial-resistance.com**).**

Background

1.1 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 community-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.

1.2 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, however they were included in this community study 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.

2 Study Design

Thirty institutions from each state and mainland territory of Australia participated in the 2010 survey. Each institution collected up to 70 *E. coli*, 20 *Klebsiella* species and 10 *Enterobacter* species from outpatients with urinary tract infections. Isolates from urinary catheters were excluded as were isolates from hospital inpatients and nursing home residents.

2.1 Methods

2.1.1 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.

2.2 Susceptibility Testing

2.2.1 Ertapenem

Ertapenem minimum inhibitory concentration (MIC) was determined by Etest® (bioMerieux) using Mueller Hinton Agar as recommended by the manufacturer.

The CLSI ertapenem interpretive breakpoints as approved June 14 2011 have been used in this report. These breakpoints are equivalent to the published EUCAST interpretive breakpoints.

Category	MIC (mg/L)	
	CLSI	EUCAST
Susceptible (S)	≤0.5	≤0.5
Intermediate (I)	1.0	
Resistant (R)	≥ 2.0	>1

Quality Control Strain: *E. coli* ATCC 25922 (acceptable range 0.004 to 0.015 mg/L)

2.2.2 Additional Antimicrobials

Additional antimicrobial susceptibility tests were performed by the Vitek®2 (bioMérieux) AST-N149 card.

Table 1: Vitek®2 AST-N149 Card

Antibiotic	Code	MIC range (mg/L)
Amoxycillin-clavulanate	AMC	2/1 – 32/16
Ampicillin	AMP	2 – 32
Cefazolin	CFZ	4 – 64
Cefepime	CPM	1 – 64
Ceftazidime	CAZ	1 – 64
Ceftriaxone	CTR	1 – 64
Ciprofloxacin	CIP	0.25 – 4
Gentamicin	GEN	1 – 16
Meropenem	MER	0.25 – 16
Ticarcillin-clavulanate	TCC	8/2 – 128/2
Trimethoprim	TMP	0.5 – 16
Trimethoprim-sulfamethoxazole	SXT	20 – 320

MICs were interpreted using CLSI criteria. The quality control strains for the Vitek®2 cards were *E. coli* ATCC® 25922 and *E. coli* ATCC® 35218.

2.2.3 Molecular Characterisation

Isolates with ertapenem MIC >0.25mg/L were screened for the presence of carbapenemases (VIM, IMP, NDM, KPC, OXA-48-like enzymes) using real-time PCR methodology.

2.3 Results

Table 2: Patient Type

Patient Type	Number	Percentage
Emergency Department	1,478	50.4
General Practice	797	27.2
Outpatient Clinic	615	21.0
Correctional Institution	3	0.1
Other	38	1.3
Total	2,931	100

Figure 1: Patient Age and Gender

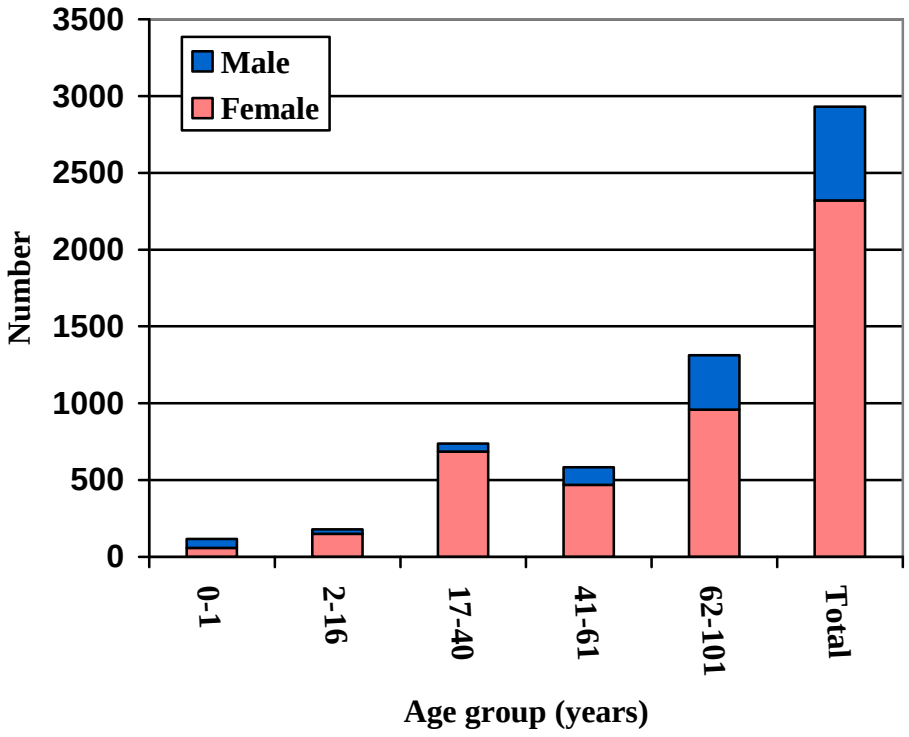


Table 3: Geographical Origin of Isolates

Region	Number of Institutions	*E. coli*	*Klebsiella species*	*Enterobacter species*	Total
Australian Capital Territory (ACT)	1	70	20	10	100
New South Wales (NSW)	7	488	140	62	690
Northern Territory (NT)	1	70	20	9	99
Queensland (Qld)	6	418	120	60	598
South Australia (SA)	3	206	59	28	293
Tasmania (Tas)	2	140	20	2	162
Victoria (Vic)	6	418	117	58	593
Western Australia (WA)	4	280	80	36	396
Total	30	2,090	576	265	2,931

Table 4 Species Identified

Genus	Species	Number	Total
Escherichia	*E. coli*	2,090	
	Total		2,090
Klebsiella	*K. pneumoniae*	458	
	K. oxytoca	98	
	K. ozaenae	2	
	Unspeciated	18	
	Total		576
Enterobacter	*E. cloacae*	136	
	E. aerogenes	114	
	E. asburiae	6	
	E. sakazakii	1	
	Unspeciated	8	
	Total		265
TOTAL			2,931

2.3.1 *E. coli* (n = 2,090)

MIC_{50} 0.008 mg/L MIC_{90} 0.016 mg/L

Susceptible: n=2,088 (99.9%) Intermediate: n=2 (0.1%) Resistant n=0 (0.0%)

Figure 2: *E. coli* Ertapenem MIC Distribution

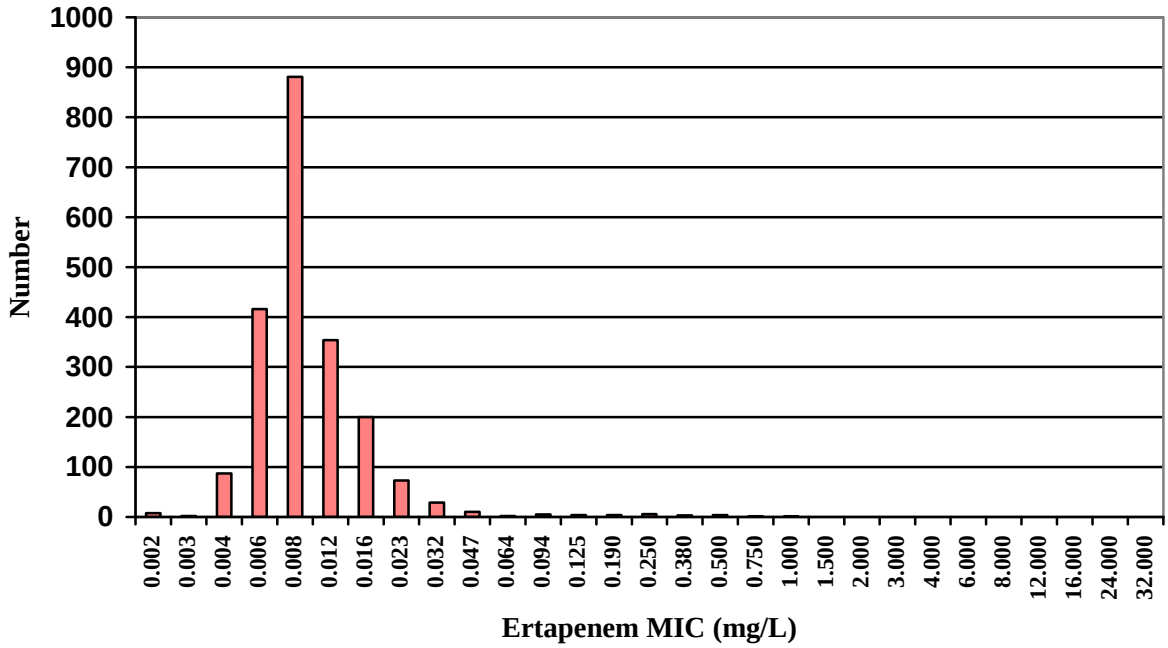
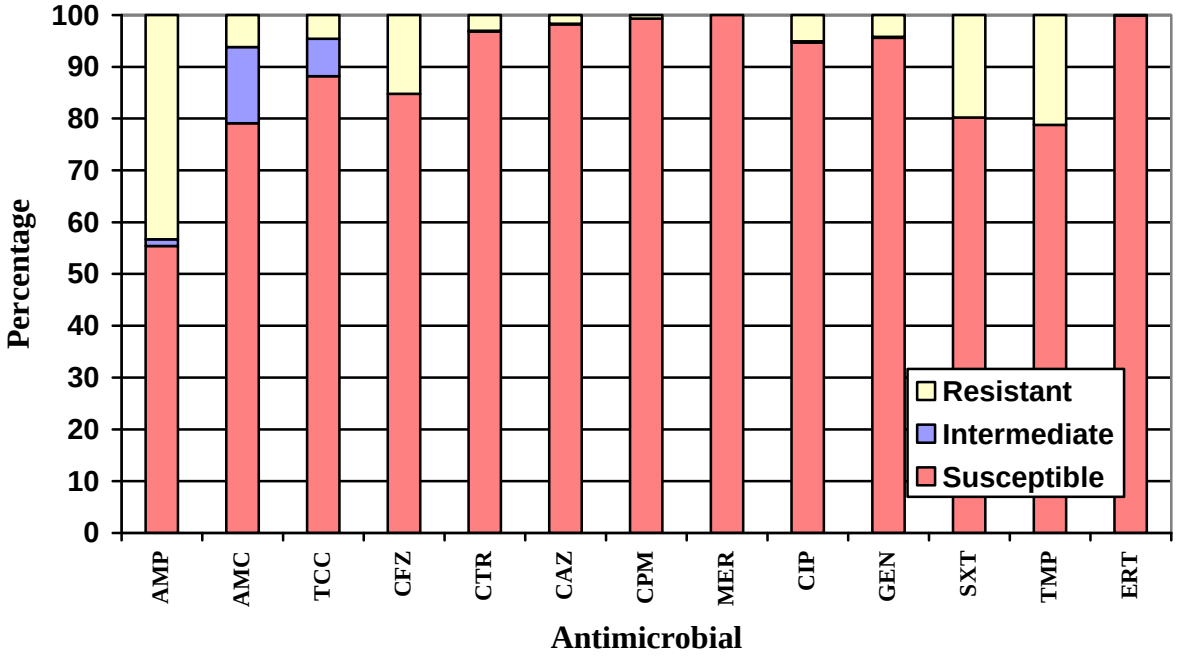


Figure 3: *E. coli* Antimicrobial Susceptibility Results



2.3.2 *Klebsiella pneumoniae* (n = 458)

MIC_{50} 0.012 mg/L MIC_{90} 0.047 mg/L

Susceptible: n=455 (99.3%) Intermediate: n=1 (0.2%) Resistant n=2 (0.4%)

Figure 4: *Klebsiella pneumoniae* Ertapenem MIC Distribution

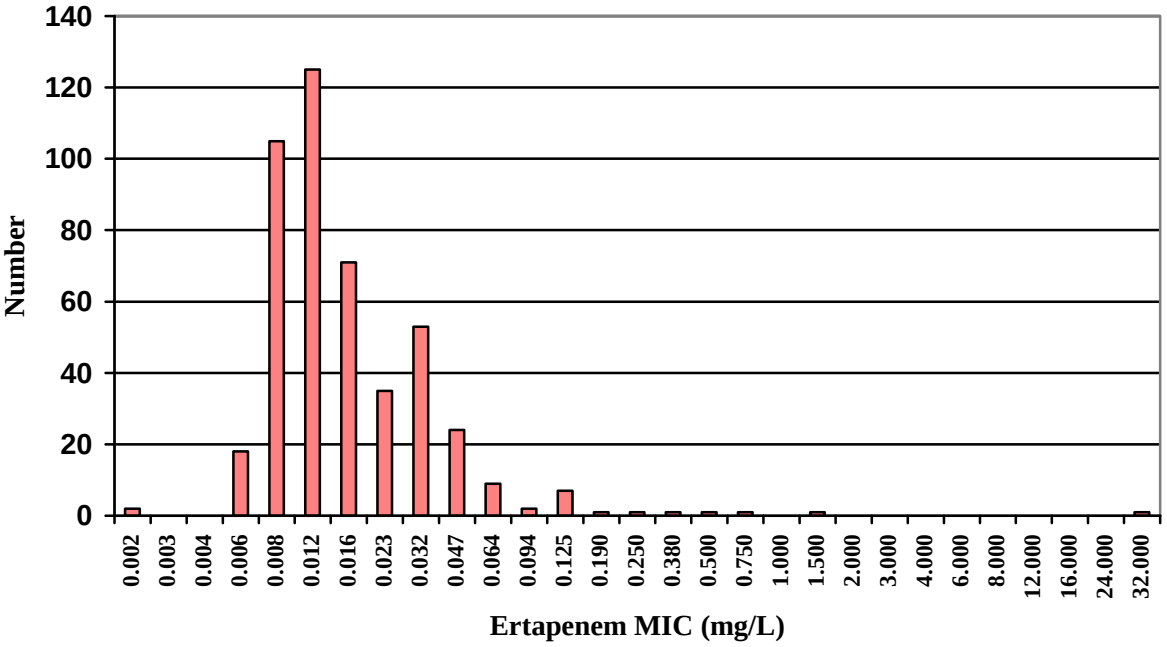
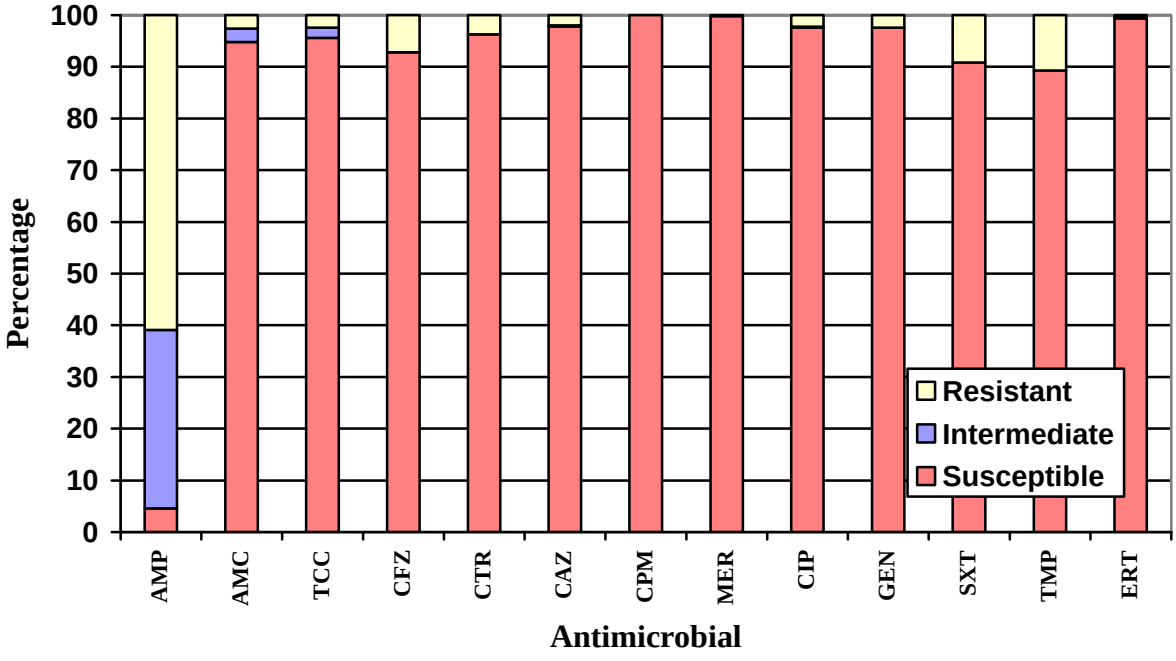


Figure 5: *Klebsiella pneumoniae* Antimicrobial Susceptibility Results



2.3.3 *Klebsiella oxytoca* (n = 98)

MIC_{50} 0.008 mg/L MIC_{90} 0.016 mg/L

Susceptible: n=98 (100%) Intermediate: n=0 (0%) Resistant n=0 (0%)

Figure 6: *Klebsiella oxytoca* Ertapenem MIC Distribution

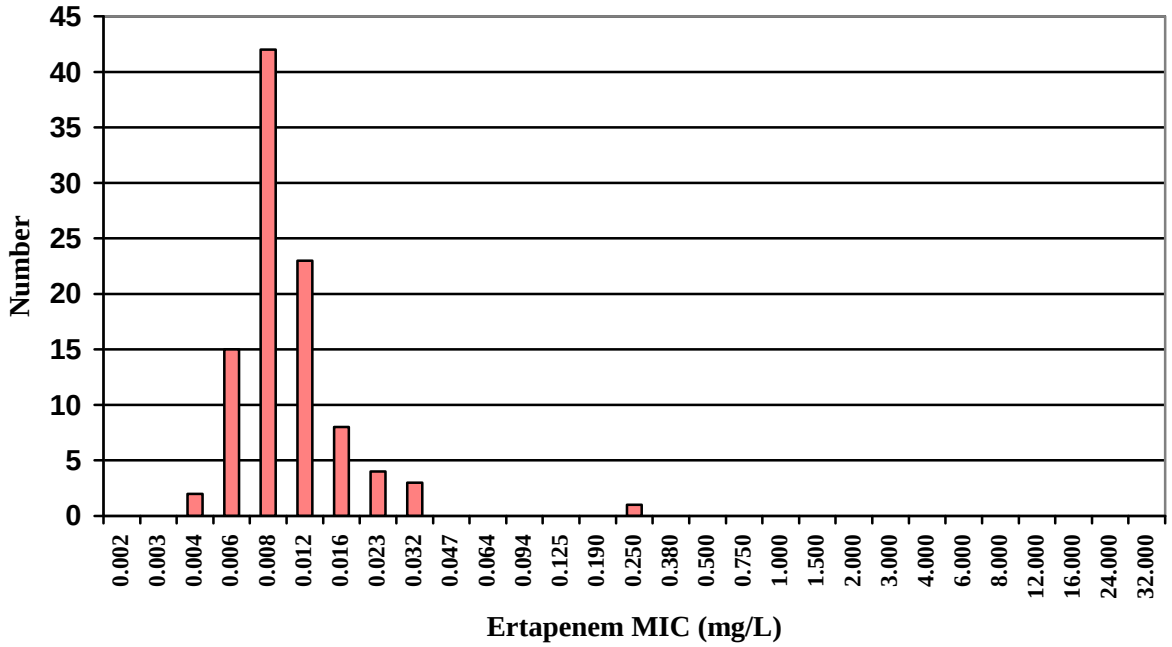
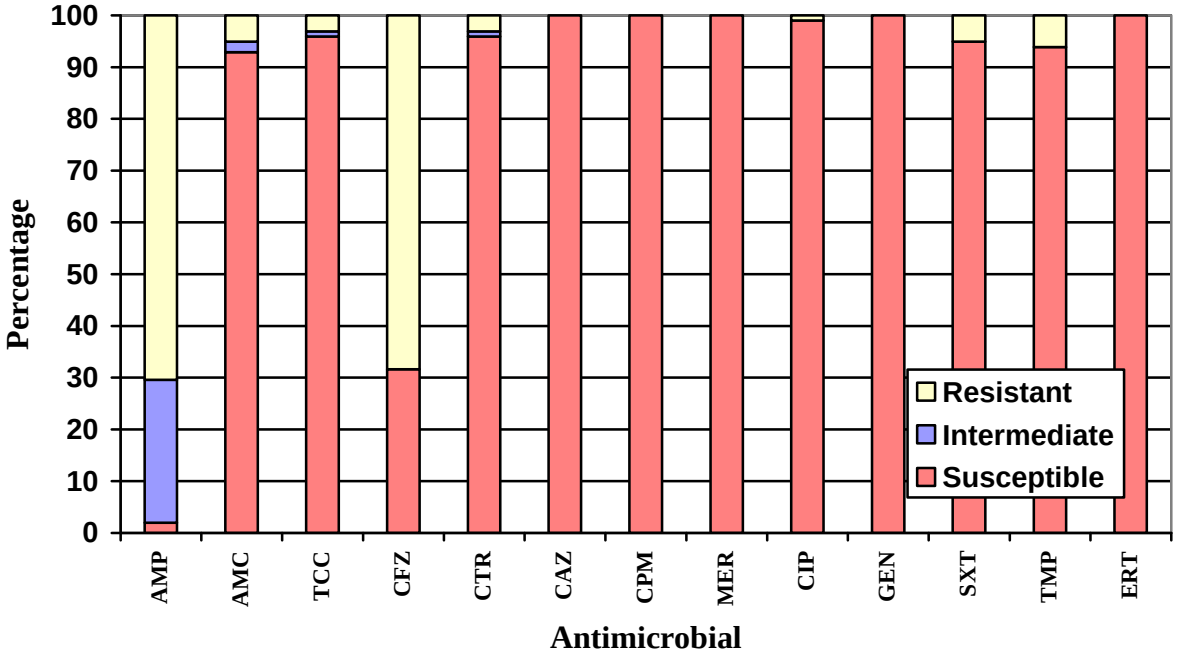


Figure 7: *Klebsiella oxytoca* Antimicrobial Susceptibility Results



2.3.4 *Enterobacter cloacae* (n = 136)

MIC_{50} 0.125 mg/L MIC_{90} 1.0 mg/L

Susceptible: n=114 (83.8%) Intermediate: n=15 (11.0%) Resistant n=7 (5.1%)

Figure 8: *Enterobacter cloacae* Ertapenem MIC Distribution

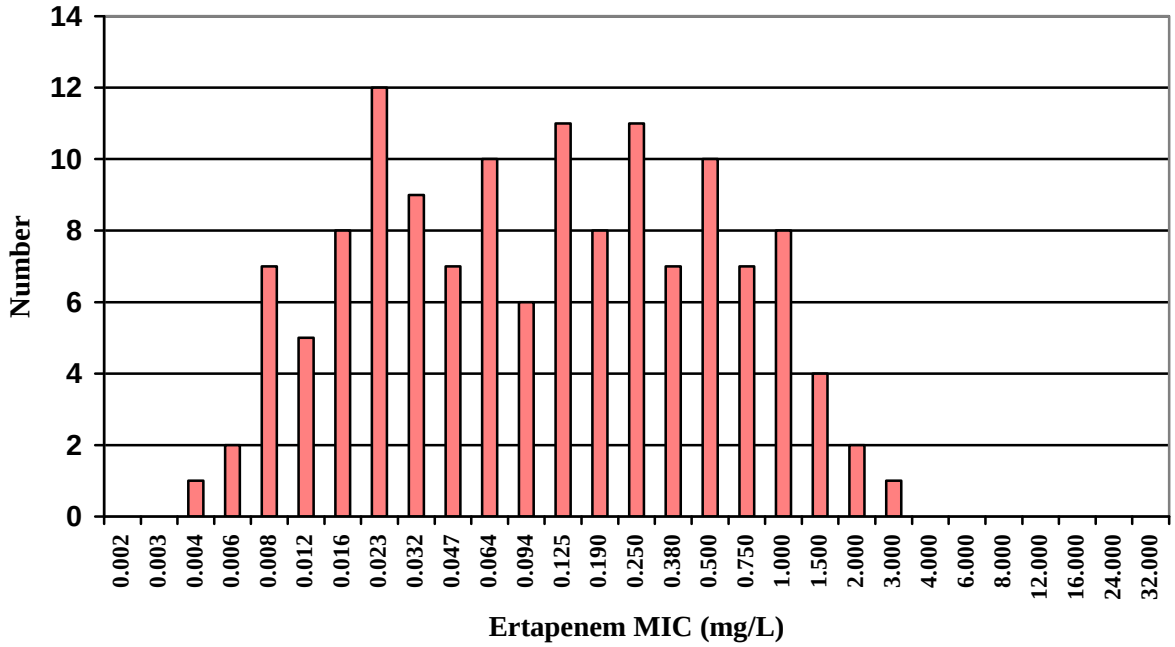
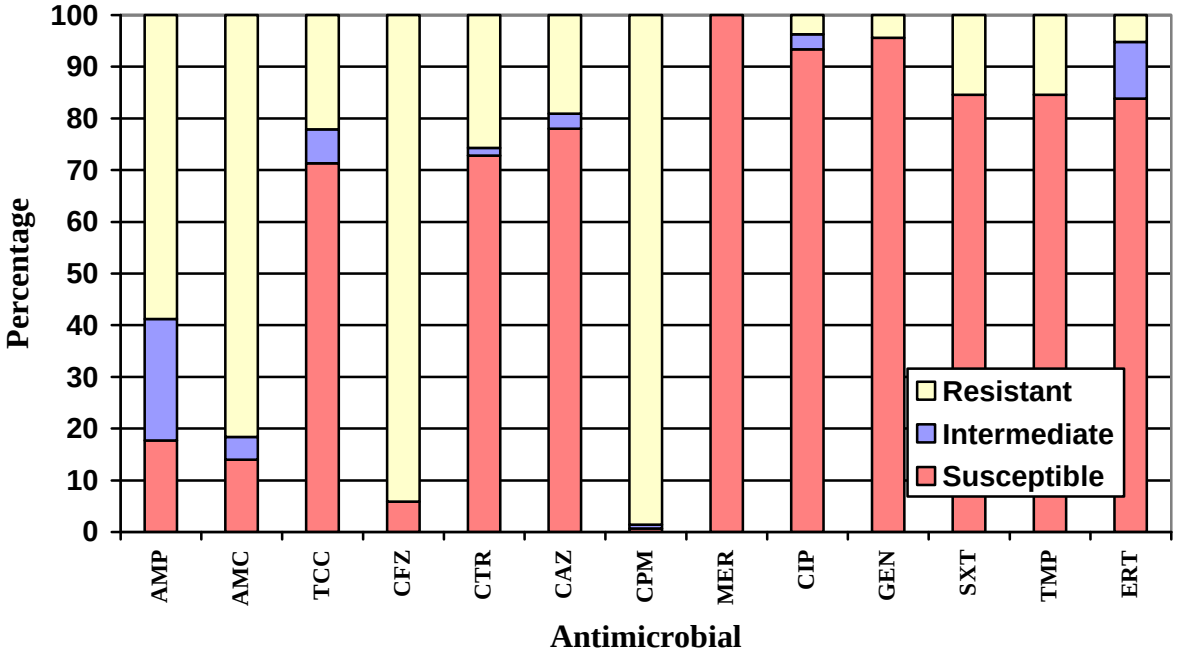


Figure 9: *Enterobacter cloacae* Antimicrobial Susceptibility Results



2.3.5 *Enterobacter aerogenes* (n = 114)

MIC_{50} 0.064 mg/L MIC_{90} 0.38 mg/L

Susceptible: n=107 (93.9%) Intermediate: n=3 (2.6%) Resistant n=4 (3.5%)

Figure 10: *Enterobacter aerogenes* Ertapenem MIC Distribution

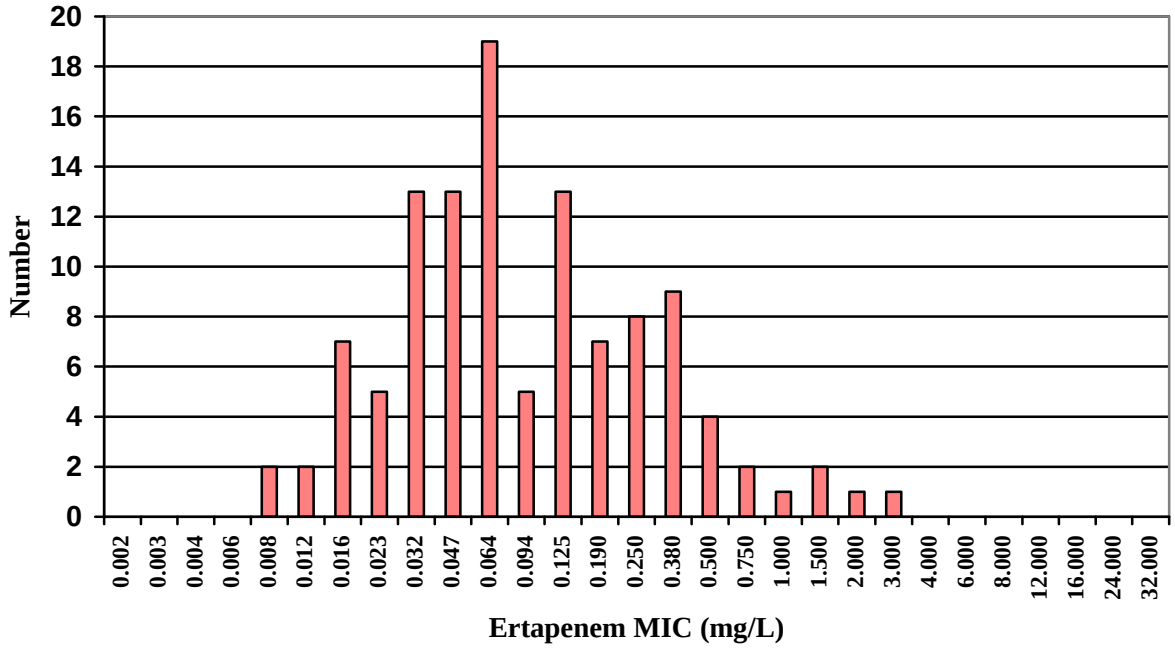
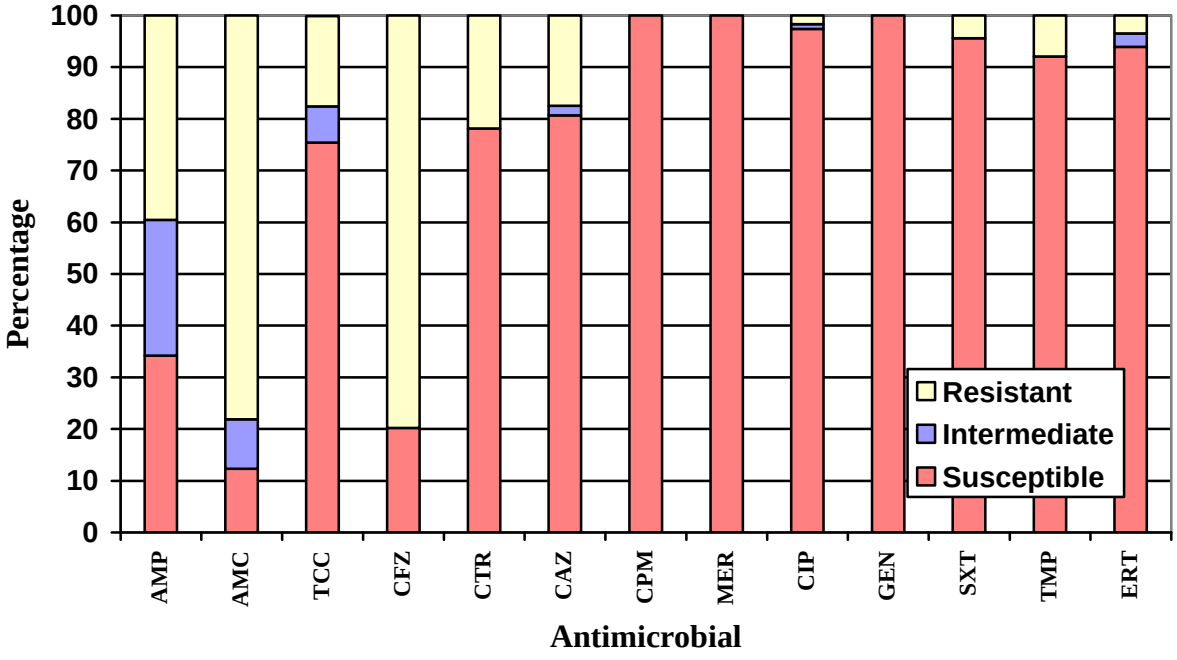


Figure 11: *Enterobacter aerogenes* Antimicrobial Susceptibility Results



2.3.6 *Miscellaneous*

***Klebsiella* (n = 20)**

	AMP	AMC	TCC	CFZ	CTR	CAZ	CPM	MER	CIP	GEN	SXT	TMP	ERT	MIC
K. ozaenae	R	S	S	S	S	S	S	S	I	S	S	S	0.012	S
K. ozaenae	R	S	S	S	S	S	S	S	S	S	S	S	0.008	S
unspeciated	S	S	S	S	S	S	S	S	S	S	S	S	0.032	S
unspeciated	R	S	S	S	S	S	S	S	S	S	S	S	0.047	S
unspeciated	R	S	S	S	S	S	S	S	S	S	S	S	0.016	S
unspeciated	R	S	S	S	S	S	S	S	S	S	S	S	0.047	S
unspeciated	R	S	S	S	S	S	S	S	S	S	S	R	0.008	S
unspeciated	R	I	I	R	S	S	S	S	S	S	S	S	0.012	S
unspeciated	R	S	S	S	S	S	S	S	S	S	R	R	0.016	S
unspeciated	I	S	S	S	S	S	S	S	S	S	S	S	0.023	S
unspeciated	R	S	S	S	S	S	S	S	S	S	S	S	0.016	S
unspeciated	R	S	S	S	S	S	S	S	S	S	S	S	0.016	S
unspeciated	R	S	S	S	S	S	S	S	S	S	S	S	0.012	S
unspeciated	R	S	S	S	S	S	S	S	S	S	S	S	0.032	S
unspeciated	R	S	S	S	S	S	S	S	S	S	S	S	0.032	S
unspeciated	R	S	S	S	S	S	S	S	S	S	S	S	0.047	S
unspeciated	I	S	S	S	S	S	S	S	S	S	S	S	0.012	S
unspeciated	I	S	S	S	S	S	S	S	S	S	S	S	0.016	S
unspeciated	R	S	S	S	S	S	S	S	S	S	S	S	0.012	S
unspeciated	R	S	S	S	S	S	S	S	S	S	S	S	0.008	S

***Enterobacter* (n = 15)**

	AMP	AMC	TCC	CFZ	CTR	CAZ	CPM	MER	CIP	GEN	SXT	TMP	ERT	MIC
E. asburiae	S	R	S	R	S	S	S	S	S	S	S	S	0.012	S
E. asburiae	R	R	R	R	R	S	R	S	S	S	R	R	0.38	S
E. asburiae	R	I	S	R	S	S	S	S	S	R	R	R	0.004	S
E. asburiae	I	R	S	R	S	S	S	S	S	S	S	S	0.064	S
E. asburiae	R	R	S	R	S	S	S	S	S	S	S	S	0.19	S
E. asburiae	R	R	S	R	S	S	S	S	S	S	S	S	0.008	S
E. sakazakii	S	S	S	R	S	S	S	S	S	S	S	S	0.032	S
unspeciated	S	I	S	S	S	S	S	S	S	S	S	S	0.016	S
unspeciated	R	R	S	R	S	S	S	S	S	S	S	S	0.125	S
unspeciated	I	S	S	S	S	S	S	S	S	S	S	S	0.19	S
unspeciated	I	R	S	S	S	S	S	S	S	S	S	S	0.094	S
unspeciated	I	R	S	R	S	S	S	S	S	S	S	S	0.094	S
unspeciated	R	R	S	R	S	S	S	S	S	S	R	R	0.032	S
unspeciated	I	R	S	S	S	S	S	S	S	S	S	S	0.125	S
unspeciated	S	S	S	S	S	S	S	S	S	S	S	S	0.008	S

2.3.7 Molecular characterisation

Abbreviations:

MBL: metallo-β-lactamase (*bla*$_{IMP}$, *bla*$_{VIM}$)

NDM: New Delhi metallo-β-lactamase

KPC: *Klebsiella pneumoniae* carbapenemase

E. coli (n = 9)

AGAR #	MIC (mg/L)	MBL	NDM	KPC	OXA-48-like
23-68	0.38	-	-	-	-
19-43	0.38	-	-	-	-
19-30	0.38	-	-	-	-
23-10	0.5	-	-	-	-
19-10	0.5	-	-	-	-
7-56	0.5	-	-	-	-
7-49	0.5	-	-	-	-
31-20	0.75	-	-	-	-
7-77	1.0	-	-	-	-

***Klebsiella pneumonia* (n = 5)**

AGAR #	MIC (mg/L)	MBL	NDM	KPC	OXA-48-like
13-23	0.38	-	-	-	-
15-74	0.5	-	-	-	-
25-76	0.75	-	-	-	-
1-71	1.5	IMP	-	-	-
28-91	32	IMP	-	-	-

***Enterobacter cloacae* (n = 39)**

AGAR #	MIC (mg/L)	MBL	NDM	KPC	OXA-48-like
27-99	0.38	-	-	-	-
2-94	0.38	-	-	-	-
22-91	0.38	-	-	-	-
19-98	0.38	-	-	-	-
19-95	0.38	-	-	-	-
19-94	0.38	-	-	-	-
4-96	0.38	-	-	-	-
16-91	0.5	-	-	-	-
27-95	0.5	-	-	-	-
26-94	0.5	-	-	-	-
12-97	0.5	-	-	-	-
28-74	0.5	-	-	-	-
5-91	0.5	-	-	-	-
20-95*	0.5				
2-98	0.5	-	-	-	-
2-93	0.5	-	-	-	-
4-95	0.5	-	-	-	-
6-94	0.75	-	-	-	-
12-100	0.75	-	-	-	-
12-96	0.75	-	-	-	-
23-100	0.75	-	-	-	-
19-92	0.75	-	-	-	-
19-91	0.75	-	-	-	-

AGAR #	MIC (mg/L)	MBL	NDM	KPC	OXA-48-like
4-97	0.75	-	-	-	-
27-96	1.0	-	-	-	-
3-91	1.0	-	-	-	-
5-99	1.0	-	-	-	-
30-97	1.0	-	-	-	-
30-95	1.0	-	-	-	-
30-50	1.0	-	-	-	-
2-92	1.0	-	-	-	-
23-98	1.0	-	-	-	-
2-91	1.5	-	-	-	-
29-99	1.5	-	-	-	-
4-99	1.5	-	-	-	-
11-97	1.5	-	-	-	-
7-2	2.0	IMP	-	-	-
5-77	2.0	-	-	-	-
22-2	3.0	-	-	-	-

* isolate not received

Enterobacter aerogenes (n = 20)

AGAR #	MIC (mg/L)	MBL	NDM	KPC	OXA-48-like
21-83**	0.38				
13-8	0.38	-	-	-	-
27-94	0.38	-	-	-	-
8-97	0.38	-	-	-	-
8-96	0.38	-	-	-	-
12-92	0.38	-	-	-	-
3-92	0.38	-	-	-	-
29-98	0.38	-	-	-	-
19-93	0.38	-	-	-	-
26-100	0.5	-	-	-	-
3-98	0.5	-	-	-	-
3-94	0.5	-	-	-	-
31-94*	0.5				
7-9	0.75	-	-	-	-
3-97	0.75	-	-	-	-
7-3	1.0	-	-	-	-
16-93	1.5	-	-	-	-
23-84	1.5	-	-	-	-
12-91	2.0	-	-	-	-
13-5	6.0	-	-	-	-

* isolate not received
** isolate non-viable

Enterobacter asburiae (n = 1)

AGAR #	MIC (mg/L)	MBL	NDM	KPC	OXA-48-like
16-97	0.38	-	-	-	-

Appendix 1

Antimicrobial Susceptibility Results

		AMP	AMC	TCC	CFZ	CTR	CAZ	CPM	MER	CIP	GEN	SXT	TMP	ERT
E. coli	S	1157	1653	1844	1772	2024	2052	2076	2090	1980	1998	1677	1647	2088
(n=2090)	I	28	308	151	0	3	3	0	0	4	5	NA	NA	2
	R	905	129	95	318	63	35	14	0	106	87	413	443	0
K. pneumoniae	S	21	434	438	425	441	448	458	457	447	447	416	409	455
(n=458)	I	158	12	9	0	0	1	0	1	1	0	NA	NA	1
	R	279	12	11	33	17	9	0	0	10	11	42	49	2
K. oxytoca	S	2	91	94	31	94	98	98	98	97	98	93	92	98
(n=98)	I	27	2	1	0	1	0	0	0	0	0	NA	NA	0
	R	69	5	3	67	3	0	0	0	1	0	5	6	0
E. cloacae	S	24	19	97	8	99	106	134	136	127	130	115	115	114
(n=136)	I	32	6	9	0	2	4	1	0	4	0	NA	NA	15
	R	80	111	30	128	35	26	1	0	5	6	21	21	7
E. aerogenes	S	39	14	86	23	89	92	114	114	111	114	109	105	107
(n=114)	I	30	11	8	0	0	2	0	0	1	0	NA	NA	3
	R	45	89	20	91	25	20	0	0	2	0	5	9	4

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