

S. Hawser², M. Hackel¹, S. Bouchillon¹, B. Johnson¹, D. Hoban¹, M. Renteria¹, J. Johnson¹, R. Badal¹, M. Dowzicky³

¹International Health Management Associates, Inc., Schaumburg, IL, USA
²International Health Management Associates Europe Sàrl, Epalinges, Switzerland
³Wyeth Pharmaceuticals, Collegeville, PA, USA

Revised Abstract

Background: ESBL producing organisms continue to be a therapeutic dilemma for physicians as many currently marketed antimicrobials are ineffective against such isolates. Only carbapenems and newer antimicrobials such as tigecycline have proven to be effective against many of these strains. Tigecycline, the first member of the glycylcyclines, was marketed in mid-2005 and has demonstrated clinical success against ESBL producing organisms. Four years of data are now available on the incidence and activity of tigecycline against these strains. **Methods:** All clinical isolates identified as *E. coli*, *K. pneumoniae* and *K. oxytoca* were confirmed as ESBL producers or non-ESBL producers using criteria established by the CLSI. MICs were determined by broth microdilution according to CLSI guidelines using identical panels. **Results:** Results of trends in incidence and antimicrobial susceptibility for tigecycline for ESBLs for each country between 2004 and 2008 are listed below:

Country	2004		2005		2006		2007		2008	
	% ESBL	MIC ₅₀	% ESBL	MIC ₅₀	% ESBL	MIC ₅₀	% ESBL	MIC ₅₀	% ESBL	MIC ₅₀
Australia	0	--	1.46	1	3.1	4	2.8	1	6.1	1
China	46	1	34.9	8	37.9	0.25	--	--	--	--
Hong Kong	--	--	0	0.12	23.4	0.25	18.1	0.5	--	--
India	67.3	1	--	--	0	--	10.5	1	53.8	1
Korea	--	--	36.7	1	13.4	2	18.6	1	18.4	1
Pakistan	69.2	4	23.5	2	34.8	--	--	--	--	--
Philippines	8.3	0.5	--	--	15.7	2	19.8	0.5	--	--
Singapore	19.4	1	66.7	--	27.1	0.5	9.2	2	25.5	0.5
Taiwan	--	--	--	--	20.8	2	21.9	1	--	--

Conclusions: With exception of Taiwan it would appear the rates of ESBLs producing organisms are going down. High rates still exist in India, China and Singapore. Tigecycline in vitro activity varies by country.

Introduction

Tigecycline is has expanded broad-spectrum antimicrobial activity and is the first marketed glycylcycline. Tigecycline inhibits protein synthesis by binding to the 30S ribosomal subunit. Although it is perceived to be bacteriostatic, its antibacterial activity is significant and has shown some bactericidal activity against key targeted pathogens [1,2]. Tigecycline was developed to provide activity against tetracycline and multi-drug-resistant Gram-positive pathogens and has demonstrated significant broad-spectrum activity against aerobic and anaerobic Gram-positive and Gram-negative microorganisms [2-4].

Tigecycline has shown to be a highly effective against multi-resistant strains that are commonly associated with serious nosocomial infections. Similar activity has been observed against *Enterobacteriaceae*, even extended-spectrum b-lactamase (ESBL) producing strains [5].

This study was designed to re-examine the *in vitro* activity of tigecycline against extended-spectrum beta-lactamase (ESBL) producing isolates collected from study centers in Europe over a period of 4 years.

Materials & Methods

- All isolates were derived from blood, respiratory tract, urine (no more than 25% of all isolates), skin, wound, fluids and few other defined sources. Only one isolate per patient was accepted.
- 10,313 Clinical isolates from 75 centers and 10 countries were collected and tested from January 2004 to November 2008.
- Custom broth microdilution panels were supplied by MicroScan (Dade MicroScan, Sacramento, CA, USA) with the following antimicrobial agents and concentrations (expressed in mg/L): amoxicillin/clavulanic acid (0.12-32); piperacillin/tazobactam (0.06-128); levofloxacin (0.008-8); ceftriaxone (0.06-64); cefepime (0.5-32); ampicillin (0.5-32); amikacin (0.5-64); minocycline (0.5-16); ceftazidime (8-32); tigecycline (0.008-16); and imipenem (0.06-16).
- MIC interpretive criteria for Tigecycline followed published guidelines established by the CLSI [7] or by the FDA where applicable [8].
- Isolates were identified to genus and species by the local laboratory. Each site tested the isolates using broth microdilution.
- Quality control of broth microdilution panels followed manufacturer's and CLSI guidelines using the following ATCC strains: *Escherichia coli* ATCC 25922; *K. pneumoniae* ATCC 700603
- ESBL Determination: *Escherichia coli*, *Klebsiella pneumoniae*, and *Klebsiella oxytoca* were screened for ESBL activity when MIC results for ceftriaxone were >1 mg/L using broth microdilution panels. ESBL activity was confirmed using the CLSI phenotypic confirmatory disk test (Oxoid, Ogdensburg, NY, USA) on Mueller-Hinton agar (Remel Inc., Lenexa, KS, USA) according to CLSI (2009) guidelines [7]. ESBL presence was confirmed by testing the following antibiotic disks: cefotaxime (30-mcg), cefotaxime/clavulanic acid (30/10-mcg), ceftazidime (30-mcg), and ceftazidime/clavulanic acid (30/10-mcg). Antimicrobial disks were manufactured by Oxoid, Inc. (Ogdensburg, NY, USA). Mueller-Hinton agar used in testing was manufactured by Remel, Inc. (Lenexa, KS, USA). An organism was interpreted as containing an ESBL if there was an increase of >5 mm in the inhibition zone of the combination disk when compared to that of the cephalosporin alone.

References

- Sum, P.E. and P. Petersen, *Synthesis and structure-activity relationship of novel glycylcycline derivatives leading to the discovery of GAR-936*. Bioorg Med Chem Lett, 1999. 9(10): p. 1459-62.
- Abbanat, D., M. Macielag, and K. Bush, *Novel antibacterial agents for the treatment of serious Gram-positive infections*. Expert Opin Investig Drugs, 2003. 12(3): p. 379-99.
- Betriu, C., et al., *In vitro activities of tigecycline (GAR-936) against recently isolated clinical bacteria in Spain*. Antimicrob Agents Chemother, 2002. 46(3): p. 892-5.
- Galles, A.C. and R.N. Jones, *Antimicrobial activity and spectrum of the new glycylcycline, GAR-936 tested against 1,203 recent clinical bacterial isolates*. Diagn Microbiol Infect Dis, 2000. 36(1): p. 19-36.
- Petersen, P.J., et al., *In vitro and in vivo antibacterial activities of a novel glycylcycline, the 9-t-butylglyclamide derivative of minocycline (GAR-936)*. Antimicrob Agents Chemother, 1999. 43(4): p. 738-44.
- Petersen, P.J., et al., *In vitro and in vivo activities of tigecycline (GAR-936), daptomycin, and comparative antimicrobial agents against glycopeptide-intermediate Staphylococcus aureus and other resistant gram-positive pathogens*. Antimicrob Agents Chemother, 2002. 46(8): p. 2595-601.
- Clinical and Laboratory Standards Institute (CLSI). 2009. Performance Standards for Antimicrobial Susceptibility Testing. Nineteenth Informational Supplement, Document M100-S19. CLSI, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.
- Tygacil®. Product Insert. 2005 (Federal Drug Administration approved). Wyeth Pharmaceuticals, Inc., Philadelphia, PA, USA.
- Hirakata, Y., Matsuda, J., et al. (2005). Regional variation in the prevalence of extended-spectrum beta-lactamase-producing clinical isolates in the Asia-Pacific region (SENTRY 1998-2002). Diagn Microbiol Infect Dis, 52(4):323-9
- Badal, R., Bouchillon, S., Hoban, D., et al. 2008. In vitro Activity of Ertapenem and Comparators Against Gram-negative Pathogens in 2007 in Asia/Pacific – The SMART Study, 48th Annual ICAAC/IDSA 48th Annual Meeting, Washington, DC, October 25-28, 2008.

Acknowledgements

This study was sponsored in part by a grant from Wyeth Pharmaceuticals. We gratefully acknowledge the contributions of the investigators, laboratory personnel and all members of the Tigecycline Evaluation Study Trials program group.

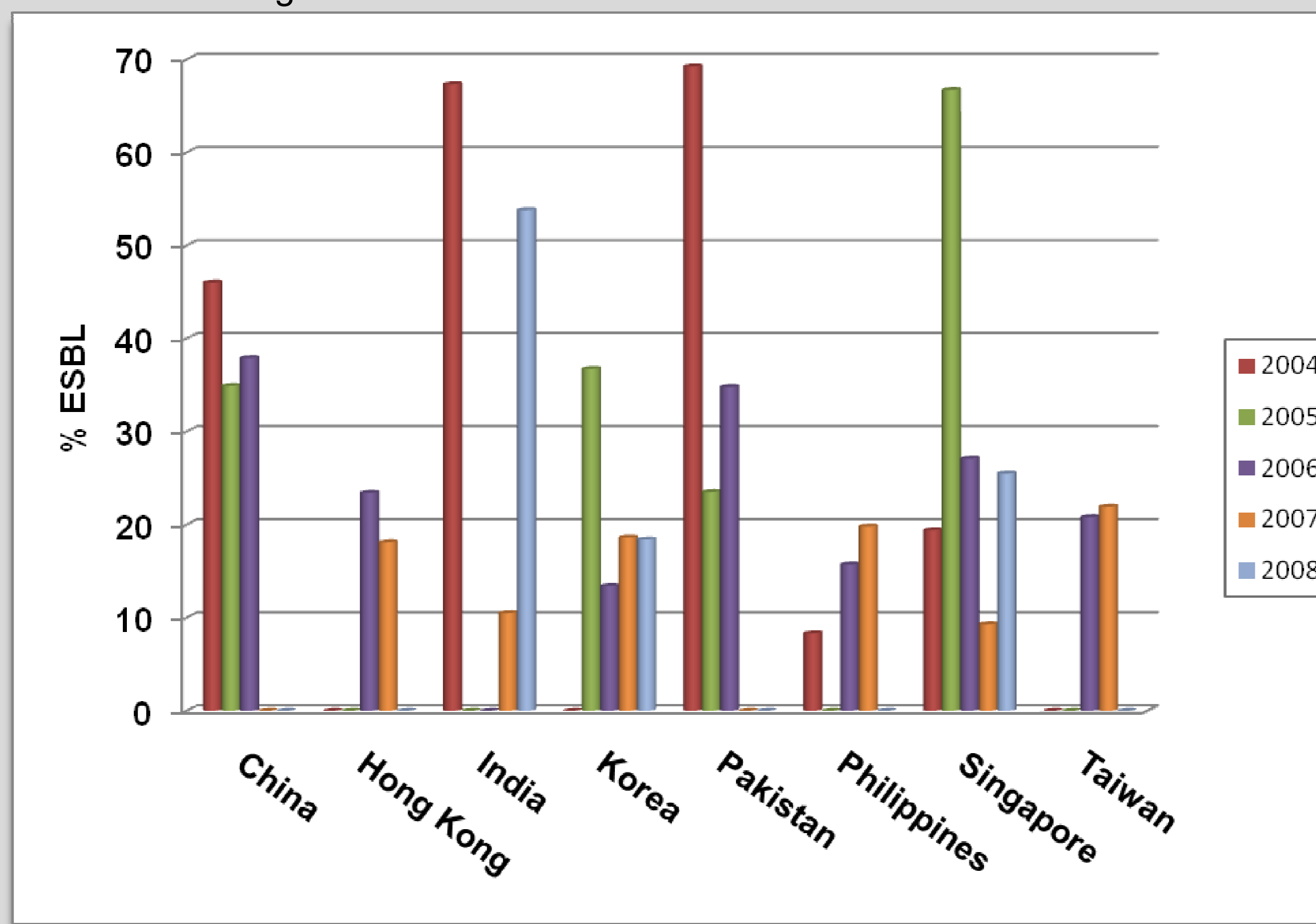
Results

Table 1. Frequency of ESBL producing *Enterobacteriaceae* from 10 Asia / Pacific Rim Countries.

	2004	2005	2006	2007	2008	Total
	(n=88)	(n=44)	(n=146)	(n=153)	(n=49)	480
Australia	0	1	6	5	3	15
China	23	15	22	0	0	60
Hong Kong	0	0	11	15	0	26
India	33	0	0	24	14	71
Indonesia	0	0	0	2	0	2
Korea	18	13	46	19	96	96
Pakistan	9	8	31	0	0	48
Philippines	4	0	8	20	0	32
Singapore	19	2	13	9	13	56
Taiwan	0	0	42	32	0	74

* Only *E. coli*, *K. oxytoca* and *K. pneumoniae* were screened for ESBLs.

Figure 1. Percent Frequency* of ESBL producing *Enterobacteriaceae* from Countries in the Asia / Pacific Rim: Longitudinal data from 2004 – 2008.



*Data from Australia and Indonesia whereby % ESBL frequencies were consistently below 10 % were excluded.

Table 2. *In vitro* Activity of Tigecycline and Comparative Agents against all ESBL (n=480) Producing Strains by Year.

Drug	2004		2005		2006		2007		2008	
	MIC ₅₀	%Sus	MIC ₅₀	%Sus	MIC ₅₀	%Sus	MIC ₅₀	%Sus	MIC ₅₀	%Sus
Tigecycline	1	97.7	2	90.9	2	98.6	1	99.3	1	98.0
Amikacin	>64	86.4	>64	86.4	>64	83.6	>64	77.1	>64	85.7
AmoxClav	>32	27.3	>32	29.5	>32	23.3	>32	31.4	>32	26.5
Cefepime	>32	55.7	>32	56.8	>32	38.4	>32	35.9	>32	32.7
Ceftazidime	>32	51.1	>32	47.7	>32	45.2	>32	52.3	>32	38.8
Ceftriaxone	>64	14.8	>64	18.2	>64	11.0	>64	9.2	>64	4.1
Imipenem*	0.5	100.0	0.5	100.0	0.25	100.0	NT	NT	NT	NT
Meropenem*	NT	NT	NT	NT	0.5	97.5	0.5	98.0	0.12	98.0
Levofloxacin	>8	42.0	>8	38.6	>8	32.2	>8	18.3	>8	16.3
Minocycline	>16	62.5	>16	54.5	>16	65.1	4	64.1	>16	51.0
PipTazo	32	87.5	64	75.0	>128	71.2	4	67.3	>128	61.2

*NT, not tested. Imipenem was tested up to 2006 and then replaced by meropenem

Table 3. *In vitro* Activity of Tigecycline and Comparative Agents** against Selected Enterobacteriaceae and ESBL Producing Strains by Year.

Organism	Drug	2004		2005		2006		2007		2008	
		MIC ₅₀	%Sus	MIC ₅₀	%Sus	MIC ₅₀	%Sus	MIC ₅₀	%Sus	MIC ₅₀	%Sus
<i>E. coli</i> n=198, 100, 416, 390, 128 Total = 1400	Amikacin	16-32	80	8-32	100	16-32	95.7	16-32	78.6	16-32	76.9
	Cefepime	8-32	100	<0.5-32	100	32-32	100	<0.5-32	100	16-32	96.2
	Ceftazidime	<0.5-64	100	<0.5-64	100	<0.5-64	86.1	<0.5-64	86.1	<0.5-64	92.3
	Imipenem	0.25-0.5	100	0.25-0.5	100	0.125-0.25	100	0.125-0.25	100	0	-
	Levofloxacin	>8-8	100	>8-8	87.1	>8-8	85.1	>8-8	80.2	>8-8	86.2
	Meropenem	>8	100	>8	100	>8	100	>8	100	>8	100
	Moxifloxacin	8-16	100	4-16	87.1	4-16	86.4	8-16	81.4	8-16	84.6
	PipTazo	32-32	100	32-32	100	8-128	100	16-128	80.9	8-128	98
	Tigecycline	0.25-0.5	100	0.25-0.5	100	0.25-0.5	100	0.25-0.5	100	0.25-0.5	100
	<i>E. coli</i> ESBL n=39, 16, 14, 89, 31 Total = 244	Amikacin	8-64	100	2-16	100	4-64	100	8-64	100	8-32
AmoxClav		>32-32	43.8	>32-32	25	32-32	83.3	32-32	100	16-32	57.1
Cefepime		>32-32	100	>32-32	71	>32-32	100	>32-32	100	>32-32	90
Ceftazidime		>64-64	100	>64-64	0	>64-64	75	>64-64	25	>64-64	7.1
Imipenem		0.25-0.5	100	0.25-0.5	100	0.125-0.25	100	0	0	0	0
Levofloxacin		>8-8	100	8-8	25	>8-8	80	>8-8	80	>8-8	90
Meropenem		0.5-0.5	100	0.5-0.5	100	0.25-0.5	100	0.25-0.5	100	0.25-0.5	100
Moxifloxacin		8-16	100	16-16	75	4-16	87.5	16-16	100	16-16	100
PipTazo		32-32	100	16-128	100	8-128	100	16-128	100	16-128	87.3
Tigecycline		0.25-0.5	100	0.25-0.5	100	0.25-0.5	100	0.25-0.5	100	0.25-0.5	100
<i>K. pneumoniae</i> n=32, 25, 47, 88, 5 Total = 168	Amikacin	8-8	100	4-64	100	<0.5-64	100	16-64	100	4-8	100
	AmoxClav	4-32	100	32-32	100	>32-32	100	>32-32	100	32-32	100
	Cefepime	<0.5-0.5	100	<0.5-8	100	32-32	100	32-32	100	8-8	100
	Ceftazidime	1-32	100	4-64	100	>64-64	100	>64-64	100	>64-64	100
	Imipenem	0.5-0.5	100	0.25-0.5	100	0.25-0.25	100	>0.5-0.5	100	0.5-0.5	100
	Levofloxacin	0.5-0.8	100	0.5-8	100	>8-8	100	8-8	100	2-2	100
	Meropenem	<0.06-0.06	100	<0.06-0.06	100	4-4	100	4-4	100	<0.06-0.06	100
	Moxifloxacin	2-2	100	4-4	100	>64-16	100	8-16	100	4-4	100
	PipTazo	2-128	100	1-128	100	>128-128	100	32-128	100	64-64	100
	Tigecycline	0.25-0.5	100	0.25-0.5	100	0.25-0.5	100	0.25-0.5	100	0.25-0.5	100
<i>K. pneumoniae</i> ESBL n=1, 4, 4, 3, 0 Total = 9	Amikacin	4-4	100	>64-64	0	4-8	100	1-2	100	1-2	100
	AmoxClav	>32-32	0	>32-32	0	>32-32	0	8-32	66.7	-	-
	Cefepime	<0.5-0.5	100	8-8	100	32-32	100	8-8	100	8-8	100
	Ceftazidime	32-32	0	>64-64	0	>64-64	100	32-64	33.3	-	-
	Imipenem	0.5-0.5	100	0.5-0.5	100	0.25-0.25	100	0	0	0	0
	Levofloxacin	>8-8	0	4-8	0	>8-8	100	1-8	66.7	-	-
	Meropenem	-	-	-	-	-	-	<0.06-0.06	100	-	-
	Moxifloxacin	2-2	100	4-4	100	16-16	100	2-8	66.7	-	-