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2011 AGAR/AstraZeneca Ceftaroline Susceptibility Study

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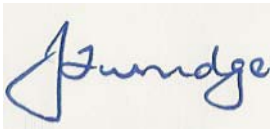

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Date: 21 August 2012

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation or special term	Explanation
ACCESS	Australian Collaborating Centre for *Enterococcus* and *Staphylococcus Species*
AGAR	Australian Group on Antimicrobial Resistance
ATCC	American Type Culture Collection
BURST	Based Upon Related Sequence Types
CA-MRSA	Community Associated Methicillin Resistant *Staphylococcus aureus*
CC	Clonal Complex
CHEF	Contour-clamped homogeneous electric field
CLSI	Clinical Laboratory Standards Institute
DNA	Deoxyribonucleic acid
HA-MRSA	Healthcare Associated Methicillin Resistant *Staphylococcus aureus*
MIC	Minimum inhibitory concentration
MLST	Multilocus sequence typing
MRSA	Methicillin resistant *Staphylococcus aureus*
MSSA	Methicillin sensitive *Staphylococcus aureus*
nuc	Gene encoding thermostable extracellular nuclease
PCR	Polymerase chain reaction
SCC*mec*	Staphylococcal Chromosome Cassette *mec*
ST	Sequence Type
WA	Western Australia

PRIMARY TEST FACILITY

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**The principal investigator is responsible for the collation of the authors' signatures on the final report. The principal investigator will be held primarily accountable for ensuring that this report represents a true and accurate record of the available data. This does not absolve other authors of responsibility, however.*

ADDITIONAL TEST SITE (IF NECESSARY)

Not Applicable

ADDITIONAL RESPONSIBLE PERSON(S)

The following personnel were also significantly involved in the conduct and evaluation of the study, data analysis, and preparation of the report:

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Responsible for: Data analysis, and preparation of the report

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Women's and Children's Hospital. Adelaide, South Australia

Responsible for: Evaluation of the study, data analysis, and preparation of the report

ARCHIVING

All original data (raw data and final report) and isolates have been retained at the primary test facility.

EXPERIMENTAL DATES

Experimental work started: 01 April 2012

Experimental work completed: 30 June 2012

SUMMARY

Background: Ceftaroline, the active metabolite of the prodrug ceftaroline fosamil, is a new cephalosporin with *in vitro* bactericidal activity against a broad range of pathogens commonly implicated in complicated skin and soft tissue infections and community-acquired pneumonia. Due to its high affinity for penicillin binding protein 2a, ceftaroline is active against methicillin resistant *Staphylococcus aureus* (MRSA). The aims of this study were to determine the activity of ceftaroline against a collection of methicillin susceptible *S. aureus* (MSSA) and methicillin resistant *S. aureus* (MRSA) isolated from hospital onset infections, and to compare the ceftaroline susceptibility of these isolates to a panel of commonly used antibiotics

Methods: Study isolates included 845 MSSA and 703 MRSA collected in the 2011 Australian Group for Antimicrobial Resistance (AGAR) Hospital-onset *S. aureus* Susceptibility Surveillance Programme. The 703 MRSA were characterised by the Australian Collaborating Centre for *Enterococcus* and *Staphylococcus Species* (***ACCESS***) Typing and Research. Ceftaroline minimum inhibitory concentrations (MICs) were determined using Etest® strips. Susceptibility testing of the comparative antibiotics was performed using the Vitek2® AST-P612 Card.

Results: All MSSA strains were inhibited at ceftaroline MIC values of ≤ 1 mg/L (USA-FDA breakpoint). Their MIC values ranged from 0.094 to 0.75 mg/L (MIC_{50} 0.25 mg/L, MIC_{90} 0.38 mg/L). The ceftaroline MICs for the MRSA strains ranged from 0.125 to 1.5 mg/L (MIC_{50} 0.75mg/L, MIC_{90} 1.0 mg/L). Ceftaroline MIC of 1.5 was recorded for 13 HA-MRSA including nine ST239-MRSA-III [3A] (Aus2/3 EMRSA), two ST22-MRSA-IV [2B] (UK EMRSA-15) and two ST36-MRSA-II [2A] isolates. All CA-MRSA were ceftaroline susceptible. Greater than 99% of *S. aureus* isolates were susceptible to vancomycin, teicoplanin, linezolid, daptomycin, rifampicin and ceftaroline.

Conclusion: Ceftaroline activity was preserved across MSSA and CA-MRSA and most HA-MRSA. MICs ranged from 0.094 to 1.5 mg/L (MIC_{50} 0.38 mg/L, MIC_{90} 0.75 mg/L). Generally, HA-MRSA were found to have slightly higher MICs than CA-MRSA. 13 HA-MRSA isolates exhibited an MIC value within one dilution above the susceptible breakpoint, and would be considered non-susceptible using Clinical and Laboratory Standards Institute definitions.

1. INTRODUCTION

The 2011 AGAR/AstraZeneca Ceftaroline Susceptibility Study was performed by the Australian Group on Antimicrobial Resistance (AGAR) along side the 2011 AGAR Hospital-Onset *Staphylococcus aureus* Susceptibility Surveillance Programme

The study objectives were:

- Determine the activity of ceftaroline against 845 methicillin susceptible *S aureus* (MSSA) and 703 methicillin resistant *S. aureus* (MRSA) isolated from hospital-onset infections.

- Compare the ceftaroline susceptibility of these isolates to a panel of commonly used antimicrobials.

AGAR was established in 1986, initially to monitor resistance in *S. aureus* throughout Australia. This surveillance role has become more important with the multiple waves of methicillin-resistant *S aureus* (MRSA) that have occurred in Australia since the 1960s. AGAR performs alternate hospital and community-onset surveys. Presently, 29 laboratories from all states and mainland territories of Australia contribute to AGAR surveys. Hospital-onset surveys have been conducted biennially since 2005. The findings of the 2011 AGAR hospital-onset survey are available on the AGAR website (http://www.agargroup.org/surveys).

2. MATERIALS AND METHODS

2.1 Bacterial strains:

From the 1st July to the 30th November 2011, 29 laboratories collected up to 100 consecutive *S. aureus* isolates from hospital inpatients (hospital stay >48 hours at the time of specimen collection). Only one isolate per patient was tested. Each *S. aureus* was judged to have come from a potentially infected site. Specimens received for the purpose of gathering surveillance data were excluded.

S. aureus were identified by morphology and positive results of at least two of the following tests: slide coagulase test, tube coagulase test, appropriate growth on chromogenic agar and demonstration of deoxyribonuclease production. Additional tests such as fermentation of mannitol, growth on mannitol-salt agar or polymerase chain reaction (PCR) for the presence of the *nuc* gene may have been performed for confirmation.

2.2 *In vitro* susceptibility test methods:

Participating laboratories performed antimicrobial susceptibility tests using the Vitek2® AST-P612 Card.

Antibiotic	Code	MIC Range
Benzylpenicillin	P	0.03 – 0.5
Oxacillin	OX1	0.25 – 4
Cefoxitin screen	OXSF	+/-
Vancomycin	VA1	0.5 - 32
Rifampicin	RA	0.5 - 32
Fusidic acid	FA	0.5 - 32
Gentamicin	GM	0.5 - 16
Erythromycin	E	0.25 - 8
Clindamycin	CM	0.25 - 8
Inducible clindamycin resistance	ICR	+/-
Tetracycline	TE	1 - 16
Trimethoprim/Sulfamethoxazole	SXT	10 - 320
Ciprofloxacin	CIP	0.5 - 8
Teicoplanin	TEC	0.5 – 32
Linezolid	LNZ	0.5 – 8
Nitrofurantoin	FT	16 – 512
Mupirocin	MUP	2 – 8
Daptomycin	DAP	0.12 – 8

CLSI breakpoints [1] were utilised for all antimicrobials excluding mupirocin and fusidic acid [2]. *Staphylococcus aureus* ATCC 29213 was used as the control strain.

All *S. aureus* isolates were referred to the Australian Collaborating Centre for *Enterococcus* and *Staphylococcus Species* (***ACCESS***) Typing and Research.

Of the 2,357 *S. aureus* referred to ***ACCESS*** Typing and Research, Ceftaroline MICs were determined on all MRSA (703 isolates) and 845 MSSA. For the MSSA, 703 isolates were selected based on the number of MRSA from each laboratory. An additional 142 MSSA were randomly selected. MICs were determined by Etest (bioMerieux) using Mueller Hinton Agar as recommended by the manufacturer. *Staphylococcus aureus* ATCC 29213 was used as the control strain.

2.3 Molecular characterisation of MRSA:

Electrophoresis of chromosomal deoxyribonucleic acid (DNA) was performed on all MRSA as previously described [3] using a contour-clamped homogeneous electric field (CHEF) DR III system (Bio-Rad Laboratories Pty Ltd). Chromosomal patterns were examined visually, scanned with a Quantity One device (Bio-Rad Laboratories Pty Ltd), and digitally analyzed using FPQuest (Bio-Rad Laboratories Pty Ltd). *Staphylococcus aureus* NCTC 8325 was used as the reference strain.

Chromosomal DNA for multilocus sequence typing (MLST) on selected isolates was prepared using a DNeasy tissue kit (Qiagen Pty Ltd). MLST was performed as previously described [4]. Sequences were submitted to http://www.mlst.net/ where an allelic profile was generated and a sequence type (ST) assigned. The clonal complex (CC) was determined using the online Based Upon Related Sequence Types (eBURST)

V3 algorithm at the same website. Clones that diverged at no more than one of the seven MLST loci were considered to belong to the same CC. Double locus variants (dlvs) were included if the linking single locus variant (slv) was present in the MLST database.

The strategy used for SCC*mec* typing on selected isolates was as previously described [5].

3. RESULTS

3.1 Ceftaroline MIC

S. aureus (n = 1,548)	Range 0.094 to 1.5 mg/L (MIC_{50} 0.38 mg/L, MIC_{90} 0.75 mg/L)
MSSA (n = 845)	Range 0.094 to 0.75 mg/L (MIC_{50} 0.25 mg/L, MIC_{90} 0.38 mg/L)
MRSA (n = 703)	Range 0.125 to 1.5 mg/L (MIC_{50} 0.75 mg/L, MIC_{90} 1.0 mg/L)

Table 1: *S. aureus* Ceftaroline MICs (mg/L)

Ceftaroline MIC (mg/L)	MSSA	MRSA	All *S. aureus*
0.094	9		9
0.125	56	1	57
0.19	212		212
0.25	432	13	445
0.38	133	80	213
0.5	2	254	256
0.75	1	216	217
1.0		126	126
1.5		13	13
Total	**845**	**703**	**1,548**

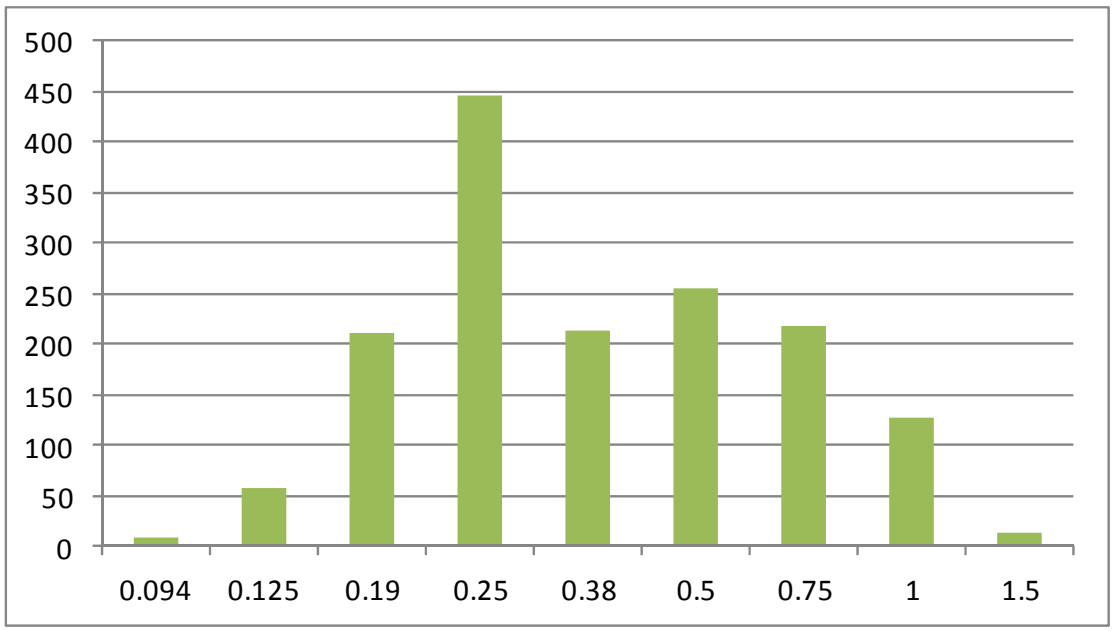

Figure 1: *S. aureus* Ceftaroline MICs (mg/L)

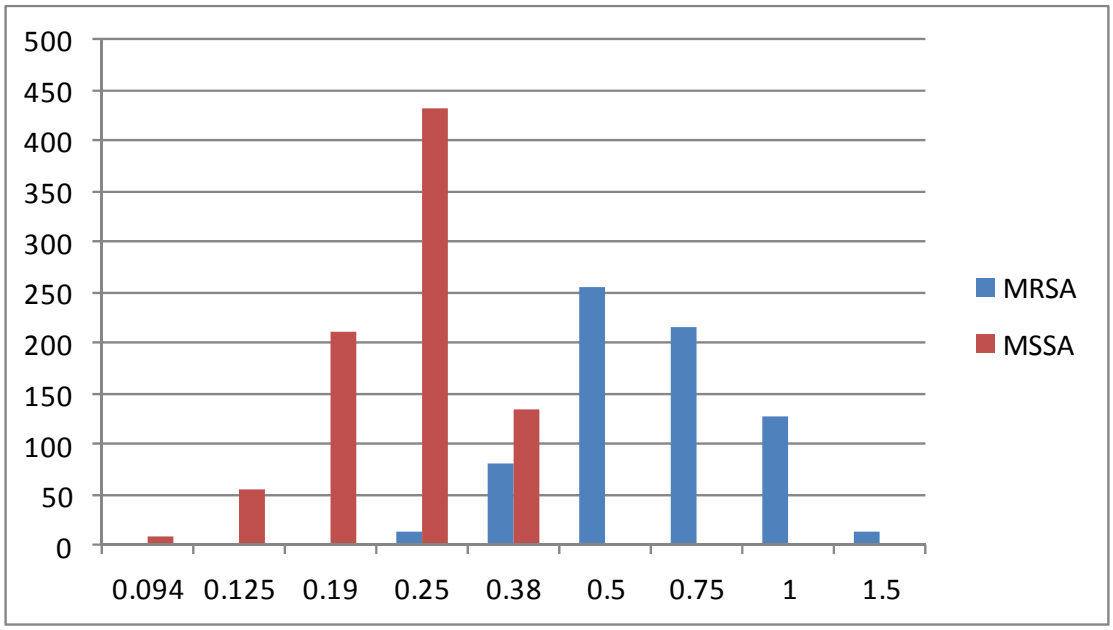

Figure 2: MRSA and MSSA Ceftaroline MICs (mg/L)

3.2 CA-MRSA vs HA-MRSA Ceftaroline MIC

MRSA (n = 703) Range 0.125 to 1.5 mg/L (MIC_{50} 0.75 mg/L, MIC_{90} 1.0 mg/L)
CA-MRSA (n = 275) Range 0.125 to 1 mg/L (MIC_{50} 0.5 mg/L, MIC_{90} 0.5 mg/L)
HA-MRSA (n = 428) Range 0.25 to 1.5 mg/L (MIC_{50} 0.75 mg/L, MIC_{90} 1.0 mg/L)

Table 2: CA-MRSA vs HA-MRSA Ceftaroline MICs (mg/L)

Ceftaroline MIC (mg/L)	CA-MRSA	HA-MRSA	Total MRSA
0.125	1		1
0.25	9	4	13
0.38	71	9	80
0.5	171	83	254
0.75	22	194	216
1.0	1	125	126
1.5		13	13
Total	**275**	**428**	**703**

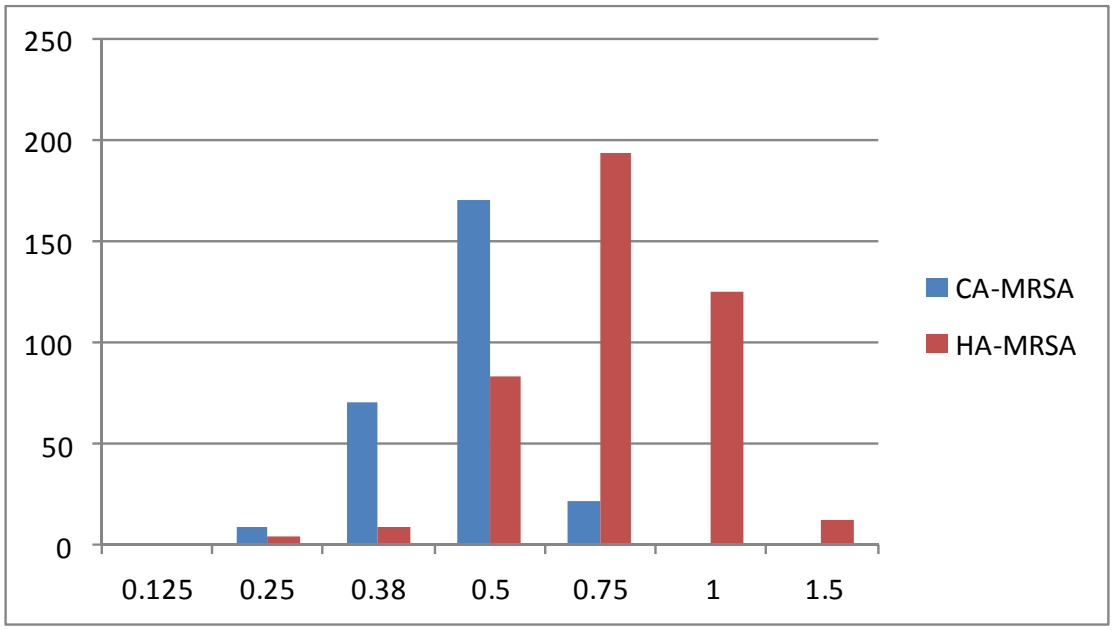

Figure 3: CA-MRSA vs HA-MRSA Ceftaroline MICs (mg/L)

3.3 CA-MRSA (Clones) Ceftaroline MIC

Table 3: CA-MRSA Clones Ceftaroline MIC (mg/L)

Clone	CC	Alternative Name	n	Ceftaroline MIC					
				0.125	0.25	0.38	0.5	0.75	1.0
ST93-IV [2B]	S	Qld MRSA	51		1	6	38	6	
ST1-IV [2B]	1	WA MRSA-1	63		1	9	47	6	
ST772-V [5C2]		Bengal Bay	3			3			
ST188-IV [2B]		WA MRSA-38	1			1			
ST573-V [5C2]		WA MRSA-10	1				1		
ST1-V [5C2]			2		1	1			
ST5-IV [2B]	5	WA MRSA-3	34		2	15	16	1	
ST73-IV [2B]		WA MRSA-65	10			4	5	1	
ST835-IV [2B]		WA MRSA-48	3			2	1		
ST5-V [5C2]		WA MRSA-90	2			1	1		
ST5-V [5C2]		WA MRSA-14	1			1			
ST575-IV [2B]		WA MRSA-25	1				1		
ST5-V [5C2]		WA MRSA-35	1			1			
ST5-V [5C2]		WA MRSA-108	1					1	
ST5-V [5C2]		WA MRSA-109	1		1				

Clone	CC	Alternative Name	n	Ceftaroline MIC					
				0.125	0.25	0.38	0.5	0.75	1.0
ST1756-V [5C2]			1				1		
ST7-V [5C2]	7		1			1			
ST8-IV [2B]	8	USA300	8			1	6	1	
ST30-IV [2B]	30	SWP MRSA	21			1	19	1	
ST45-V [5C2]	45	WA MRSA-84	25		1	15	9		
ST45-V [5C2]		WA MRSA-4	3	1	1	1			
ST45-IV [2B]		WA MRSA-75	3			1	2		
ST45-V [5C2]			2					1	1
ST45-IV [2B]		WA MRSA-23	1		1				
ST1970-V [5C2]		WA MRSA-106	1				1		
ST59-IV [2B]	59	WA MRSA-15	2				2		
ST59-IV [2B]		WA MRSA-55	1				1		
ST72-IV [2B]	72	WA MRSA-44	2					2	
ST75-IV [2B]	75	WA MRSA-8	2			2			
ST1304-IV [2B]		WA MRSA-72	1			1			
ST78-IV [2B]	88	WA MRSA-2	25			3	20	2	
ST953-IV [2B]	97	WA MRSA-54	1			1			
TOTAL			**275**	**1**	**9**	**71**	**171**	**22**	**1**

S = Singleton

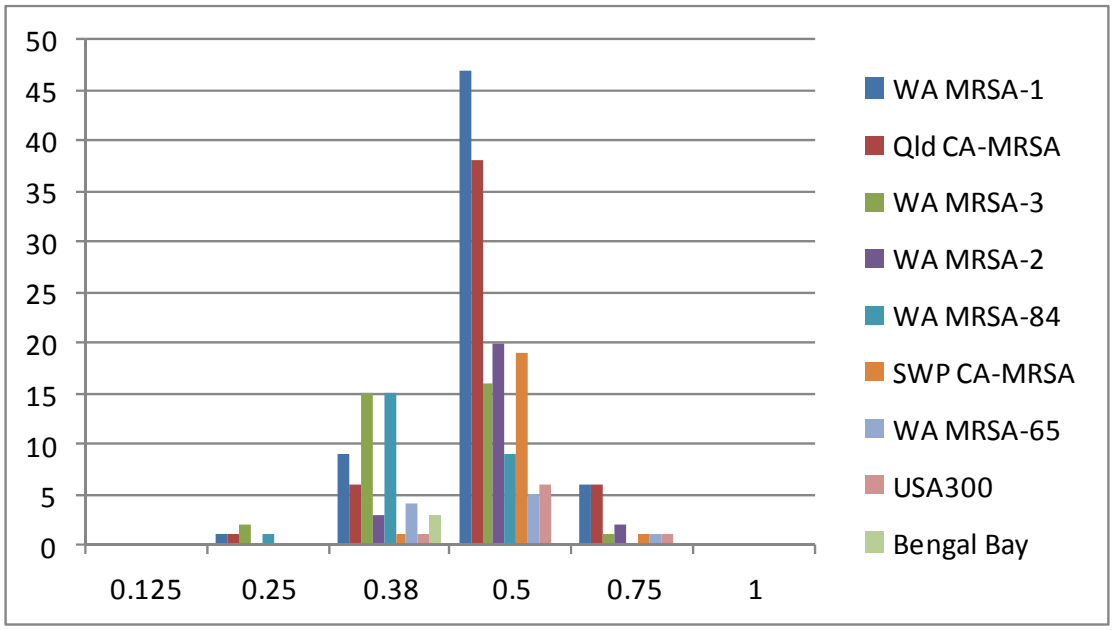

Figure 4: Major CA-MRSA Clones Ceftaroline MICs (mg/L)

3.4 HA-MRSA (Clones) Ceftaroline MIC

Table 4: HA-MRSA Clones Ceftaroline MIC (mg/L)

Clone	CC	Alternative Name	n	Ceftaroline MIC					
				0.25	0.38	0.5	0.75	1.0	1.5
ST5-II [2A]	5	New York Japan	3			1	1	1	
ST239-III [3A]	8	Aus2/3 EMRSA	211	2	5	27	91	77	9
ST22-IV [2B]	22	UK EMRSA-15	212	2	4	55	102	47	2
ST36-II [2A]	30	UK EMRSA-16	2						2
TOTAL			**428**	**4**	**9**	**83**	**194**	**125**	**13**

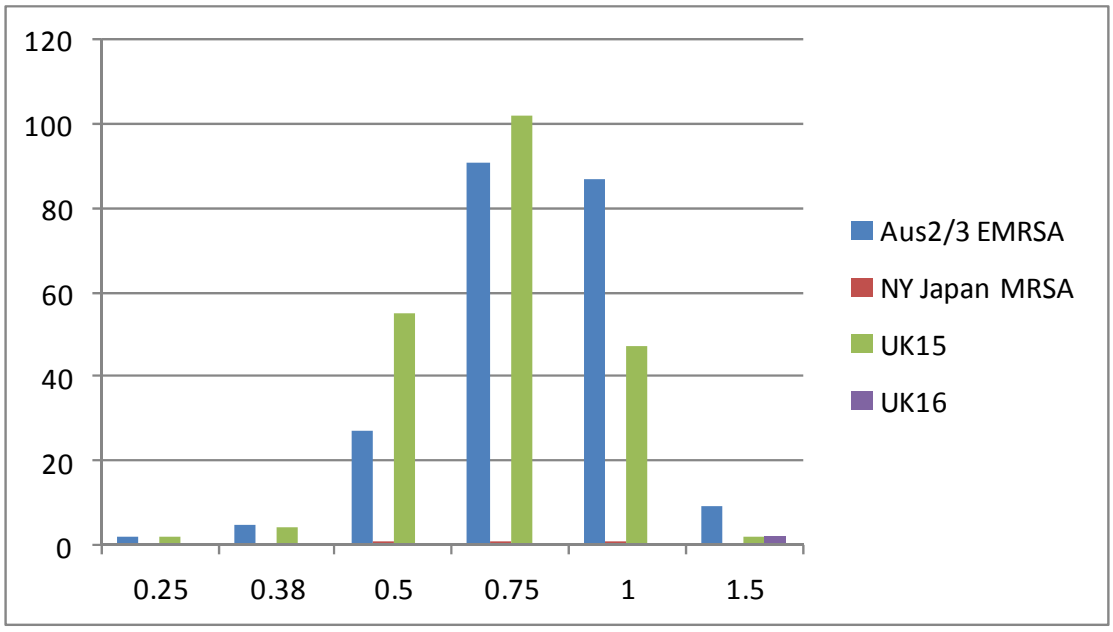

Figure 5: HA-MRSA Clones Ceftaroline MICs (mg/L)

3.5 Antibiogram

3.5.1. *S. aureus* Antibiogram

Table 5: *S. aureus* Antibiogram

Antibiotic	Susceptible	
	Number	%
Benzylpenicillin	118	7.6
Vancomycin	1,548	100
Rifampicin	1,534	99.1
Fusidic acid	1,491	96.3
Gentamicin	1,320	85.3
Erythromycin	1,000	64.6
Clindamycin	1,319	85.2
Tetracycline	1,288	83.2
Trimethoprim/Sulphamethoxazole	1,310	84.6
Ciprofloxacin	1,045	67.5
Teicoplanin	1,548	100
Linezolid	1,548	100
Nitrofurantoin	1,515	97.9
Mupirocin (High Level Resistance)	1,522	98.3
Daptomycin	1,546	99.9
Ceftaroline	1,535	99.2

Inducible clindamycin resistance: 236 (15.2%) isolates

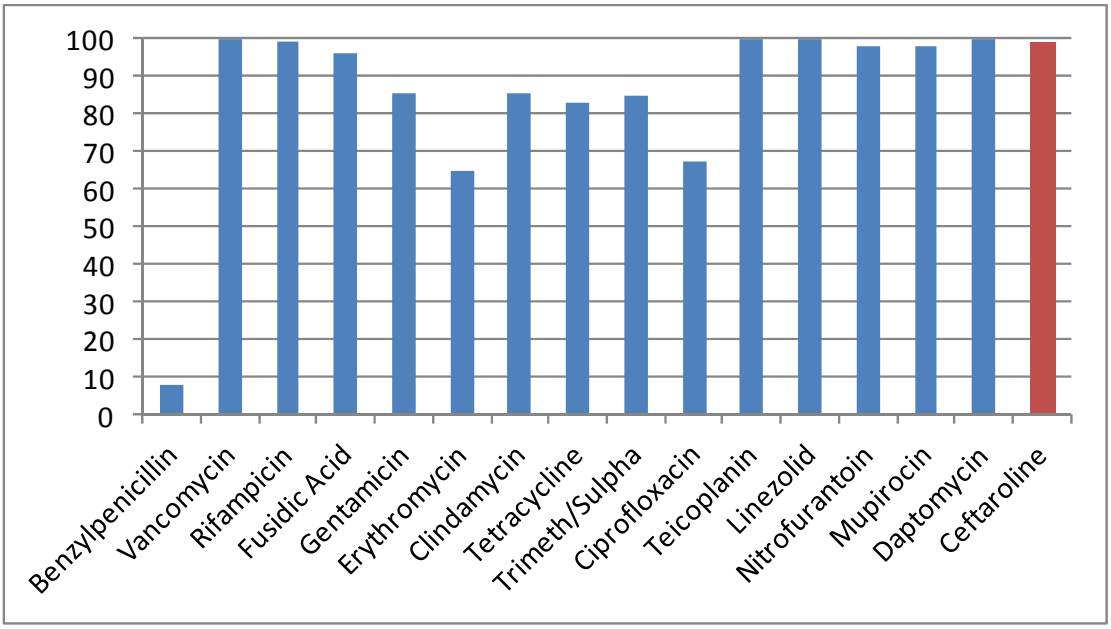

Figure 6: *S. aureus* Antibiogram

3.5.2. MSSA Antibiogram

Table 6: MSSA Antibiogram

Antibiotic	Susceptible	
	Number	%
Benzylpenicillin	117	13.8
Vancomycin	845	100
Rifampicin	845	100
Fusidic acid	813	96.2
Gentamicin	832	98.5
Erythromycin	745	88.2
Clindamycin	821	97.2
Tetracycline	819	96.9
Trimethoprim/Sulphamethoxazole	823	97.4
Ciprofloxacin	810	95.9
Teicoplanin	845	100
Linezolid	845	100
Nitrofurantoin	827	97.9
Mupirocin (High Level Resistance)	828	98.0
Daptomycin	845	100
Ceftaroline	845	100

Inducible clindamycin resistance: 48 (5.7%) isolates

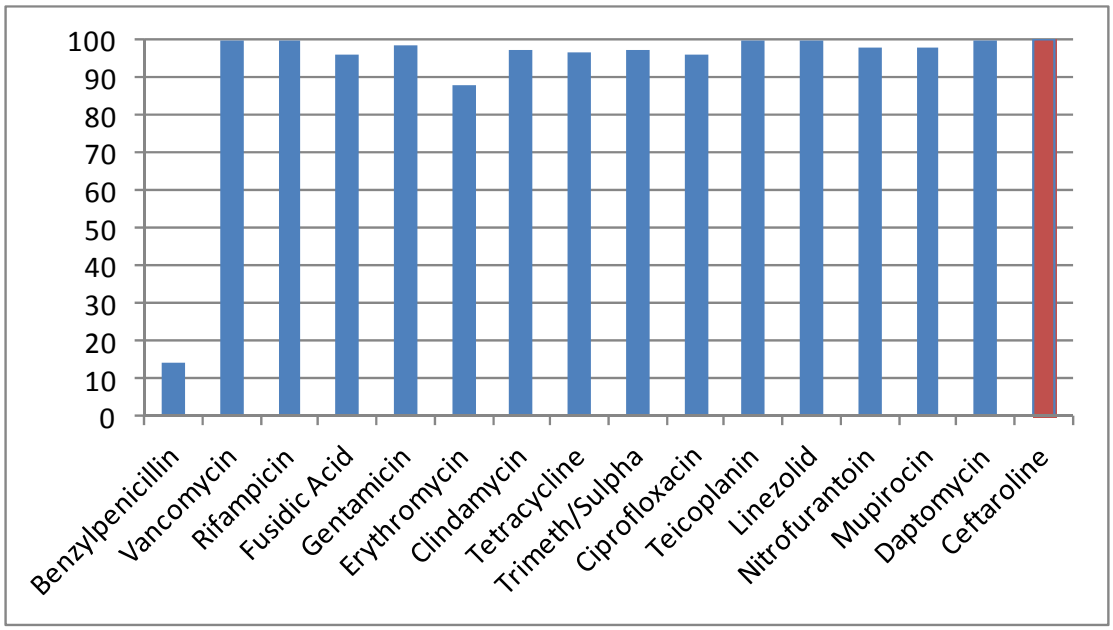

Figure 7: MSSA Antibiogram

3.5.3. MRSA Antibiogram

Table 7: MRSA Antibiogram

Antibiotic	Susceptible	
	Number	%
Benzylpenicillin	1	0.1
Vancomycin	703	100
Rifampicin	689	98
Fusidic acid	678	96.4
Gentamicin	488	69.4
Erythromycin	255	36.3
Clindamycin	498	70.8
Tetracycline	469	66.7
Trimethoprim/Sulphamethoxazole	487	69.3
Ciprofloxacin	235	33.4
Teicoplanin	703	100
Linezolid	703	100
Nitrofurantoin	688	97.9
Mupirocin (High Level Resistance)	694	98.7
Daptomycin	701	99.7
Ceftaroline	690	98.2

Inducible clindamycin resistance: 203 (28.9%) isolates

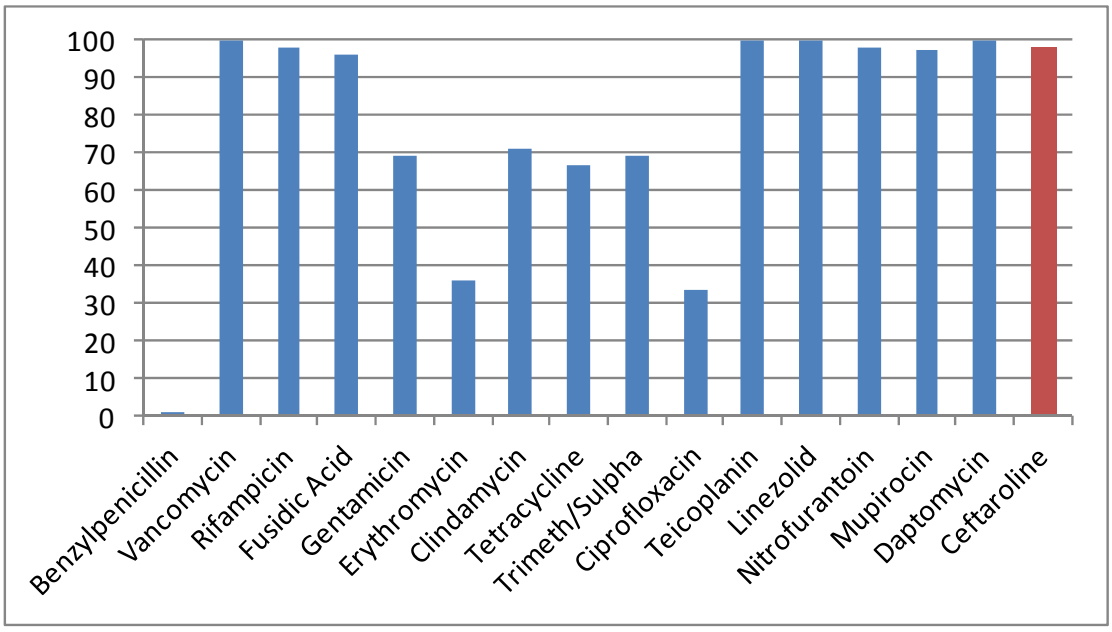

Figure 8: MRSA Antibiogram

3.5.4. CA-MRSA Antibiogram

Table 8: CA-MRSA Antibiogram

Antibiotic	Susceptible	
	Number	%
Benzylpenicillin	1	0.4
Vancomycin	275	100
Rifampicin	274	99.6
Fusidic acid	256	93.1
Gentamicin	264	96.0
Erythromycin	175	63.6
Clindamycin	261	94.9
Tetracycline	260	94.5
Trimethoprim/Sulphamethoxazole	264	96
Ciprofloxacin	224	81.5
Teicoplanin	275	100
Linezolid	275	100
Nitrofurantoin	267	97.1
Mupirocin (High Level Resistance)	267	97.1
Daptomycin	275	100
Ceftaroline	275	100

Inducible clindamycin resistance: 53 (19.3%) isolates

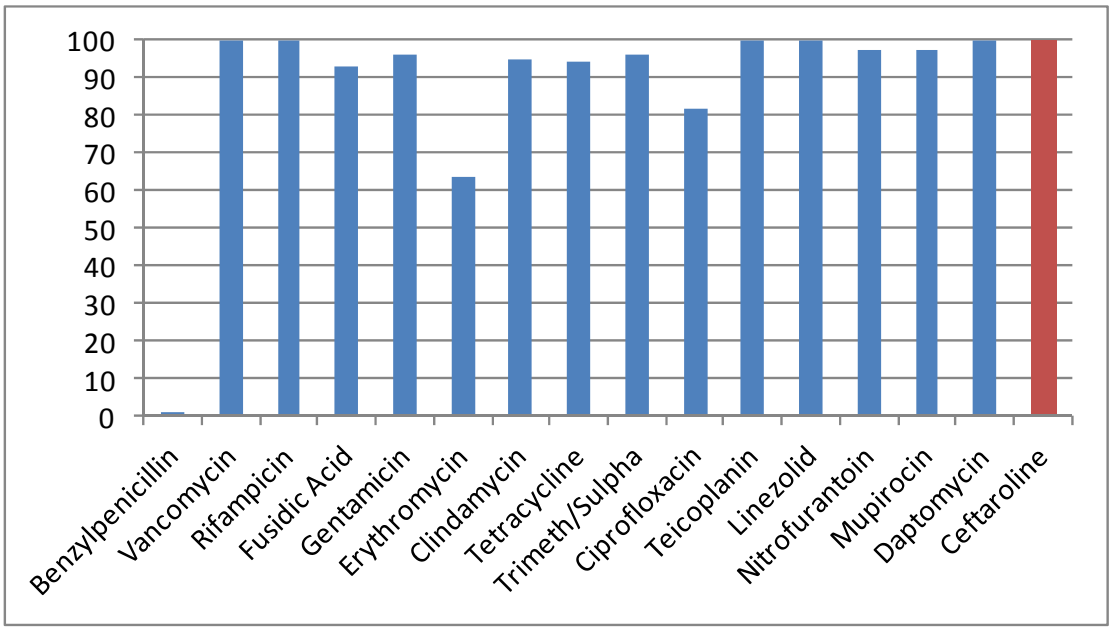

Figure 9: CA-MRSA Antibiogram

3.5.5. HA-MRSA Antibiogram

Table 9: HA-MRSA Antibiogram

Antibiotic	Susceptible	
	Number	%
Benzylpenicillin	0	0
Vancomycin	428	100
Rifampicin	415	97.0
Fusidic acid	422	98.6
Gentamicin	224	52.3
Erythromycin	80	18.7
Clindamycin	237	55.4
Tetracycline	209	48.8
Trimethoprim/Sulphamethoxazole	223	52.1
Ciprofloxacin	11	2.6
Teicoplanin	428	100
Linezolid	428	100
Nitrofurantoin	421	98.4
Mupirocin (High Level Resistance)	427	99.8
Daptomycin	426	99.5
Ceftaroline	415	97.0

Inducible clindamycin resistance: 150 (35.0%) isolates

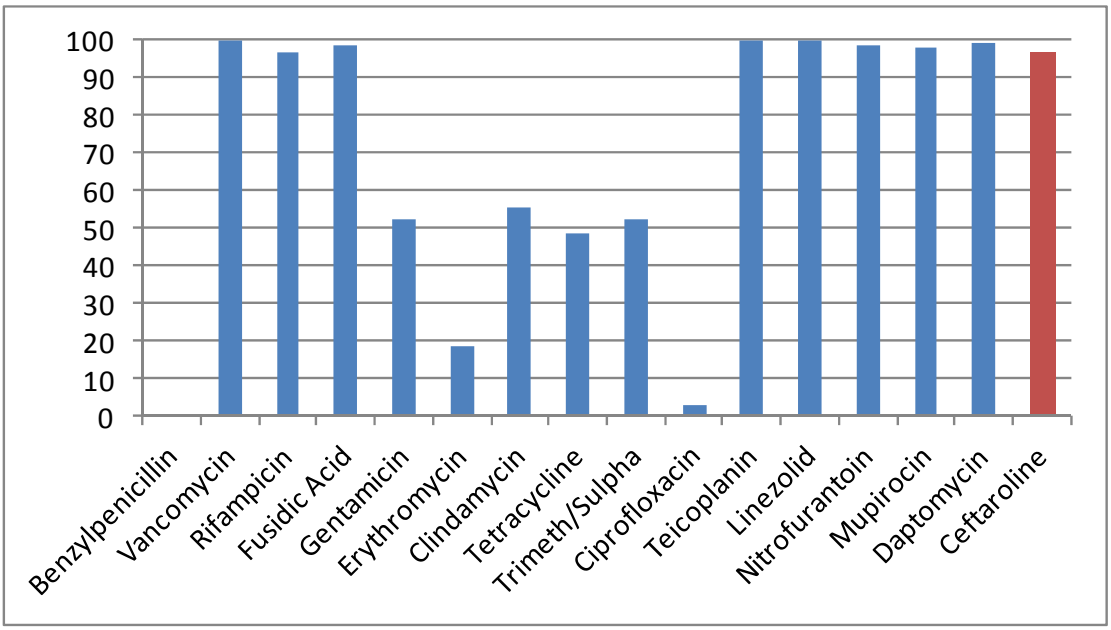

Figure 10: HA-MRSA Antibiogram

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