Characterization of Methicillin-Resistant *Staphylococcus aureus* carrying Florfenicol exporter (fexA)-mediated chloramphenicol resistance

Edet E Udo, Samar S. Boswihi, Bindu Mathew, Bobby Noronha and Tina Verghese
Department of Microbiology, Faculty of Medicine, Kuwait University. Kuwait.

Introduction and Purpose

Chloramphenicol is a broad spectrum antibiotic that inhibits bacterial protein synthesis by irreversibly binding to a receptor site on the 50S subunit of the bacterial ribosome, inhibiting peptidyl transferase. This inhibition consequently results in the prevention of amino acid transfer to growing peptide chains, ultimately leading to inhibition of protein formation [1].

The most common mechanism of chloramphenicol resistance in Staphylococcus is enzymatic inactivation by chloramphenicol acetyltransferase although drug efflux by chloramphenicol/florfenicol exporter (fexA) has recently been reported [1].

Chloramphenicol – resistant -MRSA occurred sporadically in Kuwait hospitals in the past decade. However, the number of chloramphenicol-resistant MRSA strains isolated from patients increased suddenly in different hospitals from May 2014.

This study was conducted to investigate the genotypes of the chloramphenicol-resistant isolates to ascertain whether they were new or sporadic clones that have acquired chloramphenicol resistance, and to determine the genetic basis of the chloramphenicol resistance.

Methods

Bacterial strains: Fifty four chloramphenicol – resistant MRSA isolates were obtained from nine hospitals in Kuwait from 1 May to 30 September 2014. The MRSA isolates were obtained as part of routine diagnostic microbiological investigations and were identified as MRSA at the different diagnostic laboratories. Further testing was performed at the MRSA Reference Laboratory, Kuwait. Isolates were obtained from the nose (13), skin and soft tissues (14), HVS, (8) Blood (4), Groin (5) and miscellaneous sources (10).

Antibiotic susceptibility testing: Susceptibility testing was performed by the disk diffusion method [2]. MIC was determined using Etest (bioMérieux, Marcy l'Etoile. France).

SCC*mec* typing: All isolates were typed by multiplex PCR using previously published primers and protocols [3].

Spa typing [4] and Multilocus-sequence typing (5] were performed as described previously.

DNA Microarray: Genes encoding virulence factors and antibiotic resistance were determined using the ArrayMate Reader DNA Microarray platform with Identibac *S. aureus* genotyping Kit 2.0 (Alere Technology, Jena, Germany) following protocols provided by the manufacturer.

Plasmid Analysis: Isolation of plasmid DNA, curing and DNA transfer experiments by conjugation and mobilization were performed as described previously by Udo and Jacob [6].

Results

Table 1 summarizes the phenotypic and genotypic characteristic of the 54 chloramphenicol resistant isolates. The isolates were resistant to chloramphenicol (MIC: 32- 256 mg/L), tetracycline (48 isolates), trimethoprim (45 isolates), fusidic acid (43 isolates), erythromycin and clindamycin (11 isolates), gentamicin and kanamycin (6 isolates), ciprofloxacin (3 isolates) and high level mupirocin (1 isolate).

Molecular typing classified the isolates into CC5-ST5-V-t688 (7 isolates), CC5-ST627-VI-t688 (42 isolates), CC5-ST627-VI-t450 (1 isolate), CC5-ST627-VI-t954 (1 isolate), CC8-ST239-III-t037 (2 isolates) and CC8-ST239-III-t860 (1 isolate).

Table 1. Phenotypic and genotypic characteristics of MRSA isolates

SN	MRSA Clones	#	Antibiotic Resistance	Resistance genes	Agr	Cap	Enterotoxins
1	ST5-V-t688 (WA MRSA 11/34)	6	Em, Clin, Tet, Cip	*fexA, ermC, tetK, tetM, fosB*	2	5	sed, egc
2	ST627-VI-t450 (MRSA-VI +SCC*fus*)	1	Tet, Tp, Fd	*fexA, fusC, dfrS1, tetM, fosB*	2	5	sed, egc
3	ST5-V-t688 (WA MRSA 81/ 85)	1	Em, Clin, Tet	*fexA, ermC, tetK*	2	5	egc
4	ST627-VI-t688 (MRSA-VI +SCC*fus*)	42	Tet, Tp, Fd	*fexA, fusC, dfrS1, tetM, fosB*	2	5	sed, egc
5	ST627-VI-t954 (MRSA-VI +SCC*fus*)	1	Tet, Tp, Fd	*fexA, fusC, dfrS1, tetM, fosB*	2	5	sed, egc
6	ST239-III-t037 (Vienna/Brazilian)	2	Gm,Km, Em, Clin, Tet, Tp, Fd	*cat, aacA-aphD, aphA3, tetK, tetM, ermA*	1	8	sea, sek, seq
7	ST239-III-t860 (Vienna/Brazilian)	1	Gm, Km, Em, Clin, Tet, Tp, Fd, Mup	*cat, aacA-aphD, aadD, aphA3, tetM, ermA, mupA*	1	8	ND

Abbreviations: Em, erythromycin; **Clin,** clindamycin; **Cip,** ciprofloxacin; **Tet,** tetracycline, **Tp,** trimethoprim; **Fd,** fusidic acid; **Gm,** gentamicin; **Km,** kanamycin; **Mup,** mupirocin; **Agr,** accessory gene regulator; **cap,** capsular polysaccharide; **ND.** Not detected; **fexA,** chloramphenicol/florfenicol exporter gene; **ermC,** rRNA methyl transferace gene; **tetK,** tetracycline efflux gene; **fosB,** metallothiol transferase; **fusC,** fusidic acid resistance gene; **dfrS1,** dihydrofolate reductase gene; **tetM,** ribosomal protection gene; **aacA-aphD,** gene for aminoglycoside adenyl-/phosphotransferase; **aadD,** aminoglycoside adenyltransferse gene; **aphA3,** aminoglycoside phosphotransferase gene.

Table 2. Transfer of chloramphenicol- resistance in MRSA isolates

SN	Isolates	Resistance profile	Plasmid content ;kb	*Mode of Transfer	Resistance Transferred	Plasmid Transferred, kb	Genes transferred
1	14071	Cm, Em, Clin, Tet	*c*.40.0, 28.5	C, M	None	None	None
2	14284	Cm, Gm, Km, Em, Clin, Cip, Fd, Mup	*c*.40.0, 4.4	C	Gm, Mup, Cm	c.40.0, 4.4	*cat, mupA, aacA-aphD*
				C	Gm, Mup	c.40.0	*mupA, aacA-aphD*
				C	Cm	4.0	*cat*
3	13973	Cm, Em, Clin, Tet	28.5, 2.4	M	Em	2.4	*ermC*
4	14299	Cm, Gm, Km, Em, Clin, Tet, Tp, Fd	*c*40, 4.0	M	Cm	4.0	*cat*
5	14387	Cm, Gm, Km, Em, Clin, Tet, Fd	28.5, 4.0, 2.4, 2.0	M	Cm	4.0	*cat*
6	14433	Cm, Tet, Tp, Fd	28.5	C, M	None	None	None
7	14434	Cm, Tet, Tp, Fd	28.5	C, M	None	None	None

Abbreviations: C, conjugation; **M,** mobilization

Results

Plasmid analysis

- Figure 1 shows plasmid content of representatives of the chloramphenicol resistant isolates which ranged from < 2.0kb to c40.0 kb.
- Representative were selected for curing and transfer experiments. Results of the transfer experiments are summarized in Table 2.

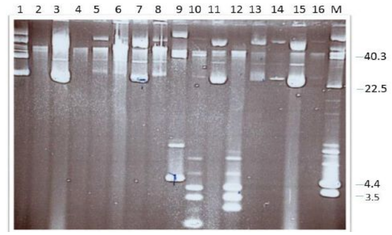

Figure 1. Plasmid DNA contents of MRSA isolates

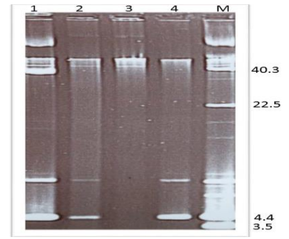

Figure 2. Transfer of *cat* mediated chloramphenicol resistance

Conclusions

- The majority of the chloramphenicol-resistant MRSA belonged to CC5-ST627-VI-t688 (42; 77.7%) followed by CC5-ST5-V-t688 (6; 11.1%), CC5-ST627-VI-t450 (1; 1.8%), CC5-ST627-VI-t954 (1;1.8%), CC8-ST239-III-t037 (2; 3.7%) and CC8-ST239-III-t860 (1;1.8%).
- Two types of chloramphenicol resistance determinants, CAT and fexA, were detected. The CC8-ST239-t037/t860 isolates harbored the CAT determinants whereas the CC5-ST5-V and CC5-ST627-VI isolates harbored the florfenicol exporter (*fexA*) determinant.
- Curing and transfer experiments located the CAT determinants on plasmids similar to pC221 and pSBK203. In contrast, *fexA* could not be lost on curing or transferred, and was presumed to be chromosomal.
- Whereas the CC8-ST239-III isolates represented sporadic isolates, the CC5-ST5-V and CC5-ST627-VI represent clones recently introduced into Kuwait.
- The study highlights the importance of molecular typing in detecting the introduction of new MRSA clones into a healthcare facility.

References

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