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MRSA - one health worldwide

Characterization of methicillin-resistant Staphylococcus aureus carrying Florfenicol exporter (fexA)-mediated Chloramphenicol resistance

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Background: Chloramphenicol resistance in *Staphylococcus aureus* can be due to inactivation by chloramphenicol acetyl transferase (CAT), effluxed by chloramphenicol/florfenicol exporter (fexA) or the 23S rRNA methyl transferase (cfr) that also mediate resistance to linezolid. Chloramphenicol - resistance was isolated sporadically in methicillin-resistant *S. aureus* (MRSA) obtained from patients in Kuwait hospitals in the past decade. However, an increasing number of chloramphenicol-resistant MRSA strains were isolated from patients in different hospitals from May 2014. This study was conducted to investigate the genotypes of the chloramphenicol-resistant isolates to ascertain whether they represented new clones or endemic clones that have acquired chloramphenicol resistance, and to determine the genetic basis of chloramphenicol resistance.

Material/methods: Fifty four chloramphenicol – resistant MRSA isolates were obtained from nine hospitals in Kuwait from 1 May to 30 September 2014 and tested for resistance to antibiotics. Molecular typing was performed using a combination of SCC*mec* typing, Spa typing and multi locus sequence typing. DNA microarray was used to determine mechanisms of antibiotic resistance. Curing, transduction, mixed culture transfer (MCT) and conjugation were used to determine the genetic location of the resistance determinants.

Results: All 54 isolates were resistant to chloramphenicol (MIC: 32- 256 mg/L). The isolates were also resistant to 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-t951 (1 isolate), CC8-ST239-III-t037 (2 isolates) and CC8-ST239-III-t860 (1 isolates). 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 by transduction, MCT and conjugation and was presumed to be chromosomal.

Conclusions: The study revealed the presence of two types of chloramphenicol resistance determinants, CAT and fexA, in MRSA circulating in Kuwait hospitals. Whereas the CC8-ST239-III isolates represented isolates obtained sporadically, the CC5-ST5-V and CC5-ST627-VI clones represent newly introduced clones. The study also emphasizes the importance of molecular typing in detecting the introduction of new MRSA clones into a healthcare facility.