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Title :
Molecular Characterization of Coagulase-Negative *Staphylococcus* and Biofilm- Associated Genes in *S. capitis*

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The coagulase-negative *Staphylococcus* (CoNS) is a group of bacteria that are gaining prominence as emerging pathogens of hospital-acquired infections. One such species is *S. capitis*, which is now the major cause of bloodstream infection especially in neonatal intensive units. The major virulence factor of *S. capitis* appears to be its ability to form a biofilm structure. A total of 200 local clinical isolates of CoNS was obtained from the Hospital Tuanku Ampuan Rahimah, Klang in between December 2010 to May 2011. Nine species of CoNS were identified with *S. epidermidis, S. haemolyticus, S. hominis* and *S. capitis* being the most prevalent strains. Identification of the isolates by biochemical tests using the Microgen Staph ID kit was less than 50% accurate while identification via the *sodA* gene sequence provided better discrimination and accuracy. The ERIC-PCR fingerprinting was then used to genotype the CoNS strains and the Discriminative Index (*D*) was calculated. At $D = 0.949$, ERIC-PCR can be used with confidence to discriminate between the *S. hominis* strains. However, low discriminative power ($D < 0.9$) was observed for *S. capitis, S. epidermidis* and *S. haemolyticus* implying that ERIC-PCR fingerprinting is not sufficient to genotype these strains. A multiplex PCR method was successfully developed to probe for the presence of *icaABCD* operon in a majority of the bacterial strains. At 88%, *S. capitis* showed the highest ability to form biofilm with a large percentage of these forming dense biofilm structures while the *icaABCD* operon was found to be present in all of the strains. Biofilm formation was however less frequent in other species, e.g. 39.2% in *S. epidermidis*, 16.7% in *S. hominis* and 3.3% in *S. haemolyticus*. Antimicrobial susceptibility test showed that for *S. capitis*, the formation of biofilm significantly increased the resistance of the biofilm cells to six types of antibiotics, similar to that reported for *S. epidermidis*. However, except for the case of ciprofloxacin, the thickness of biofilm did not appear to have any effect on the antibiotic resistance of the cells. Strain *S. capitis* B102 was selected for screening of novel biofilm-associated genes due to its ability to consistently form a very thick biofilm. Attempts to generate biofilm-defective mutants by transposon-mediated mutagenesis using the bursa aurealis system was however unsuccessful. Comparative genomics of B102 and three other *S. capitis* strains P27 (a non-biofilm former), B63 (moderate biofilm) and B145 (very strong biofilm) revealed that the *S. capitis* genome was dynamically shaped by horizontal gene transfer (HGT) via prophages, Staphylococcal Chromosome Cassettes (SCC) and plasmids. Some mobile genetic elements (MGE) present only in B102 and B145 are found to carry genes implicated in biofilm formation e.g. the *Atl* autolysin. By comparing the SNP profiles in strains with different biofilm phenotype, a list of seven candidate biofilm-associated genes was obtained. The ability of *S. capitis* to acquire additional genetic elements via HGT, and its propensity to form robust biofilm which enhances its antibiotic resistance, points to the possibility of this organism evolving into a significant pathogen.