Abstract

Background: *Staphylococcus aureus* is one of the major nosocomial bacterial agent causing foodborn diseases in humans worldwide. However, *S. aureus* is repeatedly detected in community as a consequence of cross-contamination from food handlers and food contact surfaces. The pathogenesis of *S. aureus* infections is mainly due to their abilities to form biofilms on polymer surfaces and their multi-drug-resistant characteristics. Biofilm formation also offers *S. aureus* an elevated tolerance to biocides allowing a long-term persistence of this pathogen in environments. Thus the aim of the present study was phenotypic and genotypic analysis of virulence factors of *S. aureus* strains isolated from clinical as well as CA/ environmental sources in South Assam region.

Methods: 444 several clinical (n=264) and community associated (CA)/environmental isolates (n=180) of *S. aureus* was isolated from the study area. The isolates were characterized for: susceptibility to antibiotics, methicillin-resistance (MR), biofilm production, haemolysin and enterotoxin assay and compared between both the categories. The antibiotic susceptibility profile was determined by using Kirby-Bauer Disc diffusion method. We evaluated different phenotypic techniques (Congo red agar method and Tube method) and genotypic techniques (detection of the *ica* operon and *bap* gene) related to biofilm development by clinical and CA/environmental isolates of *S. aureus.* The biofilm architecture was studied by scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM). The haemolytic activity and enterotoxin assay of the isolates were estimated by PCR detection of respective genes.

Results : The clinical isolates showed and highest resistance (100%) against Penicillin and ampicillin. This was followed by gentamycin (69%), tetracycline (50%), cefrtriaxone (46%), cefuroxime (33%), erythromycin (27%), amikacin (25%), oxacillin (23%) and ciprofloxacin

(20%). On the otherhand, the CA/environmental isolates also exhibited maximum resistance against ampicillin (100%) and penicillin (96%) followed by gentamycin (56%), tetracycline (41%), ceftriaxone (29%), oxacillin (25%), cefuroxime (23%) erythromycin (20%), amikacin (15%) and ciprofloxacin (15%). Vancomycin and linzoid exhibited similar sensitivity pattern in all the isolates and stand for maximum sensitivity (100%). Of all the *S. aureus* isolates, 23% clinical and 25% CA/ environmental isolates exhibited methicillin resistance. The MAR index of clinical *S. aureus* isolates vary from 0.1 to 0.7, while the environmental isolates have MAR index of 0.1 to 0.8, though there is no significance difference between the MAR indexes of environmental and clinical *S. aureus* samples ($p>0.05$).

Tube method detected 85% of the clinical isolates and 23% of the environmental isolates were biofilm producer *in-vitro*, whereas, in CRA method 21% clinical isolates and 22% isolates CA/environmental isolates were biofilm positive. Molecular analysis revealed presence of *icaA* and *icaD* gene in both clinical (36.5% *icaA*, 23% *icaD*) and CA/environmental isolates (24% *icaA* and 22.5% *icaD*) whereas, *icaB* and *icaC* genes were absent in all the isolates. Five (05) clinical isolates and nine (09) CA/environmental isolates found to carry *bap* gene were phenotypically biofilm producer. The CRA assay was found to be irreproducible and very subjective. More objective results were obtained using the spectrophotometric, tube method of biofilm assay. Biofilm production significantly increased in the presence of 1% glucose, 0.75% NaCl, and at neutral to slight alkaline pH (7.2). It was also observed that polyvinyl-chloride (PVC) surface supported a noble adherent site for biofilm establishment than polystyrene and glass material. There was a positive relation between biofilm formation and antibiotic (oxacillin) exposure. Both clinical and CA/ environmental isolates displayed 1.5 fold and 1.2 fold increase in biofilm production with sub-lethal doses of oxacillin exposure with respect to control. Moreover, small colony

variants (SCV) showed a higher capability to form biofilm under stressed condition, than the wild type (WT) strains.

Of the total 264 clinical isolates, 79.53% were producers of haemolysin. Among this, 50.76% isolates showed β-haemolysis (28.3% MSSA and 22.7% MRSA) and 28.78% isolates were α-haemolytic (18.56% MSSA and 10.22% MRSA). Among 180 environmental isolates, 67.22% isolates were haemolysin producer, out of which 56.11% were β-haemolytic (41.11% MSSA and 15% MRSA) and 11.11% isolates were α-haemolytic (7.22% MSSA and 3.89% MRSA) on 5% sheep blood agar. Molecular analysis revealed the presence of *hlb* gene was most prevalent (31%) than *hla* gene (25.6%) among clinical isolates. Whereas, among environmental isolates, *hla* gene (20%) gene was slightly more prevalent than *hlb* gene (19%). There was no significant correlation between clinical and CA/environmental isolates for haemolysin production ($p>0.005$).

Molecular analysis of Staphylococcal enterotoxin (SE) showed *sea* gene was most prevalent (35.01%) than *sed* gene (30.16%) among clinical isolates. Similarly in environmental isolates too, the prevalence of *sea* gene (29.65%) was more than *sed* gene (15.35%). Further no *seb* and *sec* gene carrying isolates were recovered in this study. Although there is no significant correlation between clinical and environmental isolates ($p>0.055$) but an F test signified significant correlation between *sea* and *sed* genes among all the isolates ($p< 0.05$).

Among all the isolates, 76%, 94%, 62% and 73% isolates were protease, thermonuclease, lipase and lecithinase producers respectively, as indicated by their zone of clearance.

Conclusion: *S. aureus* isolates from the clinical and CA/environmental sources exhibited several virulence factors. The frequency of harbouring virulent genes in CA/ environmental isolates was approximately a little less or similar to that of clinical isolates. The high incidence of toxigenic *S. aureus* strains portends a serious potential threat to the community. Moreover, biofilm boosts the pathogen causing them escape from antibacterial therapy. Therefore, awareness on the implications of misuse and abuse of drugs should be adopted to control spread of multi-drug resistant strains.