Title of dissertation				
Molecular Epidemiological Characterization of Methicillin-Susceptible and -Resistant Staphylococcus aureus Isolated from Skin and Soft Tissue Infections in Bangladesh				
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Staphylococcus aureus is one of the most prevalent pathogens in humans worldwide and causes a broad variety of diseases. In addition to the common skin and soft tissue infections (SSTI), this bacterium causes life-threatening infectious diseases including sepsis and necrotizing septicemia, as well as toxic diseases such as food poisoning and toxic shock syndrome. Panton–Valentine leukocidin (PVL), a bicomponent pore-forming protein encoded by *lukS-PV* and *lukF-PV* (*pvl*) carried on prophage, is prevalent in a part of methicillin-susceptible and -resistant *S. aureus* (MSSA/MRSA), and associated with increased virulence of *S. aureus* leading to severe SSTI, musculoskeletal infections, and necrotizing pneumonia. In Bangladesh, only a few studies are available for molecular epidemiological information of clinical isolates of MRSA/MSSA, and prevalence of PVL among them. The present study was conducted in 2015-2016 in Mymensingh, located in North-Central Bangladesh, to investigate the molecular epidemiological and genetic characteristics of MSSA/MRSA isolated from patients with SSTI.

A total of 430 clinical isolates of *S. aureus* were collected consecutively from patients with SSTI who visited Mymensingh Medical College (MMC) hospital, Bangladesh, for a 15-month period between October 2015 and December 2016. The main specimens of isolates were : wound exudate (47%), followed by abscess (21%), aural swab (20%), burn exudate (7%), and diabetic ulcer (5%). The age range of patients was 5–57 years, while the sex distribution (male/female) was 1.4 (248/182). Only one isolate per patient was included in the analysis. One hundred and six specimens were obtained from outpatients who had not visited other health care facilities for at least 21 days, while remaining samples were collected from inpatients. Antimicrobial susceptibility was measured by broth microdilution test using Dry Plate Eiken DP32 (Eiken, Tokyo, Japan). MICs of 18 antimicrobial agents were measured and resistance was judged according to break points mentioned in the Clinical Laboratory Standards Institute guidelines. For all the isolates, the presence of staphylococcal 16s rRNA gene, *nuc*, *mecA*, *pvl*, ACME (arginine catabolic mobile element)-*arcA*, and coagulase genotypes (coa-type) were investigated by multiplex PCR assay. For the selected 49 isolates, ST (sequence type) based on MLST (multilocus sequence typing) scheme and prevalence of genes encoding various toxins (enterotoxins, exfoliative toxins, TSST-1), virulence factors, adhesins, and antimicrobial resistance-associated proteins was analyzed by multiplex or uniplex PCRs, and sequencing as necessary.

Among 430 clinical isolates, MRSA accounted for 31% having SCC*mec* type IV (73%) and V (14%), and belonged mostly to coagulase (*coa*) genotypes IIa, IIIa, IVb, and XIa, while dominant *coa* type in MSSA was IIIa, followed by Va, IIa, and VIa. Panton-Valentine Leukocidin genes (*pvl*) were detected at higher rate in MSSA (54%) than in MRSA (24%). Based on MLST, *pvl*-positive MRSA isolates were classified into clonal complex 88 (CC88) (ST88, ST2884, ST4345), CC6 (ST6, ST4350) and CC1 (ST1, ST772), while *pvl*-negative MRSA into CC5, CC22, CC80, CC121, and CC672. The *pvl*-negative ST80 MRSA isolates had SCC*mec*-IVa (*agr*-III/*coa*-XIc, *etd*/*edinB*-positive, *fusB*-negative), indicating that they belong to the novel CC80 clade related to the European community-acquired MRSA clone. Among MSSA, genotypes ST121/*spa*-t645/*coa*-Va and ST2884 (CC88)/*spa*-t2393/*coa*-IIIa were identified in both *pvl*-positive and -negative isolates, and all the ST772 isolates harbored *pvl*. All the ST121 isolates had a variant of elastin-binding protein gene (*ebpS*-*v*) with internal 180-nucleotide deletion. The present study suggested that CC88 (ST88, ST2884) and ST772 are the putative dominant lineages of *pvl*-positive MRSA/MSSA, while novel CC80 clade is one of the main *pvl*-negative MRSA lineages distributed endemically in Bangladesh.

In summary, the present study revealed the prevalence of *mecA* and *pvl*, and the genetic characteristics of *S. aureus* clinical isolates from SSTI in Mymensingh, Bangladesh. Identification of emerging CC88 *pvl*-positive MRSA and novel CC80 *pvl*-negative MRSA clones highlight the need for further surveillance of molecular epidemiological characteristics of *S. aureus* distributed in this country.

Photos

Myself in the laboratory

With department members