# Prevalence of s. aurus in hospital canteen foods

Introduction

Being a globally important pathogen *Staphylococcus aureus* is responsible for various fatal diseases like pneumonia, blood-stream infections, skin and soft tissue infection, osteomyelitis, and endocarditis, along with toxin mediated syndrome like food poisoning and toxic shock syndrome [1, 2]. Since 1980, the rise of the pandemic waves of methicillin resistant *S. aureus* (MRSA), it is one of the most common cause of hospital as well as community acquired infection worldwide [3, 4]. Although different sources of MRSA were identified in various researches, hospital canteen foods (HCF) were remained untouched. HCF sources were being thought to be a potential and one of the major sources of MRSA. Other risk factors for HCF acquired MRSA include length of hospital stay, antibiotic exposure and colonization pressure [5-7]. HCF are the basic point of research because of its usefulness for patients, visitors as well as hospital staffs. If HCF are contaminated with such devastating bacterium, nosocomial infection might be reached to that serious condition which would be difficult to neutralize [8].

The responsible molecular processes of *S. aureus* for host specificity and disease progression is poorly understood but thought to be caused in part of gene content containing drug resistant genes especially mecA and clfA [9-12]. The presence of these gene loci in the genome of *S. aureus* indicates the resistance against different drugs. Clumping factor (CLF-A) is the product of clfA gene which is normally a fibrinogen binding protein present in the surface of S. aureus and is responsible for virulence of severe infections [13]. On the other side, mecA is responsible for methicillin resistance by altering

penicillin binding protein which indicate a low affinity to β-lactam antibiotics [14].

As there is no relevant documents about the antibiotic susceptibility of S. aureus present in HCF samples in Bangladesh, this study was designed to determine prevalence of *S.* aurus in different HCF samples along with its antibiotic resistance pattern.

# Methods

## Sample collection and maintenance

A total of 350 samples were collected from hospital canteen foods of four different medical college hospitals in Dhaka city during July, 2011 to June 2012 and transferred to the clinical laboratory of the department of Microbiology, University of Dhaka, maintaining aseptic condition. All the samples were then stored in 4°C until used.

## Isolation of *S. aureus*

The aseptically collected HCF samples were inoculated on nutrient agar media and incubated overnight at 37°C. All the bacterial colonies from nutrient culture media were cultured on Mannitol Salt Agar (MSA) media, again incubated overnight at 37°C and the isolated were presumptively identified by analyzing with standard microbiological procedure which includes colony characteristics, Gram staining, and different biochemical reactions like catalase, oxidase, citrate agar slant, urease test, triple sugar iron agar test, sulfide indole motility test, methyl red test and Voges Proskauer test.

## Antibiotic susceptibility test

Kirby-Bauer disc diffusion method was performed to determine the susceptibility and resistance to antimicrobial drugs according to the guidelines of Clinical and Laboratory Standards Institute, formerly National Committee for Clinical Laboratory Standards [15, 16]. After incubation at 37°C for 18 hours and within 24 hours, measurements of zone of inhibition were taken. The antibiotics that were used in this study include azithromycin, amoxicillin, erythromycin, ciprofloxacin, tetracycline, levofloxacin, bacitracin, oxacilin, nalidixic acid, penicillin G, gentamycin, sulfanilamide levofloxacin, chloramphenicol and vancomycin. The isolates which were resistant to at least two classes of antibiotics were considered as multi-drug resistant (MDR).

## Genotypic detection of *S. aureus*

Colonies of *S. aureus* were inoculated in LB medium and incubated overnight at 37°C. Then the culture was centrifuged at 4°C at 8, 000 g for 10 min. The pellet was then suspended in 200µl TE buffer (10 mM Tris HCl + 1 mM EDTA, pH 8. 0) with lysozyme (10 mg/ml) and incubated at 37°C for 2 hour. The bacteria was lysed with 10% SDS and proteinase K (10 mg/ml), and was incubated at 65°C for 30 min. The cell wall debris, denatured protein, and polysaccharides were removed by the addition of 5 M NaCl and CTAB/NaCl (10% hexadecyl trimethyl ammonium bromide in 0. 7 M NaCl) and incubated for 30 min at 65°C. DNA was isolated by extraction with phenol: chloroform (1: 1) and chloroform: isoamyl alcohol (24: 1). DNA was precipitated with isopropanol and sodium acetate (3 M) solutions. After washing with 70% ethanol the precipitation was suspended in 50µl of TE buffer.

Virulence determinant gene encoding clumping factor (clfA) was amplified by polymerase chain reaction (PCR) containing a total of 25 µl of PCR mixture that included 2 µl template DNA, 12. 5 µl of 2x MiFi Mix (Promega Corporation, Madison, WI, USA), 1 µl (10 pmol) of each primer and 8. 5 µl nuclease free water. Then the mixture was amplified using a PCR protocol as follows: 5 min at 94°C, 35 cycles of 1 min at 94°C, 1 min at 57°C, 1 min at 72°C, and a final elongation step of 7 min at 72°C. The amplification of clfA gene was carried out using thermocycler (Eppendrof, Germany). Amplified products were then separated using Agarose gel electrophoresis (AGE) where 1. 5% of agarose was used and visualized using UV illumination.

## Sequencing of clfA gene and molecular analysis

The amplified products were purified using the PCR clean-up system (Promega Corporation, Madison, WI, USA) according to the manufacturer's instruction. Final elution contained 50 µl of purified PCR products from which 10 µl was reanalyzed on 1. 5 % agarose gel to make sure that the purification step was performed precisely. The purified PCR products were then subjected for automated sequencing (Biorad, Germany) using the same primer pair described previously.

## Phylogenetic analysis of the clfA gene sequence

Several clfA gene sequences of *S. aureus* isolated from different countries of the world were collected from NCBI GenBank and used to prepare phylogenetic tree to predict the origin using MEGA 6. Translation of the nucleotide sequence was performed using EMBOSS Transeq tool of EMBL-EBI server [17].

# Results

## High Prevalence of *S. aureus* in HCF Samples

A total of 461 hospital canteen food samples from different sources of foods were included in this study where 64 burger, 58 chicken fry, 62 salad, 72 bread, 55 rice, 48 vegetables, 40 fish curry and 62 dishes samples were included. Of them 51. 6%, 24. 1%, 58. 1%, 33. 3%, 29. 1%, 41. 7%, 45%, 30. 6% and 39% of burger, chicken fry, salad, bread, rice, vegetables, fish curry and dishes samples were presumptively identified as positive for *S. aureus* by different microbiological and biochemical tests. From all of the *S. aureus* positive samples about 89% were coagulase positive and the rest of them were coagulase negative *S. aureus* (Table 1).

## Antibiotic resistance pattern of *S. aureus*

In this study, coagulase positive and negative *S. aureus* were highly resistant (> 60%) to azithromycin, amoxicillin and erythromycin, moderately resistant (40%-60%) to ciprofloxacin, tetracyclin, levofloxacin and bacitracin, less resistant (20%-40%) to oxacilin, nalidixic acid, penicillin G, gentamycin and sulfanilamide, and totally sensitive (> 99%) to vancomycin (Table 2).

The samples that were resistant to oxacillin and gentamycin were also resistant to tetracyclin, erythromycin, levofloxacin and amoxicillin but not vice versa.

## Molecular detection confirms the gene clfA

PCR with the above described primer demonstrated the product length of 970 bp which was also confirmed in AGE after DNA purification (Figure 1). The sequence of clfA was translated using EMBOSS Transeq tool of EMBL-EBI

server [17] to find the appropriate protein frame and analyzed by formation of phylogenetic tree which reveals the origin is Japan (Figure 2).

## Discussion

The aim of this study was to determine the prevalence of S. aureus in different HCF samples and its resistance to different existing antibiotics prevalent in Bangladesh along with identification of mecA and clfA genes in the genome of oxacillin resistant S. aureus. Among all the types of food samples the prevalence of coagulase positive S. aureus was 89%. Chicken fry and salad contain least (24. 1%) and highest (58. 1%) number of S. aureus among all types of HCF observed in this study. Salad is eaten raw with mixture of diferent vegetables on the other side chicken fry is prepared after burning a long time and that's why the deviation is responsible [18, 19]. Consuming salad randomly without proper treatment may cause serious foodborne or other staphylococcal infections among hospital canteen staff, hospital patients as well as visitors which render high chance to cause multi-infection or multi-disease among hospital indoor or outdoor patients after getting such contaminated foods. In another side, chicken fry contain more *S. aureus* of coagulase positive compared to other HCF whereas least coagulase positive *S. aureus* present in salad. This suggests that coagulase positive *S. aureus* were highly preferred in fried samples which remarks a dangerous sign of fried-food lovers to be seriously affected by staphylococcal foodborne infections.

In this study, coagulase positive *S. aureus* (CPS) from different HCF showed 14-22% resistance to oxacillin whereas, coagulase negative *S. aureus* showed 12-19%. It is clearly seen that resistance rate of CPS against 12

antibiotics was more than that of CNS, however, in case of sulfanilamide CNS was more resistant than CPS. The reason may be that coagulase might have some association with antibiotic resistance. All the positive isolates were extremely sensitive to vancomycin which might be used to treat the *S.* infections with different diseases. Only 1% of *S. aureus* from salad samples showed resistant to vancomycin which was insignificant. It has also been observed that the isolates resistant to oxacillin and gentamycin were also resistant to tetracycline, erythromycin, levofloxacin and amoxicillin but not vice versa which is consistent with other study [20]. In this study, this is clear that *S. aureus* was highly resistant to erythromycin, azithromycin, amoxicillin, ciprofloxacin, tetracycline and levofloxacin, however, slightly resistant to oxacillin, nalidixic acid, bacitracin, penicillin, gentamycin and sulfanilamide, wherever, strongly sensitive to vancomycin indicating to be effective to prevent and treat *S.* infections.

Molecular identification by PCR confirmed clfA gene and phylogenetic tree revealed the origin was Japan. As no Bangladeshi sequence of clfA was found in NCBI GenBank the alignment as well as phylogenetic tree was closely matched with a Japanese sequence. This sequence will provide necessary information for further analysis and encourage the researchers to evaluate the proper source of infection circulating in Bangladesh.

In conclusion, it can be suggested that the hospital canteens should be properly monitored to ensure the safety of foods for the patients along with the visitors as well as staffs of the canteen. As the existing antibiotics were found resistant, it is mandatory to evaluate the molecular gene specific resistance and responsible mutations to overcome such burden. It is now

essential to design new treatment method for prevention Staphylococcal infection in future.