

Original Article

Detection of Different Exotoxins Among the Isolates of *Staphylococcus aureus*

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Abstract

The study was done to detect different exotoxins among the strains of *Staphylococcus aureus* isolated in the department of Microbiology, Mymensingh Medical College in collaboration with the Department of Medicine under the Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh between the periods from July, 2006 to June, 2007. A total of 40 *S. aureus* isolates investigated in this study were identified by standard microbiological techniques. Antimicrobial susceptibility of the isolates to Oxacillin was carried out by disk diffusion method as per recommendation of the National Committee for Clinical Laboratory Standards. Any isolate showing resistance to Oxacillin was tested again by agar dilution method to determine minimum inhibitory concentration (MIC) of Methicillin. All strains were also tested for *mecA* gene by Polymerase Chain Reaction (PCR) for confirmation of Methicillin resistance. Enterotoxin (A-D) and Toxic Shock Syndrome Toxin-1 (TSST-1) were detected by Reverse Passive Latex Agglutination (RPLA) test. Out of 40 *S. aureus* isolates, 7 (17.5%) Methicillin Resistant *S. aureus* (MRSA), 1 (2.5%) Methicillin Sensitive *S. aureus* (MSSA) produced Staphylococcal Enterotoxin A (SE-A) and 1 MRSA isolate was positive for TSST-1. In case of combined toxin production among the *S. aureus* isolates, 2 (5%) MSSA were found to produce SE-A and SE-B, 2 (5%) MSSA produced SE-C and SE-D, and 1 (2.5%) MRSA, 1 (2.5%) MSSA produced SE-C and TSST-1.

Key words: Methicillin Resistant *Staphylococcus aureus* (MRSA), Methicillin Sensitive *S. aureus* (MSSA), Staphylococcal Enterotoxins, Toxic Shock Syndrome Toxin-1, Reverse Passive Latex Agglutination test

Introduction

Staphylococcus aureus is one of the most significant pathogen causing nosocomial and community acquired infections. Among the secreted Staphylococcal virulence factors, there is a growing list of exotoxins that can induce various types of

diseases' symptoms.¹ This organism produces two types of toxins with superantigen activities, Staphylococcal Enterotoxins (SEs) and Toxic Shock Syndrome Toxin-1 (TSST-1).²

Superantigen binds to major histocompatibility complex (MHC) class II on antigen presenting cells outside the classical antigen binding groove and they activate T cells by binding with the variable region of the beta-chain of T cell receptors.^{3,4} This cross-linking triggers the non-specific activation and

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proliferation of T-cells, induces production of high levels of a variety of cytokines^{5,6} and causes toxic shock syndrome, characterized by fever, rash, hypotension and multiple organ failure. Enterotoxins are responsible for many cases of food poisoning (intoxication) associated with ingestion of toxin-contaminated food.^{2,7} There are six antigenic types of SEs, namely SE-A, SE-B, SE-C, SE-D, SE-E & SE-F. In addition, new SEs such as SE-G through SE-M have recently been identified and characterized.⁸

Methicillin-resistant *S. aureus* (MRSA) have appeared as more virulent than Methicillin-sensitive *S. aureus* (MSSA) due to the expression of drug resistance property.⁹ But the ability to produce different exotoxins by MRSA and MSSA is independent of their sensitivity to Methicillin.¹⁰

There are several conventional laboratory procedures such as Enzyme Linked Immunosorbent Assay (ELISA), two dimensional gel electrophoresis, Reverse Passive Latex Agglutination (RPLA) test and Polymerase Chain Reaction (PCR) for the detection of the toxins liberated by *S. aureus*.¹¹ Among these methods, RPLA test is the most simple and sensitive method for the detection of SEs and TSST-1. The test result can be read visually thus eliminating the need for any special equipment and trained person for recording the result. In this test, latex particle are sensitized with anti-TSST-1 and SEs immunoglobulin. This sensitized latex particle can be made available commercially.¹² Detection of SEs and TSST-1 by RPLA has not yet been reported in Bangladesh. Having the described background, the present study was done as a preliminary one to detect Enterotoxins (A-D) and TSST-1 of MRSA and MSSA by RPLA test.

Methods

This observational study was carried out in the department of Microbiology, Mymensingh Medical College in collaboration with the department of Medicine under the Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh, during the period from July, 2006 to June, 2007.

A total of 40 *S. aureus* strains were investigated in this study. All of these strains were isolated from patients attending inpatient department of Mymensingh Medical College Hospital. Isolation of *S. aureus* were made by inoculating the

samples into Nutrient agar, Blood agar and MacConkey's agar media and incubated properly. Isolates were identified as *S. aureus* by colony morphology, Gram's stained smear microscopy, Catalase, Coagulase and Mannitol fermentation tests.¹³ Antimicrobial susceptibility test for Oxacillin was done for every isolate by disk diffusion method according to the recommendations of National Committee for Clinical Laboratory Standards (NCCLS).¹⁴

Any isolate showing resistance against Oxacillin (1 µg disk) by disk diffusion was tested by agar dilution method to detect minimum inhibitory concentration (MIC) of Methicillin following standard criteria.^{15,16}

All strains were also tested for *mecA* gene by Polymerase Chain Reaction (PCR) for confirmation of Methicillin resistance. The Enterotoxins and TSST-1 were detected by reverse passive latex agglutination (RPLA) test kits.

Reverse Passive Latex Agglutination (RPLA) test

Microtitre plates with V-shaped wells were used for the RPLA tests. The microtitre plate was dipped in 3% bleaching powder for 24-48 hours and then washed in distilled water three times and finally kept under UV-light for 24 hours.

One or two colonies of a *S. aureus* isolate was inoculated into a test tube containing 5 ml Brain Heart Infusion broth and incubated aerobically at 37°C for 24 hours. Finally, 5 ml of the incubated organism was taken in an eppendorf tube and centrifuged at 10,000 rpm for 5 minutes. Supernatant was used as the test specimen.

The microtitre plate was arranged so that each column consists of 5 wells and 6 columns (one for each of the SE-A, SE-B, SE-C, SE-D, TSST-1 and one for control) were used for each sample. Performing double dilution with Phosphate buffered saline (PBS) diluent, a final dilution of 1:32 was made in the last well. Latex sensitized anti-Enterotoxin A, anti-Enterotoxin B, anti-Enterotoxin C, anti-Enterotoxin D, and anti-TSST-1 (all from Denka Seiken, Japan) were added to each well for a sample, and latex control was also added. Then the plate was allowed to shake by electric shaker for few seconds and kept overnight at room temperature. In the following morning, colour of the wells was observed. No colour development indicated positive result.¹²

Result

Considering single toxin production, it was found that out of 40 *S. aureus* isolates, 7 (17.5%) Methicillin resistant *S. aureus* (MRSA) and 1 (2.5%) Methicillin Sensitive *S. aureus* (MSSA) produced Enterotoxin A. Among the 40 *S. aureus*, only 1 (2.5%) MRSA isolate was positive for TSST-1. (Table I)

Table I: Single toxin production by MRSA and MSSA strains

Type of toxin	Number of MRSA	Number of MSSA
Enterotoxin A	07 (17.5)	01 (2.5)
Enterotoxin B	00 (00)	00 (00)
Enterotoxin C	00 (00)	00 (00)
Enterotoxin D	00 (00)	00 (00)
TSST-1	01 (2.5)	00 (00)

Figures in the parentheses indicate percentages

The combined toxin production was considered among the MRSA and MSSA strains and it was found that out of 40 *S. aureus* isolates, 2 (5%) MSSA produced SE-A and SE-B, 2 (5%) MSSA produced SE-C and SE-D and 1 (2.5%) MRSA and 1 (2.5%) MSSA produced SE-C and TSST-1. (Table II)

Table II: Combined toxin production by the MRSA and MSSA strains

Combination of toxin	Number of MRSA	Number of MSSA
Enterotoxin A and B	00 (00)	02 (5)
Enterotoxin C and D	00 (00)	02 (5)
Enterotoxin C and TSST-1	01 (2.5)	01 (2.5)

Figure in the parentheses indicate percentages

Discussion

The ability of *S. aureus* to produce Enterotoxins (SEs) and Toxin Shock Syndrome Toxin-1 (TSST-1) is an important property with various clinical implications. For the determinations of SEs and TSST-1, the reverse passive latex agglutination test is the most commonly used method.¹ In the present study, 7 (17.5%) MRSA and 1 (2.5%) MSSA strains were found to produce Enterotoxin A and 1 MRSA produced TSST-1. Almost similar result was found in a study in 1993 from Japan where 6 *S. aureus* strains produced enterotoxin A, 10 strains produced enterotoxin B and 1 isolate produced Enterotoxin C.¹⁷

In case of combined toxin production, it was found that 2 (5%) MSSA isolates produced SE-A and SE-B, 2 (5%) MSSA isolates produced SE-C and SE-D, and one of each MSSA and MRSA isolates produced SE-C and TSST-1. Quite similar result was found in a study in 2003 from Marburg, Germany, where one *S. aureus* strain was positive for SE-A and SE-B, one was positive for SE-B and SE-C, and one was positive for SE-C and SE-D. They also found a combination of three toxins together.¹ The result does not correlate with that of the present study. Any contributory factor might be involved in difference in toxin production in *S. aureus* which is regulated by incubation time, oxygen and carbon dioxide concentration, osmolarity, protein concentration and pH of the environment.^{1,18-20} The production of toxin in *S. aureus* is also altered by growth phase of the bacteria. The SE-A is produced throughout log phase of growth, while SE-B, SE-C and SE-D are produced in greater quantities during the transition from the exponential to stationary phase.²¹

Analyzing the findings of the present study, it was observed that most of the MRSA isolates produced SE-A. Combined toxin production was also seen in both MRSA and MSSA isolates. Production of toxin is independent of sensitivity to Methicillin by both groups of *S. aureus* isolates.

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[Conflict of interest: None declared]