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Research Article

MOLECULAR CHARACTERIZATION OF ENTEROTOXIN PRODUCING AND ANTIBIOTIC RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATES FROM RESPIRATORY INFECTED COTTON INDUSTRIES LABOURS FROM SALEM DISTRICT, TAMIL NADU

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ABSTRACT

A total of 26 *Staphylococcus aureus* isolates from the respiratory infected cotton industries labours were subjected to the multiplex PCR amplification to detect Staphylococcal enterotoxins encoding gene. *S. aureus* isolates showed the presence of enterotoxins encoding gene i.e. *See* (50%), *Sec* (11.5%), *Sea* (8%) and *Sed* (8%). This present study also revealed that 69.2% of *S. aureus* isolates harboring *Mec*A gene confers resistance to methicillin antibiotic. About 50% of *S. aureus* isolates were found to carry *Mup*A gene confers resistance to Muprocin antibiotic. This study concludes that it is necessary to control the respiratory infections by using appropriate antibiotics in order to avoid spreading of infection as well as spreading antibiotic resistant and public health issues.

Keywords: Enterotoxin, Antibiotic resistant, Multidrug resistant, *Mec*A, *Mup*A.

INTRODUCTION

Respiratory tract infection is the most common infection reported of all human infections. Generally most of these infections are mild, transient lasting and sometimes self-limiting. However, respiratory infections are a common and important cause of morbidity and mortality worldwide. For instance, in USA alone, about 62 million persons suffer from cold annually (NIAID, 2010), while in the UK, about 8 million people are infected by some forms of chronic lung diseases. Surveillance of RT infections especially acute cases in defined populations is required to monitor prevailing pathogens while the determination population groups at risk is important for implementing strategies (Papenburg *et al*., 2010; Savage *et al*., 2011).

Staphylococcus aureus is a common pathogen associated with serious community and hospital acquired diseases and has for long been considered as a major problem of Public health (Pesavento *et al*., 2007). *S. aureus* causes a wide variety of infections ranging from mild skin infections, to life-threatening diseases such as necrotizing pneumonia and bacteremia. *S. aureus* is the most common cause of bloodstream, skin and soft tissue and respiratory tract infections (Diekema *et al*., 2000). The pathology of *S. aureus* is highly variable, and most likely different sets of virulence factors are important in different types of disease. Pathogenicity of isogenic strains observed with mutations in specific virulence genes, as well as the protective effect of immunization with specific virulence factors. In a rat model of endocartitis, fibronectin binding protein (Kuypers and Proctor, 1989; Schennings *et al.*, 1993), capsular polysaccharides (Lee *et al*., 1997) and clumping factor (Moreillon *et al*., 1995) have shown to be important for pathogenesis.

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Staphylococci can be transferred from person to person, upon transmission the organism may become established as part of the recipients normal flora and later be introduced to sterile sites by trauma or invasive procedures. Instead, the organism may be directly introduce into normally sterile sites, such as by a surgeon or nurse during surgery procedures. The person-to-person transfer of *Staphylococci* particularly those that have acquired antimicrobial resistance, most notably occur in hospitals and presents substantial infection control problems (Von *et al*., 2001).

The development of antibiotics for control of pathogenic bacteria has been of essential need in this era of drug-resistant infections (Edward *et al*., 2007). *S. aureus* has a great adaptive power to antimicrobial agents, and little by little it has been acquiring resistance to all antibiotics available in clinical practice. Due to an increasing number of infections caused by multidrug-resistant Methicillin-Resistant *Staphylococcus aureus* (MRSA) strains, therapy has become problematic. Many MRSA are susceptible only to Glycopeptide antibiotics and investigational drugs (Marta and Herminia, 2004).

In the last several years, the new antimicrobials specifically targeted against Gram-positive bacteria or MRSA strains have been launched. But the strains with decreased susceptibility to these antibiotics have been increased and therefore unfortunately, development of new antimicrobial agents in next future seems to be declining (Lorenzo *et al*., 2007). MRSA strains are increasingly encountered and cannot be treated with available β-Lactams. Most of the MSSA strains are resistant to all available quinolones and Vancomycin - Intermediate (VISA) and Vancomycin - Resistant *Staphylococcus aureus* (VRSA) strains have emerged (Kim *et al*., 2007).

The occurrence and proliferation of MRSA strains are a cause for major concern not only in the clinical environment but also in community life. Methicillin-resistant Staphylococcus aureus (MRSA) is an established pathogen that causes hospital- andcommunity-acquired infections worldwide. The prevalence rate of MRSA infections were reported to be the highest in Asia. In fact, MRSA is resistant to virtually all kinds of β-Lactam antibiotics. Though vancomycin and teicoplanin are the two glycopeptides presently used in clinics for the treatment of multi-resistant infections by Gram positive organisms, vancomycin-resistant *Staphylococcus aureus* has been developed in the United States (Hirofumi *et al*., 2005). The spread of resistance to antibiotics undermines the therapeutic utility of antibiotics in current clinical use. MRSA strains appeared in the hospital environment after introduction of the semi synthetic penicillin named as Methicillin, leaving Vancomycin as the last line of defense for MRSA treatment. However, recently appearance of Vancomycin-resistant clinical isolates, no antibiotic class is effective against multi-resistant *Staphylococcus aureus* infections (Magally *et al*., 2006).

In the current scenario, development of drug resistance (Senthilkumar and Prabakaran, 2005; Balaji and Senthilkumar, 2011; Senthilkumar *et al*., 2014a; Behailu *et al*., 2016) among the disease causing microbes to the commonly used antibiotics has provoked the research for discovery of new antimicrobial agents. Overtime, treatment of *S. aureus* infections can be challenging as the widespread use of antibiotics has led some *S. aureus* becoming more resistant to antibiotics (Archer, 1998).

Indiscriminate usage of antibiotics leads to the development of resistant in bacteria **(**Senthilkumar and Prabakaran, 2005; Behailu *et al*., 2016). Many *S. aureus* isolates showed wide range of antibiotic resistant due to antibiotic abuse. Development of new strategies to curb this increasing prevalence of respiratory infection is warranted (Nweze *et al*., 2012). We have previously reported the prevalence of virulent bacterial isolates form the respiratory infected cotton industries labours (Hemalatha *et al*., 2015). This present study is focused on the molecular characterization of enterotoxin producing and antibiotic resistant *S. aureus* isolates from the respiratory infected cotton industries labours.

Elucidation of molecular properties of MRSA has been greatly facilitated by the techniques of molecular epidemiology (Alreshidi *et al*., 2017).

MATERIALS AND METHODS

Bacterial Strains

Staphylococcus aureus (*n*=26) isolates have been illustrated elsewhere (Hemalatha *et al*., 2015) used for the antimicrobial resistant pattern and biotyping in this study.

Antibiotic susceptibility testing

The antimicrobial sensitivity of the test strains of sixteen antibacterial drugs was done using the Kirby-Bauer disc diffusion method. The commercial available antibiotic discs (Hi-Media, India) Viz. M-methicillin (30 µg), Tb-tobramycin (10 µg), A-ampicillin (10 µg), P-penicillin (10 µg), C-chloramphenicol (30 µg), Cf- ciprofloxacin **(**30 µg**)**, T-tetracycline (30 µg), G-gentamycin (30 µg), Ak-amikacin (10 µg), I-imipenem (10 µg), B-bacitracin (10 µg), Ox-oxacillin (5 µg), Of-oflaxacin (2 µg), V-vancomycin (10 µg)**,** Cd-clindamycin(10 µg) and Co-co-

trimozole (25 µg**)** were used. A lawn of test pathogen (18 h old peptone broth culture) was prepared by evenly spreading 100µl of inoculum with the help of a sterilized glass L-rod onto the entire surface of the Muller Hinton agar (Hi-Media, India) plate. Then, commercially available antibiotic discs were gently and firmly placed on the agar plates. The plates were then incubated at 37 °C for 24 h (Senthilkumar *et al*., 2014b). The diameter of the inhibition zones was measured in millimeter using a Hi-Media scale.

Molecular characterization of *S.aureus* isolates

Isolation of genomic DNA from S.aureus isolates

Genomic DNA was isolated by following the method of Senthilkumar *et al*. (2014b**).**

Determination of enterotoxin producing *S.aureus* isolates by multiplex PCR

Several enterotoxins genes, particularly (sea, seb, sec, sed and see) genes were the most identified from *S. aureus* isolates of milk origin. These genes appear to have a critical role in the pathogenicity of S. aureus in subclinical mastitis and food poisoning cases (Walid Saad Mousa, 2017).

Primers

Anticipated sizes of PCR products for the *S. aureus* enterotoxin gene-specific oligonucleotide primers used in this study were *Sea* (GSEAR-1 GGTTATCAATGTGCGG GTGG; G SEAR-2 CGGCACTTTTTTCTCTTCGG), *Seb* (*GSEBR*-1 GTATGGTGGTGTAACTGAGC; GSEBR-2 C CAAATAGTGACGAGTTAGG) *Sec* (GSECR-1 AGATG AAGTAGTTGATGTGTATGG; GSECR-2CACACTTTT AGAATCAACCG); and *Sed* (GSEDR-1 CCAATAATA GGAGAAAATAAAAG; GSEDR-2 ATTGGTATTTTTT TTCGTTC).

Multiplex PCR conditions

Each PCR reaction mixture (20 µl) contained 10 µl of Promega master mix, 1 µl (10 ng) of DNA, 20 pmol (each µl) of *sea, seb, sec, sed* and see primers and makeup to 20 µl with molecular grade water. Evaporation of the reaction mixture was prevented by addition of 100 ml of sterile mineral oil. DNA amplification was carried out in a Genei thermocycler with the following thermal cycling profile: an initial denaturation at 94 °C for 5 min was followed by 35 cycles of amplification (denaturation at 94 °C for 2 min, annealing at 57 °C for 2 min, and extension at 72 °C for 1 min), ending with a final extension at 72 °C for 7 min (Manisha *et al*., 2000).

PCR (Polymerase chain reaction) amplification of antibiotic resistant *Mup* gene and *MecA* gene *S. aureus* isolates

All isolates of *S. aureus* were subjected in PCR assay according to Braoios *et al*. (2009) and Abu (2008) procedure with some modifications. The primers was obtained from Sigma, India and used in the PCR comprised Primer 1 *Mup*A (5'-TAT ATT ATG CGA TGG AAG GTT GG-3') *Mup*B (5'-AAT AAA ATC AGC TGG AAA GTG TTG-3') for amplification of a 456 bp fragment and *Mec*A 1 (5'- AAAATCGATGGTAAAGGTTGGC-3') and *Mec*A 2 (5'- AGTTCTGCAGTACCGGATTTGC-3'), thus yielding an amplicon 533 bp.

Each PCR reaction mixture (20 µl) contained 1 µl of template DNA (Genomic DNA), 2 µl of 10 X PCR buffer, 0.5 µl of 2.0 mM of each primers, 1 µl of 25 mM of each deoxynucleotide triphosphate and 0.5 µl of Taq DNA polymerase (Con. 5U/ µl) and 15.5 µl of molecular grade water. The amplification reaction involved an initial denaturation phase at 94°C for 5 min, followed by 30 amplification cycles (denaturation at 95°C for 1 min, annealing at 59°C for 1 min and elongation at 72°C for 1 min) and a final elongation phase at 72°C for 10 min. After the reaction, 20 µL of the final product was resolved into amplified fragments by electrophoresis in 2% agarose gel at 100 V (45 mA) for 1 h. To estimate the molecular weights of fragments, a 100-bp molecular weight ladder was run on each gel.

RESULTS

Antibiotic sensitivity and resistant pattern of *S. aureus* isolates

Throat *S. aureus isolates*

A panel of 16 antibiotics was employed to study *S. aureus* antibiotic resistance pattern. Of these, ampicillin, penicillin, oxacillin and vancomycin showed 100% resistance against throat *S. aureus* isolates and followed by methicillin (88.8%) and co-trimozole (78%). Amikacin and imipenem were hundred percentages sensitive to throat *S. aureus* isolates. In this study, the lowest resistance was observed to chloramphenicol and ciprofloxacin (11.1%) and increasing percentage of resistance was observed for tetracycline and oflaxacin (66.6%).

Among the 9 *S. aureus* isolates, the highest percentage antibiotics resistance was observed in TSA2, TSA3 and TSA4 (62.5%); followed by TSA1, TSA8 and TSA9 (56.2%). The highest antibiotic resistance was observed in male samples compared than female. Absolutely 7 types of resistance patterns was observed, among M, Tb, A, P, T, B, Ox, Of, V, Co and M, A, P, T, G, Ox, Of, V, Co type of patterns was observed from 20% of *S. aureus*isolates (Table 1 and 2).

Table 1. Antibiotic susceptibility of throat *S. aureus* isolates.

S. aureus isolates/ sex	M	Tb	A	P	C	Cf	T	G	Ak	I	B	Ox	Of	V	Cd	Co	Resistance (%)
	30 mcg	10 mcg	10 mcg	10 mcg	30 mcg	30 mcg	30 mcg	30 mcg	10 mcg	10 mcg	10 mcg	5 mcg	2 mcg	10 mcg	10 mcg	25 mcg	
TSA139	S	R	R	R	S	I	S	R	I	S	R	R	I	R	R	R	56.2
TSA225	R	S	R	R	S	R	R	S	S	S	R	R	R	R	S	R	62.5
TSA339	R	R	R	R	I	S	R	S	I	S	R	R	R	R	S	R	62.5
TSA423	R	R	R	R	I	S	R	S	I	S	R	R	R	R	S	R	62.5
TSA519	R	R	R	R	I	I	R	R	I	S	S	R	I	R	S	S	50
TSA622	R	R	R	R	S	I	S	R	I	S	R	R	I	R	R	R	62.5
TSA723	R	I	R	R	I	I	R	R	S	S	S	R	R	R	S	R	56.2
TSA856	R	I	R	R	I	I	R	R	S	S	S	R	R	R	S	R	56.2
TSA944	R	S	R	R	R	S	S	I	I	S	S	R	R	R	R	S	50

Table 2. Antibiotic resistant patterns of throat *S. aureus* isolates.

Antibiotic resistance pattern	No. of *S. aureus* isolates (%)
Tb, A, P, G, B, Ox, V, Cd, Co	1(11.1%)
M, A, P, CF, T,B,Ox,Of,V,Co	1(11.1%)
M, Tb, A,P, T, B, Ox,Of, V, Co	2(22.2%)
M,Tb,A,P,T,G, Ox,V	1(11.1%)
M,Tb,A,P,G, B, Ox, V, Cd, Co	1(11.1%)
M,A,P,T,G,Ox,Of,V,Co	2(22.2%)
M ,A,P,C,Ox,Of,V,Cd,	1(11.1%)

Nasal *S. aureus isolates*

Among the 16 antibiotics, hundred percentage of resistance was observed against to four antibiotics such as methicillin, ampicillin, penicillin, oflaxacin and oxacillin. The second most antibiotic resistance was observed against tobramycin, gentamycin and vancomycin (86%). All the isolates were sensitive to imipenem and lowest resistance was observed against ciprofloxacin and bacitracin (14.2%). The increasing antibiotic resistance was observed against chloramphenicol (57.1%). Among the 7 isolates, the highest antibiotic resistance was observed from NSA5 and lowest from NSA3.

The highest percentage of antibiotic resistance was observed in female isolates compared than male isolates. Totally 5 types of resistance patterns were observed from 7 isolates, among them M, TB, A, P, C, T, G, OX, OF, V and M, TB, A, P, C, G, AK, OX, OF, V, CO types of patterns were observed from 28.5% of isolates (Table 3 and 4).

Sputum *S. aureus isolates*

About 52.5% of antibiotic resistance was observed from sputum *S. aureus* isolates. The highest percentage of resistance was observed against ampicillin and penicillin (100%) and the second most against vancomycin (90%) and followed by oflaxacin and oxacillin (80%). In this study no one antibiotic was found to be sensitive to all isolates however, the lowest resistance was observed against amikacin and co-trimozole.

The highest percentage of resistance was observed from male samples (56%) than female (46). In this study 8 types of resistance patterns was observed, among them M, TB, A, P, CF, T, I, OX, OF, V, CD and TB, A, P, OF, V patterns was observed from 20% of *S. aureus*isolates (Table 5 and 6).

Table 3. Antibiotic susceptibility of nasal *S. aureus* isolates.

S. aureus isolates	M	Tb	A	P	C	Cf	T	G	Ak	I	B	Ox	Of	V	Cd	Co	Resistance (%)
	30 mcg	10 mcg	10 mcg	10 mcg	30 mcg	30 mcg	30 mcg	30 mcg	10 mcg	10 mcg	10 mcg	5 mcg	2 mcg	10 mcg	10 mcg	25 mcg	
NSA121	R	R	R	R	R	S	R	R	I	S	S	R	R	R	S	S	62.5
NSA255	R	R	R	R	R	S	R	R	I	S	S	R	R	R	S	S	62.5
NSA345	R	R	R	R	S	S	I	I	I	S	S	R	R	S	S	S	37.5
NSA431	R	S	R	R	S	S	S	R	I	S	S	R	R	R	R	S	50
NSA543	R	R	R	R	I	R	R	R	R	S	R	R	R	R	R	S	81.2
NSA622	R	R	R	R	R	S	S	R	R	S	S	R	R	R	I	R	69
NSA723	R	R	R	R	R	S	S	R	R	S	S	R	R	R	I	R	69

Table 4. Antibiotic resistance patterns of nasal *S. aureus* isolates.

Antibiotic resistance pattern	No. of S. aureusisolates (%)
M, TB, A, P, C, T, G, OX, OF, V	2(28.5%)
M, TB, A, P, OX, OF	1(14.2%)
M, A, P, G, OX, OF, V, CD	1(14.2%)
M, TB, A, P, CF, T, G, AK, B, OX, OF, V, CD	1(14.2%)
M, TB, A, P, C, G, AK, OX, OF, V, CO	2(28.5%)

Table 5. Antibiotic susceptibility of sputum *S. aureus* isolates.

S. aureus isolates	M	Tb	A	P	C	Cf	T	G	Ak	I	B	Ox	Of	V	Cd	Co	Resistance (%)
	30 mcg	10 mcg	10 mcg	10 mcg	30 mcg	30 mcg	30 mcg	30 mcg	10 mcg	10 mcg	10 mcg	5 mcg	2 mcg	10 mcg	10 mcg	25 mcg	
SSA120	R	R	R	R	I	R	R	I	I	R	S	R	R	R	R	S	69
SSA229	R	R	R	R	I	R	R	I	I	R	S	R	R	R	R	S	69
SSA333	S	I	R	R	I	S	S	R	S	S	R	R	R	S	I	S	38
SSA451	S	S	R	R	I	I	S	R	R	S	S	R	R	R	I	R	50
SSA539	S	S	R	R	R	I	R	I	I	R	R	R	R	R	R	S	62.5
SSA622	R	R	R	R	R	S	R	R	S	I	R	R	R	R	I	S	69
SSA721	S	R	R	R	S	I	S	S	S	S	S	S	R	R	I	S	31.2
SSA834	S	R	R	R	S	I	S	S	S	S	S	S	R	R	I	S	31.2
SSA931	S	R	R	R	R	R	R	R	S	I	I	R	S	R	R	I	62.5
SSA1028	S	S	R	R	R	R	S	S	S	R	I	R	S	R	S	I	44

Table 6. Antibiotic resistance patterns of sputum *S. aureus* isolates.

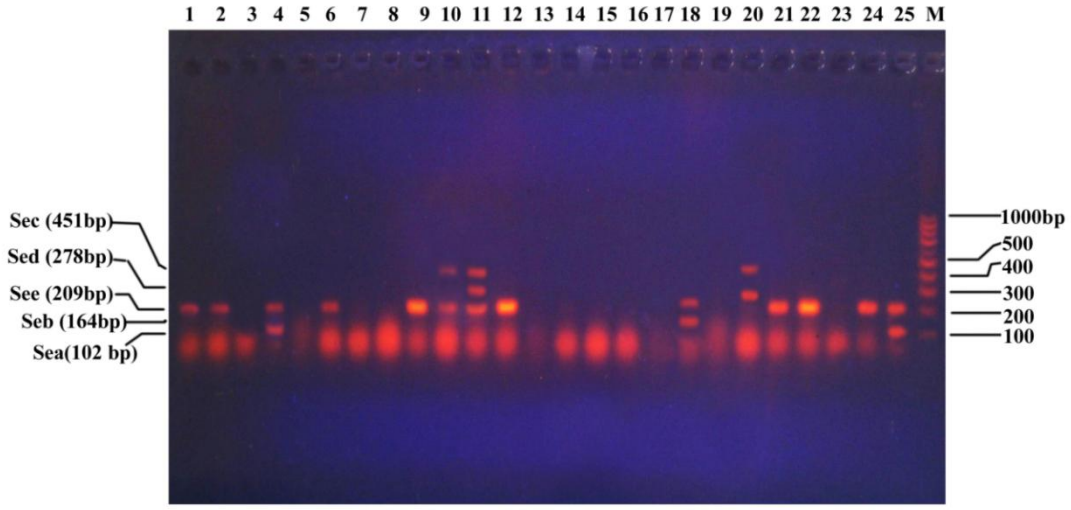
S. No.	Resistance patterns	No. of isolates (%)
1.	M, TB, A, P, CF, T, I, OX, OF, V, CD	2(20%)
2.	A, P, G, B, OX, OF	1(10%)
3.	A, P, G, AK, OX, OF, V, CO	1(10%)
4.	A, P, C, T, I, B, OX, OF, V, CD	1(10%)
5.	M, TB, A, P, C, T, G, B, OX, OF, V	1(10%)
6.	TB, A, P, OF, V	2(20%)
7.	TB, A, P, C, CF, T, G,OX,V,CD	1(10%)
8.	A,P,C,CF,I,OX,V	1(10%)

Determination of Enterotoxin encoding gene of *S. aureus* isolates

In this study PCR amplification was utilized for the determination of virulence factors. Totally 5 types of virulence primer was subjected for determination among them SEE gene was most predominant (50%) and second most SEC (11.5%) and followed by SEA and SED (8%). Among the 3 type's samples, highest percentage of virulence factors was observed from sputum samples (18.1%) and second most nasal isolates (17.4%) and followed by throat isolates (13.3%) (Table 7 and Figure 1).

Table 7. Enterotoxin encoding gene of *S. aureus.*

S. aureus isolates	Enterotoxin encoding gene				
	Sea	Seb	Sec	Sed	See
TSA1	-	-	-	-	+
TSA2	-	-	-	-	+
TSA3	-	-	-	-	-
TSA4	+	-	-	-	+
TSA5	-	-	-	-	-
TSA6	-	-	-	-	+
TSA7	-	-	-	-	-
TSA8	-	-	-	-	-
TSA9	-	-	-	-	+
NSA1	-	-	+	-	+
NSA2	-	-	+	+	+
NSA3	-	-	-	-	+
NSA4	-	-	-	-	-
NSA5	-	-	-	-	-
NSA 6	-	-	-	-	-
NSA 7	-	-	-	-	-
SSA1	-	-	-	-	-
SSA2	-	+	-	-	+
SSA3	-	-	-	-	-
SSA4	-	-	+	+	-
SSA5	-	-	-	-	+
SSA6	-	-	-	-	+
SSA7	-	-	-	-	-
SSA8	-	-	-	-	+
SSA9	+	-	-	-	+
SSA10	-	-	-	-	-



Lane:1-TSA1, Lane:2-TSA2, Lane:3-TSA3, Lane:4-TSA4, Lane:5-TSA5, Lane:6-TSA6, Lane:7-TSA7, Lane:8-TSA8, Lane:9-TSA9, Lane:10-NSA1,Lane:11-NSA2, Lane:12-NSA3, Lane:13-NSA4, Lane:14-NSA5, Lane:15-NSA 6, Lane:16 -NSA 7 Lane:17-SSA1, Lane: 18-SSA2, Lane:19-SSA3, Lane:20-SSA4, Lane:21-SSA5, Lane:22-SSA6, Lane:23-SSA7, Lane:24-SSA8 Lane:25-SSA9, Lane:M- 100 bp DNA Lader

Figure 1. Amplification of Enterotoxin encoding gene of *S. aureus.*

Determination of methicillin resistance *S. aureus* isolates

In this study all isolates of *S. aureus* were subjected to PCR analysis for amplification of methicillin resistance gene. Among them, 69.2% of isolates were resistance to methicillin antibiotic. The highest percentage of result was observed in nasal isolates (100%), second most in throat isolates (89%) and followed by sputum isolates (70%). (Table 8 and Figure 2). In this study, similar result was observed when using disk diffusion method.

Determination of Muprocin resistance *S. aureus* isolates

All isolates were subjected to PCR analysis for determination of Muprocin resistance isolates. Among the 26 isolates, 50% of isolates were resistance to Muprocin antibiotic. The highest percentage of isolate were observed from nasal isolates (86%), second most (55.5%) and followed by sputum samples (20%) (Table 9 and Figure 3).

Table 9. Determination of methicillin and muprocin resistance gene of *S. aureus.*

S. aureus isolates	Methicillin and muprocin resistance gene	
	*Mec*A	*Mup*
TSA1	-	+
TSA2	+	+
TSA3	+	+
TSA4	+	-
TSA5	+	+
TSA6	+	-
TSA7	+	-
TSA8	+	-
TSA9	+	+
NSA1	+	-
NSA2	+	+
NSA3	+	+
NSA4	+	+
NSA5	+	+
NSA 6	+	+
NSA 7	+	+
SSA1	+	+
SSA2	+	+
SSA3	-	-
SSA4	-	-
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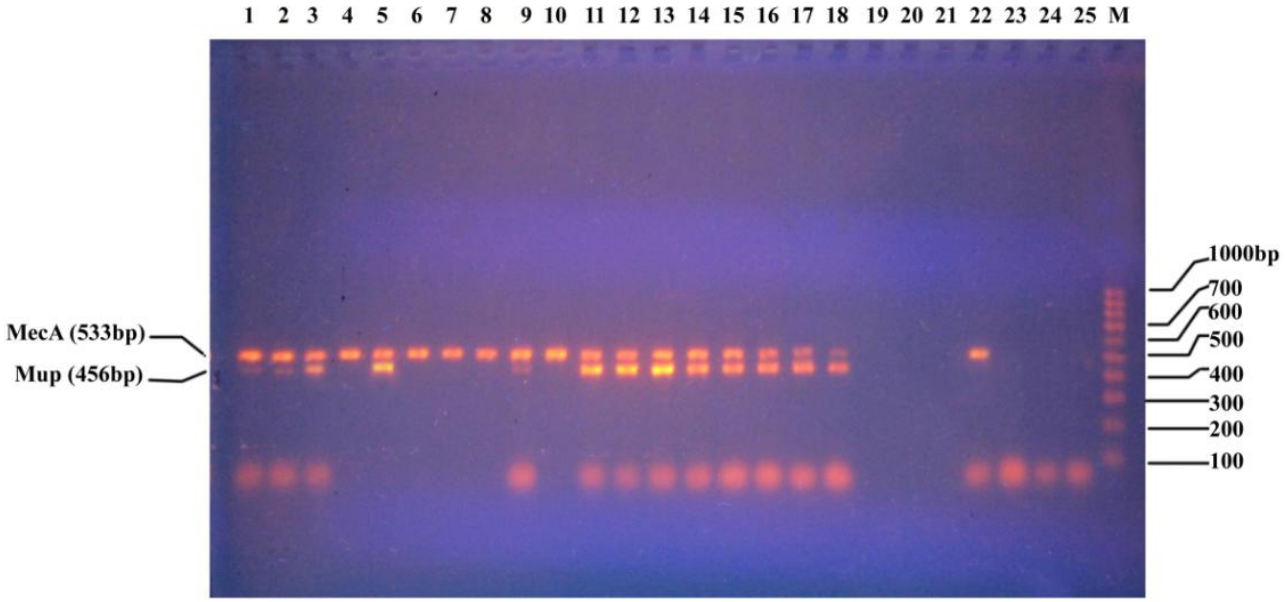
SSA6	+	-
SSA7	-	-
SSA8	-	-
SSA9	-	-
SSA10	S	S



Lane:1-TSA1, Lane:2-TSA2, Lane:3-TSA3, Lane:4-TSA4, Lane:5-TSA5, Lane:6-TSA6, Lane:7-TSA7, Lane:8-TSA8,

Lane:9-TSA9, Lane:10-NSA1,Lane:11-NSA2, Lane:12-NSA3, Lane:13-NSA4, Lane:14-NSA5, Lane:15-NSA 6, Lane:16 -NSA 7

Lane:17-SSA1, Lane: 18-SSA2, Lane:19-SSA3, Lane:20-SSA4, Lane:21-SSA5, Lane:22-SSA6, Lane:23-SSA7, Lane:24-SSA8

Lane:25-SSA9, Lane:M- 100 bp DNA Lader

Figure 3. Multiplex PCR for amplification of methicillin and muprocin resistance gene of *S. Aureus* isolates.

DISCUSSION

The most notable virulence factors associated with *S. aureus* are the heat-stable enterotoxins, that cause the sporadic food-poisoning syndrome or food borne outbreaks and the toxic shock syndrome toxin-1 (TSST-1), which diminishes the immune response of a colonized host (Kerouanton *et al*., 2007; Vancraeynest *et al*., 2007). In the present study, based on the multiplex PCR amplification virulence factors of *S. aureus* enterotoxin gene identified were *See* (50%), *Sec* (11.5%), *Sea* (8%) and *Sed* (8%). Among the 3 types of clinical samples, the highest percentage of virulence factors observed from sputum (18.1%), and second most nasal (17.4%) and followed by throat (13.3%) *S. aureus* isolates. Therefore, sputum *S. aureus* isolates could be easily transmitted to other individuals than other clinical sample isolates.

Antibiotic resistance is acquired by *S. aureus* through extra chromosomal plasmids carrying resistance genes or transposons carrying additional genetic information or mutations in chromosomal genes (Jonathan *et al*., 2005). There is a vivid increase of *S. aureus* that are resistant to multiple antibiotics which poses an increasingly serious problem in many hospitals, and it is responsible for numerous hospital outbreaks (Duckworth, 1993; Allen *et al*., 1994; Francais, 1997). In concurrent with these previous studies, this present study revealed the presence of multidrug resistant resistance among the throat (20%), nasal (28.5%) and sputum (20%) *S. aureus* isolates.

Antibiotic resistance has increased rapidly during the last decade, creating a serious threat to the treatment of infectious diseases. Data from the Canadian Nosocomial Infection Surveillance Program have revealed that the incidence of MRSA, as a proportion of *Staphylococcus aureus* isolates, increased from 1% in 1995 to 8% by the end of 2000 (John , 2002). Summaiya *et al*. (2007) have been used pus, sputum and other clinical samples for the isolation of Staphylococcal species. They identified 20 numbers of *Staphylococcus aureus* and 11 of MRSA from pus samples and 3 of *S. aureus* and one of MRSA from sputum samples.

This present study revealed that 69.2% of isolates harboring *Mec*A gene confers resistance to methicillin antibiotic. The highest percentage of *Mec*A gene was observed in nasal isolates (100%), second most in throat isolates (89%) and followed by sputum isolates (70%). Similarly, Kang-Ju Kim *et al*. (2005) reported the presence of MRSA isolates through the amplification of *mec*A gene and compared with the β-lactamase activity. Further, Durmaz *et al*. (1997) reported that among 73.8% of *Staphylococcus aureus*, 31.3% were MRSA, and 26.2% were CNS out of 513 Staphylococcal species.

Methicillin-resistant *Staphylococcus aureus* (MRSA) cause nosocomial infections and is associated with increased rates of illness and death (Cosgrove *et al*., 2003). Strains of MRSA with decreasing susceptibility to vancomycin were documented in the Qassim region of Saudi Arabia (Abdullateef *et al*., 2012). In concurrent, the present study also revealed the presence of methicliin and vancomycin resistant *S. aureus* isolates.

About 50% of isolates were found to carry *Mup*A gene confers resistance to Muprocin antibiotic. The highest percentage of isolate was observed from nasal isolates (86%), second most (55.5%) and followed by sputum samples (20%).

With increasing pressure to prevent MRSA infection, it is possible that there will be increased use of mupirocin for nasal decolonization of MRSA. High-level mupirocin resistance in *S. aureus* is mediated by a plasmid-encoded *mup*A gene. This gene can be found on conjugative plasmids that carry multiple resistance determinants for other classes of antimicrobial agents. High-level resistance has been associated with decolonization failure, and increased resistance rates have been associated with increased mupirocin use. Institutions that are considering the implementation of widespread mupirocin use should consider these resistance issues and develop strategies to monitor the impact of mupirocin use (Jean *et al*., 2009). Of the 161 *S. aureus* isolates identified, 73 (45.3%) were MRSA, and 82.2% were mupirocin-susceptible 17.8% were mupirocin-resistant (Ghada and Yasmin, 2016).

The present study disclosed prevalence of multidrug resistant and virulent *S. aureus* isolates among the cotton industry labours. Therefore, it is necessary to control the infection by using appropriate antibiotics in order to avoid spreading of bacterial infections as well as spreading antibiotic resistant and public health issues (Senthilkumar *et al*., 2017).

The increase of antibiotic resistance of microorganisms to conventional drugs has necessitated the search for new, efficient and cost effective ways for the control of infectious diseases. The results of different studies provide evidence that some medicinal plants might indeed be potential sources of new antibacterial agents (Kone *et al*., 2004). The use of medicinal plants is part of the Indian tradition. Many local regions all over India have a great variety of vegetation used by the local population to treat and prevent diseases (Bonyadi *et al*., 2009). The extracts of *Aerva lanata* leaves (Arun *et al*., 2014) and *Phyllanthus amarus* (Arun *et al*., 2012), silver metals with the *A. leucophloea* bark extract (Murugan *et al*., 2014) and brevicin (Senbagam *et al*., 2015) have a broad spectrum of antibacterial activity against bacteria including *S. aureus* and support the traditional use of these plants and bacterial metabolites as medicines.

CONCLUSION

Emerging antibiotic resistant among bacterial pathogens threaten the extraordinary health benefits that have been achieved with antibiotics. The necessary preventive measures or alternate drugs are needed well in order to control the bacterial respiratory infections, and spreading of infection as well as antibiotics resistant.

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