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Research Article

PREVALENCE OF TSST PRODUCING COAGULASE-NEGATIVE *STAPHYLOCOCCUS AUREUS* IN WOUND SAMPLES AND CHARACTERIZATION OF MRSA AGAINST TEA EXTRACT

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ABSTRACT

Objective: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a potential pathogen for hospital-acquired infections. This study was conducted to determine the prevalence of MRSA using tea extract.

Methods: All *S. aureus* isolates obtained from wound samples were studied for antibiotic resistance pattern using 23 different antibiotics. Based on coagulase negative, *S. aureus* isolates were identified for toxic shock syndrome toxin (TSST) gene and analyzed using PCR method. The antibacterial activities of tea extract were tested against MRSA using agar well-diffusion method.

Results: A total of 100 wound samples were collected from hospital, where 75% of samples showed presence of *S. aureus.* About 100% resistance to cefoperazone, ampicillin, penicillin, rifampicin, novobiocin, and vancomycin antibiotics was observed. The isolates showed less resistance <50% toward chloramphenicol (30%), ciprofloxacin (25%), gentamycin (52%), amikacin (38%), and imipenem (33%). Twenty-five isolates were selected for MRSA characterization based on multiple drug resistance pattern. Coagulase-negative *S. aureus* isolates showed presence of TSST gene. Tea extract (2%) showed effective antibacterial activity against MRSA strains.

Conclusion: The study showed the presence of MRSA at higher level and suggesting to out further epidemiological study on such infections. However, cost-effective and easily available tea extract was found to be the best antimicrobial agent for preventing such bacterial infection and to reduce the risk of emerging resistance.

Keywords: Wound samples, β-lactamase, Methicillin-resistant *Staphylococcus aureus*, Coagulase negative, Toxic shock syndrome toxin, Tea extract.

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INTRODUCTION

Staphylococcus aureus is among the leading Gram-positive bacteria causing diseases in humans and animals. *S. aureus* can cause wide range of illnesses ranging from minor skin infection to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteremia, and sepsis [1]. MRSA is a bacterium responsible for causing several difficult-to-treat infections in humans. MRSA is a strain of *S. aureus* bacteria that are resistant for larger group of antibiotics called beta-lactams. In hospitals, patients with open wounds, invasive devices, and weakened immune systems are at greater risk for infection [2]. Beta-lactam antibiotics are the preferred drugs for serious *S. aureus* infections. Since the introduction of methicillin, the occurrence of MRSA strains has increased steadily, and nosocomial infections have become a serious problem worldwide. TSS is very rare but is a potentially fatal illness caused by bacterial toxin. Some strains of *S. aureus*, which produce exotoxin TSST-1, are the causative agents for TSS and other strains produce enterotoxin, which is the causative agent of *S. aureus* [3]. The increasing prevalence of multidrug-resistant organisms with no treatment option available for MRSA has become a global problem.

In plant sciences, many plant extracts have shown effective antibacterial activities. However, tea extract from the leaves of plant was found to be the best source, because of its wide range of antioxidant, anti-inflammatory, anticarcinogenic, and antibacterial activities against many pathogens [4]. Green tea extract is very high in polyphenols, flavonoids, catechin, and proanthocyanidins [5]. Catechins are powerful antioxidants, which are being investigated for their ability to prevent cancer and heart disease [6]. Green tea has various advantages over black tea because it undergoes minimal oxidation during the processing, which further prevents various bioactive components from being oxidized [7]. The tea extract shows wide range of activities such as antibacterial, antiviral, antioxidative, antimutagenic, and anticarcinogenic [8]. Thus, the present study provides initiative for the prevention of emerging trends toward antimicrobial resistance among wound isolates of *S. aureus* and provides a platform to initiate epidemiological studies for staphylococcal infections.

METHODS

Pus samples were collected from Namakkal government headquarters hospital and private hospitals in and around Namakkal district, Tamil Nadu, India. Pus was collected from abscesses by needle puncture and from necrotic material using standard culture method. In case of wound pus, sample was collected from patients using sterile swab. Then, it was transferred into sterile test tubes containing brain heart infusion (BHI) broth. All samples were handled aseptically and transferred to research laboratory for bacteriological investigation within 1–2 h of sample collection.

Loopful of culture from peptone water was streaked onto Mannitol salt agar (MSA) plate. The plates were incubated at 37°C for 24–48 h. The clinical isolates were identified on the basis of colony characteristics, Gram staining morphology, and biochemical tests (indole, methyl red, Voges–Proskauer, citrate utilization, triple sugar iron agar, nitrate reduction, urease, gelatin hydrolysis, beta-galactosidase (ONPG),

oxidation-fermentation, alkaline phosphatase, starch hydrolysis, DNase, and carbohydrate fermentation test with maltose, mannitol, glucose, galactose, fructose, and sucrose).

Antibiotic sensitivity and resistance pattern were done by disc diffusion method in which the zone of growth inhibition of the test organism around the antibiotic disc was compared with the standard sensitive organism. The isolated colonies were inoculated in nutrient broth and were incubated at 37°C for 24 h. After incubation, the turbidity was observed. Then, a sterile swab was dipped in the incubated culture and the excess fluid was removed by pressing the swab against test tube wall. Mueller-Hinton agar (commonly used for antibiotic susceptibility testing) plates were prepared and sterilized at 121°C for 15 min and the culture was swabbed onto the plates with sterile swab. Plates were left at room temperature to remove excess of moisture and with the help of sterile forceps, different antibiotics were placed on the agar and kept at refrigerator 30 min for pre-diffusion of disc. Then, the plates were incubated at 37°C for 24 h. Following incubation, the zone of inhibition was noted in diameter and results were interpreted using standard chart. The standard antibiotics used were methicillin, penicillin, chloramphenicol, gentamycin, oxacillin, tobramycin, ampicillin, amikacin, trimaxazole, erythromycin, rifampicin, streptomycin, cefoperazone, levofloxacin, ciprofloxacin, tetracycline, imipenem, sparfloxacin, novobiocin, bacitracin, vancomycin, ofloxacin, clindamycin, and ceftriaxone.

Beta-lactamase production was assayed using the following method, in which the broth culture of test organism was spot inoculated onto Mueller-Hinton agar plate containing 1% starch and penicillin, then incubated overnight at 37°C. The plates were flooded with freshly prepared phosphate-buffered saline containing potassium iodide solution. All *S. aureus* isolates were subjected for the detection of slime production using Congo red agar plate method [9]. The BHI agar was supplemented with 5% sucrose and Congo red. Congo red was prepared as concentrated aqueous solution, mixed with BHI media, and autoclaved at 121°C for 15 min. The isolates were streaked to a length of 1.5 cm on the prepared plate and incubated at 37°C for 48 h and the results were recorded.

The determination of proteolytic activity in *S. aureus* was examined for the formation of zone of clearance around the colonies. Casein hydrolysis was tested on Mueller-Hinton agar (MHA) containing 10% (W/V) skimmed milk powder by streaking 10 µl of culture suspension onto the plates and incubated at 37°C for 24 h. The presence of transparent zone around the colonies showed positive for caseinase activity. The coagulase test was performed for the organism by picking the colony from nutrient agar plate with a sterile glass rod and emulsified in two drops of saline placed on the slide. A drop of undiluted human plasma was added to the emulsion and mixed gently; the prompt clumping of the organism indicates the presence of bound coagulase. The absence of clumping indicates coagulase negative.

The coagulase-negative culture, one to two loopful of cells from blood agar plates were used for DNA extraction using phenol chloroform isolation method [10]. Relative molecular mass of the PCR products was obtained by size comparison with 100 bp ladder marker (Sigma). The sequence of primers used was 5'-ATGGCAGCATCAGCTTGATA-3' and 5'-TTTCCAATAACCACCCGTTT-3' (Genei, Bangalore, India). Amplification was Aldrich, St. Louis, Missouri, United States carried out in a PCR machine using the following steps: 1 cycle at 94°C for 1 min followed by 30 cycles of denaturation for 1 min at 94°C, annealing of primers for 1 min at 53°C, and primer extension for 1 min at 72°C with autoextension. After 30 cycles, the PCR tubes were incubated at 72°C for 5 min before cooling to 4°C. Visualization of amplified products was carried out by electrophoresis using agarose gel in 1 × TBE buffer (TBE is 10.8 g of Tris, 5.5 g of boric acid, 0.93 g of EDTA, and pH 8.3, adjusted to 1000 ml with water) and ethidium bromide staining.

Tea extract was prepared with 2 g of tea powder in 100 ml of boiling water standing for 10 min, after cooling it was filtered and the filtrate was used for the study. MRSA was cultured in peptone water for 18 h and from it 0.2 ml of test culture was taken and added with 10 ml of MHA and poured into sterile petri plates. After solidifying the agar media, wells were made on the agar using sterile stainless steel cork borer and were filled with 150 µl of tea extract. The plates were incubated overnight at 37°C and the diameter of resulting zone of inhibition was measured.

RESULTS

In the current study, 100 pus samples were collected from hospital patents. The samples were from 10 to 50 age groups of 10 y intervals (Table 1). Out of 100 processed samples, 75 samples showed positive results for the presence of *S. aureus*. Maximum infections (more than 75%) were observed in the age group of 41–60 y followed by 21–30 y. However, only 58.82% isolates were obtained in the age group of 21–40 y.

The isolated pus samples were further identified using biochemical and carbohydrate fermentation test (data not shown) and confirmed as *S. aureus*. Out of 75 different isolates, only 25 isolates showed multidrug resistance and all these 25 isolates showed antibiotic resistance to more than 86.9% of antibiotics (Table 2).

Based on the multiple drug resistance pattern studied, 25 isolates were processed for MRSA characterization. The test showed that 15 strains were positive, which was evident with the presence of clear colorless zone around the bacterial growth and it is also an indication of

Table 1: Distribution of study objects over age groups

S. No.	Age groups (y)	Number of samples processed	Number of isolates obtained	% of isolates
1.	10–20	07	05	71.42
2.	21–30	16	12	75.00
3.	31–40	17	10	58.82
4.	41–50	50	40	80.00
5.	51–60	10	08	80.00

Table 2: Multiple drug resistance pattern of MRSA

S. No.	Name of the isolates	Number of drugs showing resistance	Total number of antibiotics	% resistance
1.	S6	20	23	86.90
2.	S10	20	23	86.90
3.	S11	21	23	91.30
4.	S14	21	23	91.30
5.	S16	22	23	95.60
6.	S18	21	23	91.30
7.	S21	20	23	86.90
8.	S27	20	23	86.90
9.	S29	20	23	86.90
10.	S33	20	23	86.90
11.	S35	22	23	95.60
12.	S36	22	23	95.60
13.	S37	21	23	91.30
14.	S38	21	23	91.30
15.	S42	21	23	91.30
16.	S50	20	23	86.90
17.	S53	20	23	86.90
18.	S55	21	23	91.30
19.	S59	21	23	91.30
20	S62	22	23	95.60
21.	S64	20	23	86.90
22.	S67	21	23	91.30
23	S68	20	23	86.90
24	S69	20	23	86.90
25	S70	22	23	95.60

MSRA: Methicillin-resistant *Staphylococcus aureus*

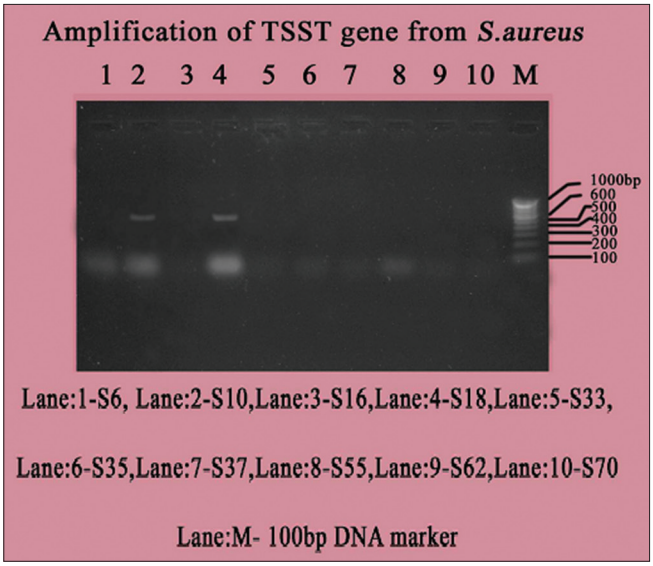

Fig. 1: Detection of TSST gene from MRSA isolates. TSST: Toxic shock syndrome toxin, MRSA: Methicillin-resistant *Staphylococcus aureus*

β-lactamase production and detected by the addition of iodine solution. The remaining 10 strains (MRSA strains) were further studied for TSST gene encoding and only two isolates (S10 and S18) showed TSST positive using specific primers (Fig. 1).

The antibacterial activity of tea extract against MRSA isolates was carried out using different concentration of tea extract (1%, 1.5%, and 2.5%) and 2% was found to be the optimal concentration for effective antibacterial activity on MRSA (Table 3).

DISCUSSION

The morphological characterization of the culture from the collected pus samples was carried out with various selective media such as nutrient, MacConkey, and MSA medium. It was found that the prevalence of MRSA has rapidly increased from 1993 at its tertiary care center from 12% in 1992 to 80.89% in 1999 [11].

Antibiotic resistance is very common in India and the most focusing area. The prevalence of MRSA and susceptibility profile was found with the the study conducted by Indian Network of Antimicrobial Resistance group at 15 Indian territory [12]. Antibiotic resistance patterns of *S. aureus* strains isolated from clinical and food sources are also very common in Libya. Less than 50% of Libyan clinical strains were resistant to penicillin and were β-lactamase producers. However, almost 75% of Libyan strains originating from food were resistant to penicillin and were positive for β-lactamase. Fortunately, none of the Libyan *S. aureus* strains were resistant to methicillin or vancomycin [10]. The β-lactamase activities of *S. aureus* isolated from healthy individuals were quite low compared to those isolated from hospital cases. There was association of β-lactamase-producing *S. aureus* with gender and age in domiciliary condition. Similarly, in hospital isolates, no association of gender and age was observed with the occurrence of β-lactamase-positive *S. aureus*.

All MRSA isolates were subjected for the production of slime and detected using Congo red method [9], where 13 strains were found to be strongly positive, four strains as moderately positive, five strains showed to be weakly positive, and three strains showed negative results. It is reported that there are more chances of cross-infections between hospitalized patients and nursing students, than the medical students who spent less time in comparison to the nursing students who spend more time with the hospital patients and reported 90–95% of beta-lactamase producers among *S. aureus* and CoNS isolates

Table 3: Antibacterial sensitivity pattern of MRSA isolates against tea extract

Isolates	Tea extract concentration and zone of inhibition±SD (mm)			
	1.0 (%)	1.5 (%)	2.0 (%)	2.5 (%)
S6	4±0.5	6±1.0	18±1.0	16±1.5
S10	6±1.0	7±0.5	20±1.2	17±1.3
S16	3±0.3	5±1.1	15±1.3	14±1.6
S18	5±1.0	8±0.6	21±1.0	13±1.0
S33	6±0.4	6±0.9	19±1.7	15±1.4
S35	7±1.2	8±1.0	19±1.0	11±1.0
S37	2±0.5	4±0.5	16±1.9	17±1.2
S55	8±1.1	10±1.1	18±1.0	11±1.2
S62	3±0.8	5±1.2	17±1.0	10±1.0
S70	5±0.3	4±0.4	19±1.1	12±1.1

MSRA: Methicillin-resistant *Staphylococcus aureus*; SD: Standard deviation

obtained from the healthy hospital staff and from the patients who were undergoing cardiac surgery [1]. The slime-producing strains also ranged from 55% to 65% of the isolates. The protease production was carried out from the positive results and 25 MRSA isolates indicated the formation of clear colorless zone around the bacterial growth. The coagulase production carried out from the positive results, showed that 25 MRSA isolates could occur only form the uncoagulated bacterial growth and was reported to be as coagulase-negative *S. aureus*. It is found that most coagulase-negative strains are resistant to β-lactam antibiotics and produce β-lactamase [13].

In comparison to the current study, TST gene encoding TSST-1 was detected in only three (7.5%) of 40 Libyan *S. aureus* clinical strains and in none of the food strains [10]. These three strains were also positive for TSST-1 using TST-RPLA kit. In Taiwan, PCR assay using TSST-1 specific primers was employed [14]. It was found that only 3 strains (4.8 %) out of 62 strains of *S. aureus* obtained from clinical sources were found positive for carrying TST.

It was also observed that the antibacterial activity of tea extract was not demonstrated in all types of tea or in tea grown in all geographical locations for instance 20% extract of Nigeria Lipton tea showed little or no effect on *Proteus* sp., but the same concentration of Kenya tea produced mean inhibition zone of 6.1 mm. The zone of inhibition produced by Kenyan tea on test organism was found to be larger, when compared with zone of inhibition produced using Nigerian Lipton tea. This may be due to the fact that it contains more active ingredients (phytochemical substances) than the Nigerian tea, which resulted in the inhibitory effect on the test organism [15].

CONCLUSION

As a conclusion, this study demonstrates that MRSA is a problem in India and especially in Namakkal district of Tamil Nadu. More number of MRSA isolates were found to be multidrug resistant, for which tea extract was found to be best and easily available source for its prevention and treatment.

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