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 cephalorazone, ampicillin, penicillin, rifampicin, novobiocin, and vancomycin antibiotics was observed. The isolates showed less resistance <50% toward chloramphenicol (50%), ciprofloxacin (25%), gentamycin (52%), amikacin (38%), and imipenem (35%). 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 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.

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 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),...
isolates were subjected for the detection of S. aureus. 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 left at refrigerator 30 min for pre-diffusion of disc. Then, the plates were incubated at 37°C for 24 h. After 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, ceftazidime, levofloxacin, ciprofloxacin, tetracycline, imipenem, sparfloxacin, novobiocin, bacitracin, vancomycin, ofloxacin, clindamycin, and cephradine.

Beta-lactamase production was assayed using the following method, in which the broth 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 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).

| Table 2: Multiple drug resistance pattern of MRSA |
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| **S. No.** | **Name of the isolates** | **Number of drugs showing resistance** | **Total number of antibiotics** | **% of resistance** |
| 1. | 56 | 20 | 23 | 86.90 |
| 2. | 510 | 20 | 23 | 86.90 |
| 3. | 511 | 21 | 23 | 91.30 |
| 4. | 514 | 21 | 23 | 91.30 |
| 5. | 516 | 22 | 23 | 95.60 |
| 6. | 518 | 21 | 23 | 91.30 |
| 7. | 521 | 20 | 23 | 86.90 |
| 8. | 527 | 20 | 23 | 86.90 |
| 9. | 529 | 20 | 23 | 86.90 |
| 10. | 533 | 20 | 23 | 86.90 |
| 11. | 535 | 22 | 23 | 95.60 |
| 12. | 536 | 22 | 23 | 95.60 |
| 13. | 537 | 21 | 23 | 91.30 |
| 14. | 538 | 21 | 23 | 91.30 |
| 15. | 542 | 21 | 23 | 91.30 |
| 16. | 550 | 20 | 23 | 86.90 |
| 17. | 553 | 20 | 23 | 86.90 |
| 18. | 555 | 21 | 23 | 91.30 |
| 19. | 559 | 21 | 23 | 91.30 |
| 20. | 562 | 22 | 23 | 95.60 |
| 21. | 564 | 20 | 23 | 86.90 |
| 22. | 567 | 21 | 23 | 91.30 |
| 23. | 568 | 20 | 23 | 86.90 |
| 24. | 569 | 20 | 23 | 86.90 |
| 25. | 570 | 22 | 23 | 95.60 |

MSRA: 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 S16) 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 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 β-lactamase producers among S. aureus and CoNS isolates 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 uncogaulase 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 three 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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