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Original Research Article

Evaluation of Anti-Microbial Activity of *Ziziphus mauritiana* (Ber), *Ocimum sanctum* (Tulsi) and *Ficus religiosa* (Peepal) on *Staphylococcus aureus* Strains Isolated from Environment of Various ICUs

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ABSTRACT

Acetone and Diethyl ether leaf extracts of 3 medicinal plants traditionally used in medicine were studied for their antimicrobial activity against. Total of 270 samples (240 swabs and 30 expose plates) were collected out of which 39 (14.44%) Staphylococcus aureus strains were isolated from different instruments and surfaces of various ICUs. The antimicrobial activity of plant extracts was determined by using disc diffusion method. The plant extracts showed varied levels of antimicrobial activity against these S. aureus isolates. Acetonic Extracts of Ocimum sanctum (Tulsi) showed good activity against most of the isolates whereas the extracts of Ziziphus mauritiana (ber) showed moderate activity against most of the isolates. The plant extracts showed variable initial sensitive pattern values against these S. aureus isolates being maximum zone size for O. santum (16.5mm) followed by Z. mauritiana (13.5mm) for acetonic extract and for Diethyl ether extract maximum zone size was for Z. mauritiana (14.7mm) followed by O. sanctum (13.5mm). The present study thus suggests the use of these medicinal plants in the treatment of various diseases caused by drug resistant S. aureus strains.

Key Words: Medicinal plants, Antimicrobial activity, Staphylococcus aureus, MDR (multi-drug resistance), Nosocomial infections

INTRODUCTION

Hospital acquired infections are a serious problem in patient care and they adversely affect the mortality and morbidity. Mainly the affected areas are critical care units and acute wards where the patients are critical and immuno compromised. Nosocomial infections complicate the primary disease process and create problems like septicaemia and Acute Respiratory Distress Syndrome. They remain endemic in critical care wards and lead to epidemic outbreaks. In the ICU, the accumulation of a number of immuno compromised patients, their nursing and invasive procedures provide a favourable environment to the growth and transmission of nosocomial infections. Outbreaks of HAI are frequent and may spread between health care facilities (HCF) through patient transfers. Also HAI cause disability, reduce quality of life and create emotional stress. [1]

The environment of critical care unit and health care workers play an important role in transmission of nosocomial infections. Sites that are frequently touched by hands are thought to provide the greatest risk for patients, and those situated right beside patients provide the biggest risk of all. Air conditioning vents, ventilator circuits, door handles, medicine trolley, suction apparatus, and mobile phones etc.

get colonized with bacteria and disseminated in the hospitals which lead to nosocomial infections. Hospital acquired infection caused by multidrug resistant Gram-Positive organisms such as *Staphylococcus aureus* and *Enterococcus* species are a growing problem in many health care institutions. [2]

Staphylococcus aureus is a gram-positive, round-shaped bacterium that is a member of the Firmicutes, and it is a member of the normal flora of the body, frequently found in the nose, respiratory tract, and on the skin. [3] *Staphylococcus aureus* is one of the most important and devastating pathogens among HAIs, and many studies have shown that nosocomial *S. aureus* infection, especially bloodstream infection (SA-BSI) causes a tremendous burdens on the healthcare system. [4] Both community-associated and hospital-acquired infections with Staphylococcus aureus have increased in the past 20 years, and the rise in incidence has been accompanied by a rise in antibiotic-resistant strains- in particular, methicillin-resistant S aureus (MRSA) and, more recently, vancomycin-resistant strains. Hospital environment and health care workers can be the carriers of *Staphylococcus aureus* and *Methicillin resistant Staphylococcus aureus.* [5]

Antibiotics provide the main basis for the therapy of microbial infections. Since the discovery of these antibiotics and their uses as chemotherapeutic agents there was a belief in the medical fraternity that this would lead to the eventual eradication of infectious diseases. However, overuse of antibiotics has become the major factor for the emergence and dissemination of multidrug resistant strains of several groups of microorganisms. Thus, in light of the evidence of rapid global spread of resistant bacterial isolates, the need to find new antimicrobial agents is of paramount importance. For this reason, researchers are increasingly turning their attention to herbal products, looking for new leads to develop better drugs against MDR microbe strains. [6]

A number of phytotherapy manuals have mentioned various medicinal plants for treating infectious diseases due to their availability, fewer side effects and reduced toxicity. There are several reports on the antimicrobial activity of different herbal extracts. There has also been a considerable effort to discover plant-derived antibacterial active against *Staphylococcus aureus* strains, which have developed resistance to many antibiotics. [6]

Driven by this the present study was planned to investigate antibacterial activity of acetone and diethyl ether extract of Ocimum sanctum (Tulsi), Ziziphus mauritiana (Ber) and Ficus religiosa (peepal) against Staphylococcus aureus strains isolated from environment of various ICUs.

Aims and Objectives

1. To isolate and identify Staphylococcus aureus from the surfaces of various objects and instruments in the ICUs.
2. To carry out resistotyping of the isolated S.aureus from the Intensive care unit.
3. To evaluate the antibacterial effect of acetonic and diethyl ether leaf extract of Ocimum sanctum (Tulsi), Ziziphus mauritiana (Ber), Ficus religiosa (peepal).

MATERIALS AND METHODS

The present study was carried out in the Department of Microbiology, MGM Medical College, Kamothe, Navi Mumbai over a period of two years from May 2016 to August 2018. Ethical clearance was obtained from Institutional Ethics Committee prior to study.

Following samples were collected from Paediatric ICU (PICU), Medicine ICU (MICU) and Surgery ICU (SICU)

Category A: This includes air Samples from the AC vents. These air samples were collected by exposed plate method near the AC vent. For isolation of S.aureus from AC vents, Blood Agar plate was exposed near the AC vent at a distance of 30 cm for a period of 15 min.

Category B: This includes swabs collected from the ventilator circuit, suction apparatus, medicine trolley, door handles, tap handles, mobile phones, bed sheets, floor, wall and mobile phones. The swabs obtained from category B were inoculated on Blood Agar and Mannitol salt Agar. Staphylococcus aureus were identified by colony morphology and various biochemical tests. [7]

Colonies obtained after overnight incubation of plates of category A and B were observed and the colony characters were noted. The smears were prepared from the colonies on clean grease free slides and were heat fixed. They were stained by Gram's stain. Antibiotic susceptibility testing of all the isolates was done by Kirby Bauer disc diffusion method on Mueller Hinton agar (Himedia) as recommended by CLSI. [8] Leaf extract of three plants Ocimum sanctum (tulsi), Ziziphus mauritiana (ber) and Ficus religiosa (peepal) was prepared by using two solvents, acetone being polar solvent and diethyl ether as non polar solvent and then 5mm Whatman filter paper disc (pre-sterilized) was dipped in plant extract and dried inside the laminar flow. These discs were used to evaluate the efficiency of these leaf extracts by disc diffusion method on Mueller Hinton agar on which isolated colonies of S.aureus were lawned and incubated for 24 hrs. at 37°C along with plant extract discs and zone size was measured on next day.

RESULTS

In the present study three ICUs including PICU, MICU, SICU were studied for the S.aureus load. Swabs collected from various places (ventilator circuit, suction apparatus, medicine trolley, door handles, bed sheets, floor, wall and HCW's mobile phones) showed presence of S.aureus. Table 1 shows rate of isolation of S.aureus from various sites of PICU, MICU, SICU. Among the various places studied, door knobs (23.33%) and mobile phones of Health Care Worker's (30%) show maximum colonization of S.aureus.

Table 1: Rate of isolation of S.aureus from Various sites of all the ICUs

SR.No.	Samples	PICU	MICU	SICU	TOTAL %
1.	Bed sheet	1/10 (10%)	1/10 (10%)	1/10 (10%)	3/30 (10%)
2.	Door	2/10 (20%)	2/10 (20%)	3/10 (30%)	7/30 (23.33%)
3.	Vacuum suction	1/10 (10%)	1/10 (10%)	0	2/30 (6.67%)
4.	Floor	1/10 (10%)	2/10 (20%)	1/10 (10%)	4/30 (13.33%)
5.	Wall	1/10 (10%)	1/10 (10%)	1/10 (10%)	3/30 (10%)
6.	Ventilator	1/10 (10%)	1/10 (10%)	2/10 (20%)	4/30 (13.33%)
7.	Medical Trolley	1/10 (10%)	1/10 (10%)	2/10 (20%)	4/30 (13.33%)
8.	HCW Mobile Phone	3/10 (30%)	3/10 (30%)	3/10 (30%)	9/30 (30%)
9.	Expose Plate	1/10 (10%)	2/10 (20%)	0	3/30 (10%)

Total of 39 (14.44%) S. aureus strains were isolated from different sites of various ICUs of 270 samples collected (240 swabs and 30 expose plates)

Table 2: Spectrum of S.aureus (n=39) Isolated from ICUs

SR.No.	ICUs s	Total isolates of S.aureus
1.	PICU	12/39 (30.76%)
2.	MICU	14/39 (38.89%)
3.	SICU	13/39 (33.33%)

Table 2 shows that of total Staph. aureus isolated from various ICUs most predominant presence was seen in MICU (38.89%) of all the ICUs.

Table 3 shows the antibiotic sensitivity pattern of S.aureus isolated from various ICUs of which maximum sensitivity was shown to Augmentin (84.61%), Cefuroxime (79.49%) and Clindamycin (71.80%) and high resistance was shown to Penicillin (56.41%), Roxithromycin (53.85%) and Tetracycline (51.28%).

Table 3: Antibiotic Sensitivity Pattern of *S.aureus* Isolated from ICUs (n=39)

SR.No.	Antibiotics	Sensitive (S)	Intermediate (I)	Resistant (R)
1.	Augmentin (AMC)	33 (84.61%)	2 (5.13%)	4 (10.25%)
2.	Cefoxitin (CX)	27 (69.23%)	3 (7.69%)	9 (23.07%)
3.	Penicillin (P)	15 (38.46%)	2 (5.13%)	22 (56.41%)
4.	Clindamycin (CD)	28 (71.80%)	1 (2.56%)	10 (25.64%)
5.	Roxithromycin (RO)	15 (38.46%)	3 (7.69%)	21 (53.85%)
6.	Tetracycline (TE)	18 (46.15%)	1 (2.56%)	20 (51.28%)
7.	Azithromycin(AZM)	24 (61.54%)	1 (2.56%)	14 (35.90%)
8.	Co-Trimoxazole (COT)	19 (48.72%)	4 (10.25%)	16 (41.03%)
9.	Cefuroxime (CXM)	31 (79.49%)	2 (5.13%)	06 (15.38%)
10.	Ciprofloxacin (CIP)	21 (53.85%)	2 (5.13%)	16 (41.02%)
11.	Cefazolin (CZ)	26 (66.66%)	1 (2.56%)	12 (30.77%)
12.	Gentamicin (GEN)	20 (51.28%)	3 (7.69%)	16 (41.03%)

Table 4: Antimicrobial Activity of Three Plant Extract of Polar Solvent (Acetone) on S.aureus isolated (n=39)

SR.No.	Medicinal Plant	Zone size <=5mm	Zone size between 5mm-10mm	Zone size >10 mm
1.	Ocimum sanctum (Tulsi)	4/39 (10.26%)	13/39 (33.33%)	22/39 (56.41%)
2.	Ziziphus mauritiana (Ber)	6/39 (15.38%)	15/39 (38.46%)	18/39 (46.15%)
3.	Ficus religiosa (peepal)	9/39 (23.07%)	18/39 (46.15%)	12/39 (30.76%)

Table 4 shows that among all the 3 medicinal plants used Ocimum sanctum Acetonic leaf extract showed sensitivity to most the S.aureus strains isolated (56.41%) and Ficus religiosa leaf extract showed least sensitivity (30.76%) whereas Ziziphus mauritiana showed moderate sensitivity (46.15%).

Table 5: Antimicrobial Activity of Three Plant Extract of Non-Polar solvent (Diethyl ether) on S.aureus isolated (n=39)

SR.No.	Medicinal Plant	Zone size <=5mm	Zone size between 5mm-10mm	Zone size >10 mm
1.	Ocimum sanctum (Tulsi)	11/39 (28.21%)	18/39 (46.15%)	10/39 (25.64%)
2.	Ziziphus mauritiana (Ber)	17/39 (43.58%)	14/39 (35.89%)	8/39 (20.51%)
3.	Ficus religiosa (peepal)	25/39 (64.10%)	13/39 (33.33%)	1/39 (2.56%)

Table 5 depicts that the diethyl ether leaf extract of Ocimum sanctum showed most sensitivity among all the medicinal plants to S.aureus isolates (25.64%) and least sensitivity is shown by Ficus religiosa (2.56%) whereas moderate sensitivity was shown by Ziziphus mauritiana (20.51%).

Table 6: Maximum Size of Zone of Inhibition by Three Plant Extract in Both Polar and Non-Polar Solvent

SR.No.	Plant Extract	Solvent	Zone of Inhibition (mm)
1.	Ocimum sanctum (Tulsi)	Acetone	16.5 mm
	Ocimum sanctum (Tulsi)	Diethylether	13.5 mm
2.	Ziziphus mauritiana (Ber)	Acetone	13.5 mm
	Ziziphus mauritiana (Ber)	Diethylether	14.7 mm
3.	Ficus religiosa (Peepal)	Acetone	11.5 mm
	Ficus religiosa (Peepal)	Diethylether	10.5 mm

Table 6 shows that the maximum size of zone of inhibition in Acetone leaf extract was shown by Ocimum sanctum 16.5mm and for Diethyl ether leaf extract by Ziziphus mauritiana 14.7mm.

DISCUSSION

In the present study three ICUs including PICU, MICU, SICU were studied for the S.aureus load. Swabs collected from various places (ventilator circuit, suction apparatus, medicine trolley, door handles, bed sheets, floor, wall and HCW's mobile phones) showed presence of S.aureus.

Table 1 shows rate of isolation of S.aureus from various sites of PICU, MICU, SICU. Among the various places studied, door knobs (23.33%) and mobile phones of

Health Care Worker's (30%) show maximum colonization of S.aureus. In a similar study done by Sales V.M. and Oliveira E *et al* in the year 2014 showed the maximum colonization in respirators. In this study out of 6 swabs which were taken from the respirators 5 showed growth. [9]

In a prior study done by Oelberg DG, Joyner SE *et al* in the year 2000 showed that the most commonly contaminated sites in all were personnel hands, computers, blood gas analyzers, door and telephone handles, control buttons and door knobs. [10] In another study by Ulger F., Esen S *et al* in the year 2009 aiming to access contamination of mobile phones of healthcare workers in operating rooms and ICUs showed that 94.5% of the mobile phones were harbouring bacterial pathogens. [11]

Door knobs and mobile phones are the most commonly handled objects by the Health Care Workers. In a study done by Ustun C., Cihangiroglu M. *et al* in the year 2010 on mobile phones showed growth of 9.5% of MRSA. [12] This finding is very significant as they can act as source of infection in the ICUs. In a similar study previously carried out by Tajeddin E., Rashidan *et al* in 2016 showed that the maximum colonization was on ventilator, bed and patient table. [13]

Total of 39 (14.44%) S. aureus strains were isolated from different sites of various ICUs of all 270 samples collected (240 swabs and 30 expose plates). Table 2 shows that of total Staph. aureus isolated from various ICUs most predominant presence was seen in MICU (38.89%) of all the ICUs. *Staph. aureus* being an important nosocomial pathogens their presence in the ICUs needs to be monitored.

In similar study done by French G and Otter J *et al* in 2004 showed that MRSA was cultured from 43% of beds of individuals not known to be suffering from MRSA infection and in another study done by Trick W.E & Temple R.S *et al* 2002 show that VRE was cultured from 13% of surfaces in rooms of patients not known to be colonized with VRE. [14,15]

Tajeddin E., Rashidan *et al* in 2016 also showed the predominant organisms to be *S.aureus* (8.10%) and CONS (19%). [13] Brady R.R. and Varran J. *et al* in 2009 investigated the contamination of clinicians' mobile phones in developed countries, like USA and UK, reported a level of overall mobile phone contamination (pathogenic and non-pathogenic organisms) ranging from 75 % to 96 %. The most common isolated organisms were coagulase-negative staphylococci (CONS) and *Micrococcus*; while between 9 % and 25 % of mobile phones were contaminated by other pathogenic bacteria known to cause HAIs, including methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* (MSSA & MRSA). [16] S.aureus infection may be due to endogenous strains from the patient's native flora. There is emerging evidence that strains are often transmitted among hospitalized patients. These nosocomial strains are increasingly antibiotic resistant and strains with vancomycin resistance being also reported.

Table 3 shows the antibiotic sensitivity pattern of S.aureus isolated from various ICUs of which maximum sensitivity was shown to Augmentin (84.61%), Cefuroxime (79.49%) and Clindamycin (71.80%) and high resistance was shown to Penicillin (56.41%), Roxithromycin (53.85%) and Tetracycline (51.28%). In this study 23.07% *S.aureus* were found Methicillin-Resistant. This finding carries very high significance because of its potential to cause nosocomial infection. In a similar study done Deepa S. and Abishek MU *et al* in 2014 the GPCs isolated from ICUs environment show high resistance to Ampicillin, Cefotaxime, and Cefoxitin. Among the Staphylococci isolates 72.2% were MRSA. The GPCs isolated in this study were highly sensitive to Chloramphenicol and Amikacin. [17]

The hospital environment was highlighted as a potential reservoir of multi drug resistant S.aureus. The higher contamination rate in ICU is coherent with the physical structure, high number of equipments and the conditions of intensive care patients, who tend to have more risk factors and higher infection rates. In such environment the risk of being infected by multidrug resistant S.aureus may increase in the presence of colonized patients or if the length of stay exceeds the average of 15 days.

Table 4 and 5 show the antimicrobial activity of the Polar (acetonic) and Non-polar (Diethyl ether) leaf extract of the three medicinal plants taken in the study against S.aureus isolates from the ICUs. In the study polar leaf extract of Ocimum sanctum was most effective with highest 22 of 39 (56.41%) of isolates showing sensitivity of greater then 10mm followed by Ziziphus mauritiana 18 of 39 (46.15%) showing high sensitivity and least effective was Ficus religiosa 12 of 39 (30.76%). The non- polar (Diethyl ether) leaf extract of Ocimum sanctum showed highest sensitivity to the S.aureus isolates 10 of 39 (25.64%) followed by Ziziphus religiosa 8 of 39 (20.51%) isolates and least effective was Ficus religiosa only 1 of 39 (2.56%) isolate showed sensitivity greater then 10mm. In another study done by Firdaus Jahan et al in 2011 with 5 leaf extract including the 3 leaf extract taken in this study in ethanolic solvent showed that the most effective was *Syzygium cumini* and good effectiveness was also shown by the leaf extract of plants taken in this study Ocimum sanctum and was one of the most effective along with Ziziphus mauritiana. [6] In another study done by Ahmad I.et al in 2001 showed similar finding and presented that Ocimum sanctum, Ziziphus mauritiana and Ficus religiosa are very effective medicinal plants and have a very good antimicrobial activity. [18] Dubey R.et al reported good antibacterial activity of aqueous, methanolic and saponin extracts of *Ziziphus mauritiana* barks against *S.aureus* and other human vaginal pathogens. [19] Ocimum sanctum and Ficus religiosa have a good antimicrobial effect because phytochemical analysis of these plants revealed the presence of glycosides, phenols and tannins in good amount, these substances are known for their antimicrobial properties, whereas antimicrobial activity of Ziziphus mauritiana is due to presence of Saponins, glycosides and flavonoids.

Table 6 show the maximum size of zone of Inhibition of all the 3 leaf extract in both polar and non-polar extracts. In polar extract (acetonic) maximum size of zone of inhibition was shown by Ocimum sanctum 16.5mm followed by Ziziphus mauritiana 13.5mm and least was for Ficus religiosa 11.5mm, in non-polar leaf extract maximum size of zone of inhibition was shown by Ziziphus mauritiana 14.7mm followed by Ocimum sanctum 13.5mm and least was for Ficus religiosa 10.5mm. In a similar study done by Firdaus Jahan et al in 2011 showed that among the 3 plant extract in the present study maximum size of zone of inhibition for MDR and non-MDR S.aureus strains was Ocimum sanctum 15.66mm and 13.66mm respectively. [6]

The variations in the result may be due to the solvent used, incubation temperature and duration, media used for making dilutions, amount of inoculums added, plant species and parts used and moreover, methods used.

CONCLUSION

In this study ICUs were screened for the colonization of S.aureus.

S.aureus isolated in this set up which indicates poor quality of cleaning and disinfection. This was informed to the concerned staff and more vigilance was guaranteed by the ICU in charges.

Isolation of *S.aureus*, and especially the Methicillin Resistant strains was the most serious point of concern. This was informed and immediate control measures were taken.

Antibiotic sensitivity pattern of the isolated S.aureus indicate that all these strains show some degree of resistance to the routinely used as well as higher antibiotics. The frequent and rampant use of antibiotics in the healthcare settings builds up the selective pressure. Hence most of the strains isolated in these ICUs were multidrug resistant.

Indiscriminate use of antibiotics lead to development of MDR strains and these strains in the ICUs are the major risk for the critical patients of ICUs.

The plants used in the present study showed promising antibacterial activity against the resistant S. aureus strains. Thus, the study suggests the use of these plants in the treatment of various diseases caused by resistant bacteria.

Hence, the Authors insist on proper cleaning, disinfection of all the areas and material used in ICUs. Strict execution of Antibiotics policy, its monitoring and stringent measures for disinfection of various areas of ICUs is of utmost importance in order to minimize the microbial load in the critical care set up. Further, the potential of the plants used in the study must be explored more and more, in order to develop an alternate therapy for the treatment of infections caused by antibiotic-resistant bacteria.

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