

**“DETECTION OF METHICILLIN RESISTANT
STAPHYLOCOCCUS AUREUS (MRSA) IN TERTIARY CARE
HOSPITAL OF NORTH KARNATAKA.”**



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2019



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This is to certify that this thesis “**DETECTION OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) IN TERTIARY CARE HOSPITAL OF NORTH KARNATAKA.**” is a bonafide work of **Dr. Basavaraj C Metri** and was carried out under our supervision and guidance in the Department of Microbiology, Shri B. M. Patil Medical College, Hospital & Research Centre, Vijayapura, Karnataka, India.

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I declare that the thesis entitled “**DETECTION OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) IN TERTIARY CARE HOSPITAL OF NORTH KARNATAKA.**” has been prepared by me under the guidance of **Dr B.V. Peerapur**, Professor and Head, Department of Microbiology, Raichur institute of medical sciences(RIMS), Raichur, Karnataka, India. (Former professor and Head, Department of Microbiology, Shri B. M. Patil medical college, Hospital and researchcentre, Vijayapura) No part of this thesis has formed the basis for the award of any degree or fellowship previously.

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
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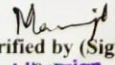
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Abstract:

Introduction : Methicillin resistant *Staphylococcus aureus* strains emerged after the introduction of methicillin. It leads to various type of skin infections, osteoarthritis and respiratory tract infections and other infections in humans. The objectives of the study included 1.To evaluate different screening tests for detection of Methicillin resistant *Staphylococcus aureus* . (MRSA) 2.To know the antimicrobial profile of MRSA strains. 3.To detect of *mec A* gene by molecular methods(PCR) and Molecular identification of MRSA by detection of SCCmec .4.To compare different phenotypic methods with PCR for identification of MRSA.

Methods: The current study was conducted in Microbiology Department, SBMPMCHRC, Vijayapur.A total of 383 *S.aureus* were identified and antimicrobial susceptibility pattern studied for three years period. Statistical analysis: Values were expressed in terms of Mean \pm SD. Analysis was done by using SPSS software version 16. MRSA were detected byThe Cefoxitin Disc Diffusion Test and The Oxacillin Disk Diffusion Method. Genotypic detection of MRSA was done by identification of *mec A* gene by PCR . Molecular characterisation of MRSA isolates were carried out by detection of various staphylococcal cassette chromosome *mec* (SCCmec) by Multiplex PCR.

Results: The current study revealed the prevalence of MRSA in the tertiary care center in this part of India is very high(48.6%). The prevalence of MRSA was more prevalent in males. More number of MRSA were from pus samples (76%). Higher rate of MRSA isolates were from department of Surgery (56.5%). Linezolid (89% sensitive), tetracycline (86%) vancomycin (83%) showed better results against MRSA. Out of 186

isolates of MRSA, oxacillin and cefoxitin detected 80%, 96% MRSA respectively. Results of cefoxitin disc diffusion by cefoxitin is in agreement with the PCR for identification of *mecA* gene.. We found a higher number of multidrug-resistant MRSA (76.8%) in our hospital. This study shows that the prevalent MRSA strains in Vijayapur are *SCCmec-III* and *SCCmec-II*.

Conclusion: In the current study, the prevalence of MRSA in the tertiary hospital in this part of India is very high. Therefore, it is mandatory to choose suitable antibiotics with respect to their antimicrobial pattern for treating the infections .Results of disc diffusion test by cefoxitin is in agreement with *mecA* gene detection by PCR, and therefore the disc diffusion test by cefoxitin is ideal for detection of MRSA and the test can be better alternative to PCR for detection of MRSA in resource poor settings. We found a higher number of multidrug-resistant MRSA in our hospital. If we look into the Indian setting , it seems the burden drug resistant-MRSA is increasing over time. This study shows that the prevalent MRSA strains in Vijayapur are *SCCmec-III* and *SCCmec-II*.

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Abbreviations

- MRSA :Methicillin resistant *Staphylococcus aureus*
- MSSA: Methicillin sensitive *Staphylococcus aureus*
- *S aureus* : *Staphylococcus aureus*
- SCC :Staphylococcal cassette chromosome
- *CoNS* : *Coagulase Negative Staphylococci*
- TSST : Toxic shock syndrome toxin
- MIC : Minimum inhibitory concentration
- ABC : ATP Binding Cassette
- MFS : Major facilitator super family
- PCR : Polymerase chain reaction
- CLSI : Clinical and laboratory standards institute
- CFU : Colony forming unit
- HCW : Health care worker

1. Introduction

INTRODUCTION:

Staphylococcus aureus was first described by Sir Alexander Ogston in 1882. This centuries-old pathogen still causes significant morbidity and mortality despite huge advances in medical care. Indeed, infections due to *S. aureus* continue to grow in number and complexity as a consequence, ironically, of advances in patient care and of its ability to adapt to a changing environment.¹⁻²

Tahnkiwale SS⁴ in his article has stated that “*Staphylococcus aureus* is one of the most frequent bacterial pathogens in humans. It causes skin infections, osteoarthritis and respiratory tract infections and other infections in humans.”³ “*Staphylococcus aureus* has been reported to be the a major cause of community and hospital acquired infection”.⁴

According to Anupurba S⁶ “Methicillin resistant *Staphylococcus aureus* (MRSA) strains emerged after the introduction of methicillin into the clinical practice”.⁵ “MRSA strains were initially described in 1961 and emerged in the last decade as one of the most important nosocomial pathogens. Infected and colonized patients provide the primary reservoir and transmission is mainly through hospital staff. The risk factors which contribute to MRSA are excessive antibiotic usage, prolonged hospitalization, intravascular catheterization and hospitalisation in intensive care unit. With the increased incidence of MRSA, the effectiveness of penicillin and cephalosporins is questioned. In fact many strains of MRSA exhibit resistance to beta -lactams and aminoglycosides.”⁶

“The worst feature of MRSA has been simultaneous drug resistance to many of the antibiotics, chronic carrier stage among health care workers and greater resistance of the strains.”⁷

The current study was undertaken to analyze various techniques for detection of MRSA and know the prevalence of MRSA and their antimicrobial profile.

2. Objectives

OBJECTIVES OF STUDY:

- To evaluate different screening tests for detection of Methicillin resistant *Staphylococcus aureus* . (MRSA)
- To know the antimicrobial profile of MRSA strains.
- To detect of *mec A* gene by molecular methods.(PCR) and Molecular characterisation of MRSA strains by detection of SCCmec .
- To compare different phenotypic methods with PCR for identification of MRSA.

3. Review of Literature

REVIEW OF LITERATURE

Boucher HW.¹ stated that “Micrococcus, which when limited in its extent and activity, causes acute suppurative inflammation (phlegmon), produces, when more extensive and intense in its action on the human system, the most virulent forms of septicaemia and pyaemia...”. “This quote from Sir Alexander Ogston in 1882 describes several facets of *Staphylococcus aureus* that continue to plague physicians in modern times. This centuries-old pathogen still causes significant morbidity and mortality despite huge advances in medical care. Indeed, infections due to *S. aureus* continue to grow in number and complexity as a consequence, ironically, of advances in patient care and of its ability to adapt to a changing environment”.¹

Deresinski S⁸ has written “It was only 1 year after an Oxfordshire constable, Albert Alexander, became the first recipient of penicillin, that Rammelkamp reported the identification of isolates of *Staphylococcus aureus* resistant to this miracle drug. Infections caused by penicillin-resistant *S. aureus* were initially limited to hospitalized patients and were only later detected in the community, where they eventually became common. In an historical reprise, the identification of methicillin-resistant *S. aureus* (MRSA) was reported within 1 year after the 1960 introduction of this semisynthetic penicillin, and once again, an organism that was initially present only in hospitals later became prevalent in the community”.⁸⁻¹¹

According to first edition of Bergey’s manual of systemic bacteriology¹² the family *Micrococcaceae* includes genera: *Staphylococcus*, *Micrococcus*, *Planococcus*, *Stomatococcus*. Application of modern taxonomic concepts to classify *Staphylococci* can

be said to have begun with studies of Baird Parker in 1965. According the CDC“ the genus *Staphylococci* is now classified into 32 species and 15 subspecies based on chemical composition of their cell wall components and other properties”.¹³

Staphylococcus measure 0.5-1.5µm and divide incompletely in three planes to form pairs, tetrads, short chains and clusters of variable size resembling bunch of grapes. [Greek = *Staphyle* mean bunch of grapes and *kokkos* = berry]. *Staphylococci* are non-motile, non-sporing, but can occasionally be capsulated. They are generally aerobes and facultative anaerobes, catalase positive, oxidase negative, utilize carbohydrates fermentatively.¹⁴

Colony morphology⁹:

Staphylococci produce distinctive colonies on a variety of selective and nonselective agar media. The commonly used selective media include mannitol-salt agar, lipase-salt-mannitol agar, phenylethyl alcohol, Trypticase soy agar and Baird-parker agar base supplemented with egg yolk tellurite enrichment.^{9,15} These media inhibit the growth of gram-negative bacteria, but allow the growth of *staphylococci* and other gram positive bacteria. *Staphylococci* grow well in various types of broth media, including tryptose phosphate broth, brain-heart infusion broth and nutrient broth.¹⁵

Habitat:¹⁶

S.aureus is commensal found in humans and its primary habitat is the squamous epithelium of the anterior nares; axilla, perineum and vulval skin which accounts for 67%. The superficial layers of skin are acidic due to lactic acid in sweat. *Staphylococci* can grow at this pH and most strains can grow in presence of 10% NaCl and some even at

15% NaCl. There is approximately 30% chance of infection by *S.aureus* in normal healthy population. It can be transmitted from person to person, which upon may become established as part of the recipients normal flora. Carriers play a key role in epidemiology and in the pathogenesis of infection and they are at greatest risk both for development of hospital and community acquired infections. Majority of *Staphylococcal* infections originate as endogenous heat and dry resistant, thus can persist for long periods on fomites which in turn serve as source of infection.

Cellular components (Virulence factors):

The peptidoglycan and teichoic acid are the major components of the *staphylococcal* cell wall¹⁶. The biological activities of peptidoglycans include endotoxin-like properties, complement activation, generation of chemotactic factors, inflammatory skin reaction^{9,16}.

Slime is a complex extracellular substance produced by many *staphylococci*. *S. epidermidis* and *S. capitis* produce copious amount of this substance. Continued production of slime by a growing clone of cells attached to a polymer surface results in encasement and formation of connective cell-slime clusters biofilm^{9,16,17}.

Once established, the bacterial biofilm may act as a penetration barrier to antibiotics. Biofilm is the material which embeds sessile bacteria adherent to prosthetic surfaces. The mechanism by which coagulase-negative *staphylococci* (CoNS) attach to prosthetic material is a complex and multiple process^{9,16,18}. One important element in this pathway is the *ica* operon, a gene cluster encoding the production of PIA which mediate intercellular adherence of bacteria and the accumulation of multilayered biofilm^{9,16,19}.

Verwey(1940) described an antigenic substance present in the cell walls of *Staphylococcus aureus* which is responsible for agglutination. In 1958, Jensen designated it as PROTEIN-A. This unique protein has the ability to bind the F_C region of IgG molecules. It is bound to the cell wall peptidoglycan and also shed into the medium during growth. The presence of Protein-A on *Staphylococcus aureus* provides the basis for a novel bacteriological technique Co-agglutination, used for identification and detection of bacterial antigen in the body fluids²⁰

In 1978 Todd described a multisystem illness Toxic shock syndrome(TSS) characterized by fever, hypotension, erythroderma, vomiting, diarrhea, etc. Initially it was noticed among menstruating women, who used tampons. Subsequently it has been reported in both males and females. This disease since then ascribed to infection with specific toxigenic strains of *Staphylococcus aureus*²¹.

In 1981 two groups Berg doll, Reiser and cross & Schlivert, Shades and Don – reported “isolation and characterization of unique toxins produced by *Staphylococcus aureus* isolated from toxic shock syndrome patient. These were designated as pyrogenic exotoxin type-C and *staphylococcal* enterotoxin-F. Further studies indicated that these were identical and the toxin is now designated as Toxic Shock Syndrome Toxin (TSST)”

22,23

Risk factors and prevention :²⁴

According to CDC “risk factors for MRSA are excessive antibiotic usage, prolonged hospitalization, intravascular catheterization and hospitalisation in Intensive Care Unit²⁵”. Prevention of *S.aureus* infections according to CDC (1994) recommendations²⁵ for

preventing the spread a consensus panel's definition and management guidelines are eradication of *Staphylococcal* carriage, hospital infection control measure to prevent nosocomial infection, including pre-operative antibiotic prophylaxis²⁵, proper handwashing, appropriate patient isolation, prompt evaluation and intervention when an outbreak occurs, adherence to standard guidelines on disinfection and sterilization and occupational health program for health care providers²⁵.

Antibiotic resistance among MRSA:

Sakoulas G in his article writes that “Historically, the development of antimicrobial resistance in *Staphylococcus aureus* has been rapid. Resistance to penicillin in *S. aureus* was noted only a year after its introduction, and, in the early 1950s, three quarters of *S. aureus* strains in large hospitals in many countries had become penicillin resistant. Currently, 90%–95% of clinical *S. aureus* strains throughout the world are resistant to penicillin. In 1959, the first antistaphylococcal penicillin—methicillin—was introduced. Within 2 years, the first methicillin-resistant *S. aureus* (MRSA) strain emerged”²⁶

In the year 1928 Alexander Fleming made an epoch making discovery. He noted that colonies of *staphylococci* around a mould penicillium, a common laboratory contaminant, were undergoing lysis²⁷. It was the oxford group of workers led by Flory and Chain did lot of experimental works and finally in 1941 the first trials with systemic penicillin began and the result was tremendous.

The *staphylococcus* on which the effect of penicillin was originally seen, had the capacity to produce an enzyme capable of destroying penicillin. Abraham and his associates reported about this enzyme in the year 1940 prior to introduction of penicillin

into human use. The factor responsible for this resistance was first designated as an enzyme penicillinase. When its specific action was noticed as the breaking of β -lactam ring of penicillin, the name was changed to β -lactamases.^{27,28}

Erythromycin came at a time when antibiotic resistant *Staphylococcus aureus* presented a serious clinical problem and this drug proved effective against drug resistant *staphylococcal* infections. However too soon by the year 1953, it became clear that, strains quickly became resistant. The introduction of semisynthetic β -lactamase resistant penicillin — Methicillin in 1960 has made major therapeutic break through for *staphylococcal* infections. But unfortunately the first methicillin resistant *staphylococcus* termed MRSA, was isolated in London in just one year later in 1961, soon after the introduction of Methicillin drug in Britain by Jevons(1961)²⁹.

Methicillin resistance mechanism:⁸

Deresinsky in his article on MRSA states that “The mechanism of resistance to methicillin was uncovered in 1981 with the identification of reduced-affinity penicillinbinding proteins in MRSA. The altered protein, PBP2a (PBP2 in the United Kingdom), retains effective transpeptidase activity while having reduced affinity for penicillin and other available b-lactam antibiotics. PBP2a exhibit both a reduced rate-constant for acylation by b-lactams and elevated dissociation constants. These 2 factors, acting together, prevent acylation of PBP2a and thus result in b-lactam resistance. PBP2a is encoded by the *mecA* gene.⁸ The mobile *mecA* gene complex is comprised of *mecA* together with its regulator genes, *mecI* and *mecR*, and resides within a genomic island, the staphylococcal cassette chromosome *mec* (SCC*mec*) that constitutes 1%–2% of the

~2.9 million–bp *S. aureus* chromosome⁸. SCCmec also contains the insertion sequence, IS431mec, as well as recombinases necessary for site-specific integration and excision. Some SCCmec types also contain various additional genetic elements, such as Tn554 (which encodes resistance to macrolides, clindamycin, and streptogramin B) and pT181 (which encodes resistance to tetracyclines) . The expression of PBP2a is induced by the binding of β -lactam antibiotics to a cytoplasmic membrane sensor-transducer receptor encoded by the *mecR1* gene, triggering a signal leading to the proteolytic release of the *mecI* repressor from the operator region of the *mecA* gene. Phenotypic resistance to methicillin is variably expressed, and population analysis demonstrates that each MRSA strain has a characteristic growth profile at each concentration of methicillin examined . In contrast to this heterogeneously expressed resistance to methicillin, homogeneous resistance requires the interaction of additional factors, such as the *femA–F* genes that are involved in peptidoglycan synthesis” .

REVIEW OF PREVIOUS STUDIES:

Prevalence:

In a study conducted by K Rajadurai et al³⁰ in 2006, *S.aureus* isolates, 250 (31.1%) out of 803 clinical samples were MRSA. Almost all strains were resistant to Penicillin, Ampicillin, Gentamicin, Cotrimoxazole, Cefotaxime. Multidrug resistance was observed among 63 % and all isolates were sensitive to Vancomycin.

In a study conducted by Vijay Mohan et al³¹ in 2014, “Out of total 61 strains of *S.aureus* isolated, 16(22.2%) were found to be Methicillin resistant; which were sensitive to Amikacin, Clindamycin, Vancomycin, Linezolid and Rifampin”. Ameer Abbas et al³²

in 2015 conducted study on prevalence and antibiogram of hospital and community acquired MRSA in Rajasthan. Of the 500 strains of *S.aureus* isolated from different clinical samples, 201 were MRSA (40.2%), with major sensitivity to Vancomycin and Linezolid. The study showed 16.38% resistance to Teicoplanin.

Sex-wise distribution:

Goyal A. et al³³ in 2013 conducted study which revealed 58% male preponderance below 20 years of age group. No resistance Vancomycin, Linezolid, Teicoplanin. Fomda BA et al³⁴ in 2014 conducted study on nasal carriage of Methicillin resistant *Staphylococcus aureus* among healthy population of Kashmir, showing 53% male preponderance among the age group of 21–30 years respectively. All the isolates were sensitive to Clindamycin, Vancomycin, Linezolid, Teicoplanin.

Sumit Kumar et al³⁵ in 2015 conducted a study on prevalence of MRSA in patients admitted in a tertiary care hospital of North India showing 55% male preponderance compared to females. Common age group affected was 21-40 years. Antibiotic susceptibility showed 32% resistance to Ciprofloxacin, 21% resistance to Amikacin, 45% resistance to Clindamycin. All the strains were sensitive to Vancomycin, Teicoplanin, Linezolid.

Phenotypic detection methods:

Karami S. et al³⁶ in 2011 conducted a study evaluation of five phenotypic tests for identification of MRSA. Out of 294 isolates, MRSA was detected with specificity of 98.9%, 97.9% by Cefoxitin Disc Diffusion method, CHROMagar MRSA respectively.

Priya et al³⁷ in 2011 conducted a study on detection of MRSA strains and susceptibility

patterns. PCR *mecA* was taken as the gold standard in this study. Out of 200 isolates, 70 were MRSA positive by *mecA* gene detection. These isolates were 100%, 99.2% found specific by Cefoxitin Disc Diffusion method and CHROMagar MRSA respectively. Comparison of Cefoxitin disc diffusion method and *mecA* for MRSA detection were correlated, which confirmed Cefoxitin as the surrogate marker for *mecA* gene. CHROMagar MRSA had an accuracy in detecting MRSA isolates.

Zaidi et al³⁸ in 2013 conducted a study on comparison of Chromogenic agar medium and Disc diffusion test for detection of HA-MRSA from patients. Out of 148 samples, 96 were culture positive for MRSA, 84.6% were detected specific by Cefoxitin disc and 100% by CHROMagar MRSA. Isolates positive by disc diffusion were confirmed by Chromogenic agar. CHROMagar MRSA generated positive results in lesser period of time in this study with higher sensitivity and specificity. Poojary et al³⁹ in 2015 conducted a study on rapid identification of MRSA using Chromogenic media compared with conventional methods. Out of 246 samples, 40(16.26%) were culture positive for MRSA, detected by CHROMagar MRSA and disc diffusion method. 83.7%, 93% specificities were observed with Cefoxitin disc diffusion and CHROMagar MRSA. Turn around time for CHROMagar was reduced compared to disc diffusion methods in the study, concluding CHROMagar as reliable test for the early identification of MRSA and to initiate decolonization measures.

Type of predominant sample:

Anupurba S et al⁶ in 2013 conducted a study on MRSA. Out of 549 strains of *S.aureus* isolated from different clinical specimen, 52.5% were isolated from pus

samples. 55% were found to be MRSA. Of the total isolates, 80% were found to be resistant to , Ciprofloxacin, Gentamicin, Penicillin and Tetracycline. Many strains were multidrug resistant. Uma Chaudhary et al⁴⁰ in 2012 conducted a study on comparative study of community and health care associated Methicillin resistant *Staphylococcus aureus* infections, where maximum isolates were from pus samples (43.2%) received from various departments.

In the study conducted by Bilal Ahmed Mir et al⁴¹ in 2013 on antimicrobial susceptibility patterns and prevalence in MRSA and coagulase negative *Staphylococcus aureus* in a tertiary center, Jammu and Kashmir. MRSA were isolated in majority from pus samples received from various departments contributing to 52% of total samples.

Sensitivity pattern by disc diffusion:

Kunsang Ongmoo Bhutia et al⁴² in 2012 conducted a study on occurrence and antimicrobial susceptibility of CA-MRSA, HA-MRSA in Sikkim, out of 38.65% MRSA strains isolated, maximum resistance of 10.5% was observed with Linezolid. All the isolates were sensitive to Vancomycin, Teicoplanin. 92% isolates were resistant to Cotrimoxazole, 97% resistant to Amoxicillin, 31% resistant to Ciprofloxacin. Hajera M et al⁴³ in 2014 conducted a study on antimicrobial patterns and prevalence and of MRSA from a tertiary center, in which 100% resistance was observed with Penicillin, Cefoxitin, 87% with Amoxicillin and Clavulanate combination, 4.2% with Clindamycin, 6.7% with Linezolid, 2% with Vancomycin, 1.6% with Teicoplanin respectively.

Apoorva Tripathi et al⁴⁴ in 2015 conducted a study on prevalence and antimicrobial susceptibility patterns of Methicillin resistant *Staphylococcus aureus* in

central India, with 100% sensitivity to Clindamycin, Vancomycin, Linezolid, Teicoplanin. Isolates were 100% resistant to Penicillin and Cefoxitin, 98% resistant to Amoxicillin, 47% to Cotrimoxazole, 56% to Ciprofloxacin respectively.

4. Materials and Methods

MATERIALS AND METHODS:

The study was conducted in the Department of Microbiology, Shri B.M Patil Medical College Hospital and Research center, Vijayapur. *S. aureus* isolated from all the clinical samples formed the material for study. Clinical samples like pus, urine, sputum, blood and other body fluids of patients attending Shri B M Patil Medical College and Hospital were selected for study.

Sample size :With 5% margin of error and at 95% level of confidence and prevalence rate of 40%, the calculated sample size $n=383$ using statistical formula

$$n=(1.96)^2 p(1-p)/d^2$$

Hence a minimum of 383 *S.aureus* were identified and antimicrobial susceptibility pattern studied for three years period

Statistical analysis: Values were expressed in terms of Mean \pm SD. Analysis was done by using SPSS software version 16. $P \leq 0.05$ was considered statistically significant.

Inclusion criterion: Samples which yielded pure growth of *S. aureus* were included.

Exclusion criterion: Samples which did not yield *S. aureus* were excluded from the study.

Specimens were screened by preliminary Gram's stain and then inoculated on 10% sheep blood agar and MacConkey's agar. *S. aureus* were identified by conventional techniques. Antimicrobial susceptibility testing of the isolates was performed by Kirby Bauer disc diffusion method using following discs. penicillin-G (10 unit); cloxacillin (30 μ g); cephalixin (30 μ g); cefuroxime (30 μ g); tetracycline (30 μ g) ; erythromycin

(15µg); gentamicin (10µg); ciprofloxacin (5µg); pefloxacin (5µg); Cefoperazone /salbactam(75 µg/ 30 µg); azithromycin(15µg); linezolid (15µg). Vancomycin(30µg); piperacillin/tazobactam(100µg/10 µg); amoxicillin/clavulanic acid (20 µg /10 µg). The data were recorded and analyzed at the completion of the study as per recommendations of the CLSI.⁴⁵

Detection of MRSA:

- The Cefoxitin Disc Diffusion Test:

The Cefoxitin disc diffusion method was carried out on Mueller-Hinton agar by using a 30 µg cefoxitin disc. Inoculum was prepared and compared with 0.5 McFarland turbidity constant. Mueller-Hinton agar was inoculated and excess was removed. Cefoxitin 30mcg discs were applied with forceps and pressed gently to ensure even contact with the medium. The plates were incubated for 18 – 24 hours at 37°C. Interpretation was done using the Kirby-Bauer charts. An inhibition zone diameter of \leq 21 mm was reported as methicillin resistant.⁴⁶

- The Oxacillin Disk Diffusion Method:

The Oxacillin disk (1 µg) diffusion method was carried out on Mueller-Hinton agar which was supplemented with 4% NaCl to detect MRSA according to the CLSI guidelines. The isolates were considered as resistant when the diameter of inhibition was \leq 10 mm.⁴⁷

Genotypic detection of MRSA by PCR (mec A gene):⁴⁸⁻⁵⁰ DNA Extraction Procedure was done by Modified Proteinase-K method. MRSA strains were amplified by

conventional PCR. Following set of PCR primers were used which were specific to Methicillin resistant *S.aureus*.¹

Forward Primer : 5'- TGC TAT CCA CCC TCA AAC AGG -3'

Reverse Primer : 3'-AAC GTT GTA ACC ACC CCA AGA -5'

AMPLIQON RED 2X Mastermix was used which contain following reagents

Tris-HCL pH 8.5, (NH₄)₂SO₄, 3mM MgCl₂, 0.2% Tween 20, 0.4mM of each dNTP ,
0.2 units/μl Ampliqon Taq DNA Polymerase

The PCR conditions were as follows

Initial denaturation (94⁰ C, 5min), Denaturation (94⁰ C, 1 min), Annealing(50⁰ C,
1 min)

Extension (72⁰ C, 2 min), Final extension(72⁰ C for 5 min)

Reagents with their company names : PCR Master mix: Ampliqon Oligonucleotide

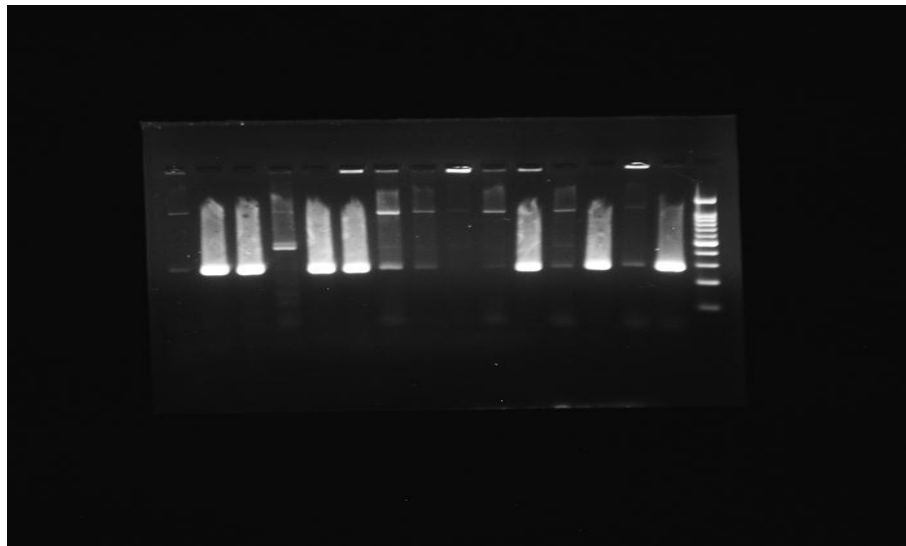
Primers: Bioserve India pvt Ltd

Instruments:

Thermal cycler: Applied Biosystems, USA. Electrophoresis apparatus: Bio bee
Tech, Bangalore. Gel Documentation system: Major Science, USA.

Interpretation:

- The PCR was carried out for MRSA strains with MRSA specific primer set. After PCR, the agarose gel electrophoresis was done where PCR amplified products were run on a 2% agarose gel.
- After running the electrophoresis, the amplified products will get separated on the gel according to the product size which was determined while choosing a primer.
- We had chosen a primer set which gives amplified product of size 280 base pair. So the well which gives DNA band of 280 base pair is considered positive, whereas the well which does not have any DNA band is indicated as negative.
- The size or the position of the DNA band can be known by running the DNA ladder simultaneously with each gel.



Results of *mecA* gene

Lane 1: Molecular weight marker

Lane 2: MRSA ATCC 43300

Lane 3: MSSA ATCC 25923

Lane 4, 6, 11,12,14 and 15: MRSA isolates from clinical samples(280 BP)

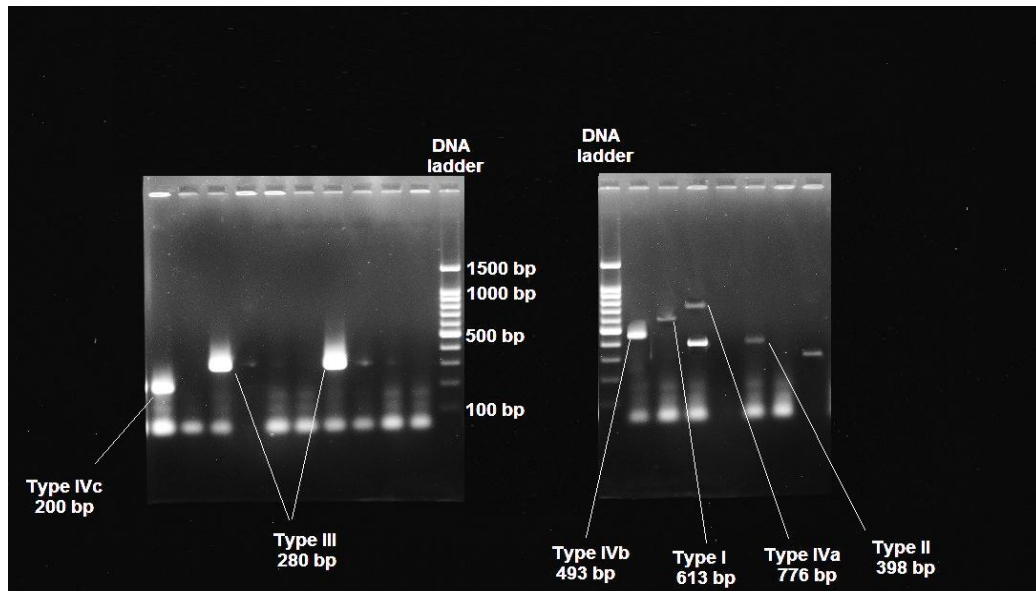
Lane 5,7-10,13,16: MSSA isolates from clinical sample

Multiplex PCR⁴⁸⁻⁵² was carried out for detection of SCCmec types, using following primers.

Table 1: SCCmec types and their primers.

Primer Name	Primer Sequence 5' --> 3'	Primer Length (bp)	Amplicon size in base pair
mecA F	TGCTATCCACCCTCAAACAGG	21	286
mecA R	AACGTTGTAACCACCCCAAGA	21	
Type I F	GCTTTAAAGAGTGTCGTTACAGG	23	613
Type I R	GTTCTCTCATAGTATGACGTCC	22	
Type II F	CGTTGAAGATGATGAAGCG	19	398
Type II R	CGAAATCAATGGTTAATGGACC	22	
Type III F	CCATATTGTGTACGATGCG	19	280
Type III R	CCTTAGTTGTCGTAACAGATCG	22	
Type IVa F	GCCTTATTCGAAGAAACCG	19	776
Type IVa R	CTACTCTTCTGAAAAGCGTCG	21	
Type IVb F	TCTGGAATTACTTCAGCTGC	20	493
Type IVbR	AAACAATATTGCTCTCCCTC	20	
Type IVc F	ACAATATTTGTATTATCGGAGAGC	24	200
Type IVc R	TTGGTATGAGGTATTGCTGG	20	
Type IVd F	CTCAAATACGGACCCCAATACA	23	881
Type IVdR	TGCTCCAGTAATTGCTAAAG	20	
Type V F	GAACATTGTTACTTAAATGAGCG	23	325
Type V R	TGAAAGTTGTACCCTTGACACC	22	

SCCmec typing in MRSA isolates.



5. Results

RESULTS:

Table 2: Distribution of *S. aureus* according to age.

AGE (YRS)	N	%
<1	4	1.0
1-10	48	12.5
11-20	33	8.6
21-30	71	18.5
31-40	46	12.0
41-50	63	16.4
51-60	55	14.4
>80	63	16.4
Total	383	100.0

MEAN±SD=38.4±21.7

Figure 1: Distribution of *S. aureus* according to age.

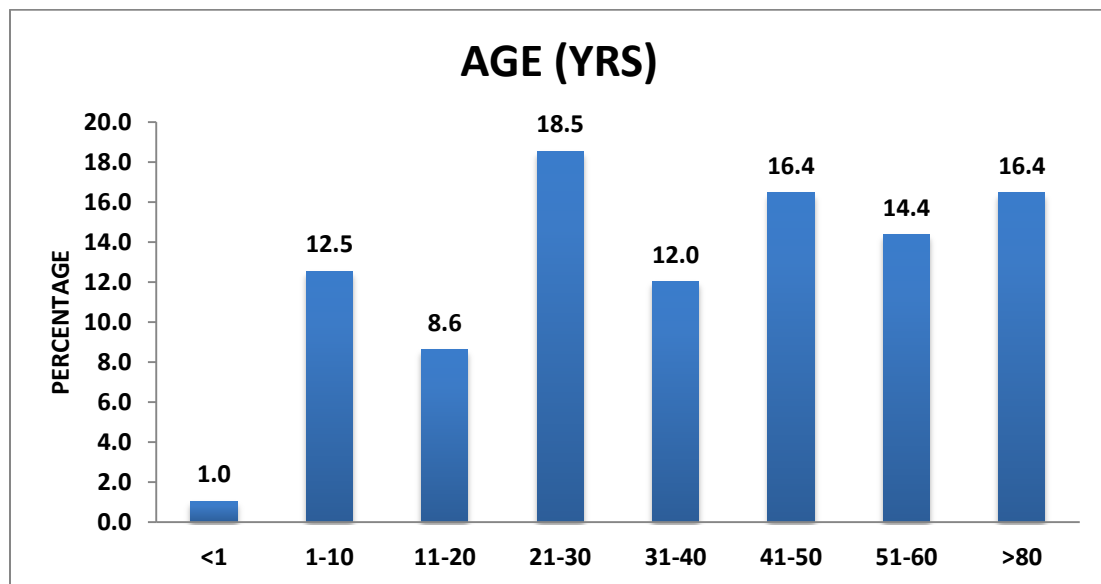


Table 3: Distribution of *S. aureus* according to sex.

SEX	N	%
Male	237	61.9
Female	146	38.1
Total	383	100.0

Figure 2: Distribution of *S. aureus* according to sex.

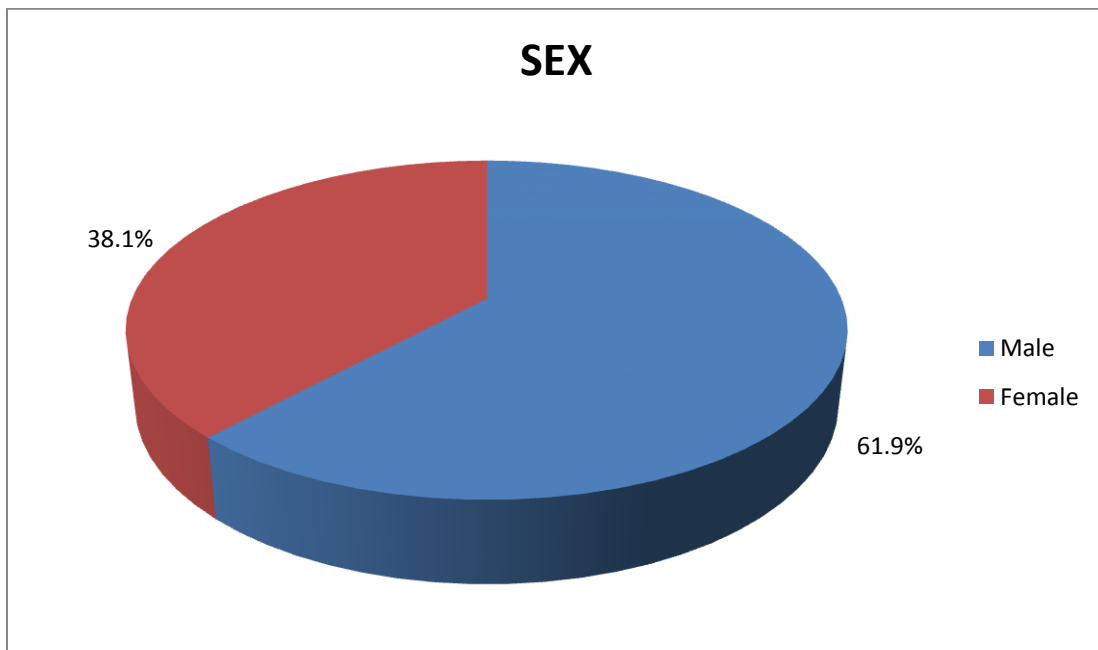


Table 4: Association Of Age WithSex Among*S. aureus*.

AGE (YRS)	Male		Female		p value
	N	%	N	%	
<1	2	0.8	2	1.4	0.013*
1-10	33	13.9	15	10.3	
11-20	16	6.8	17	11.6	
21-30	34	14.3	37	25.3	
31-40	24	10.1	22	15.1	
41-50	46	19.4	17	11.6	
51-60	39	16.5	16	11.0	
>80	43	18.1	20	13.7	
Total	237	100.0	146	100.0	

Figure 3 : Association Of Age With Sex Among *S. aureus*.

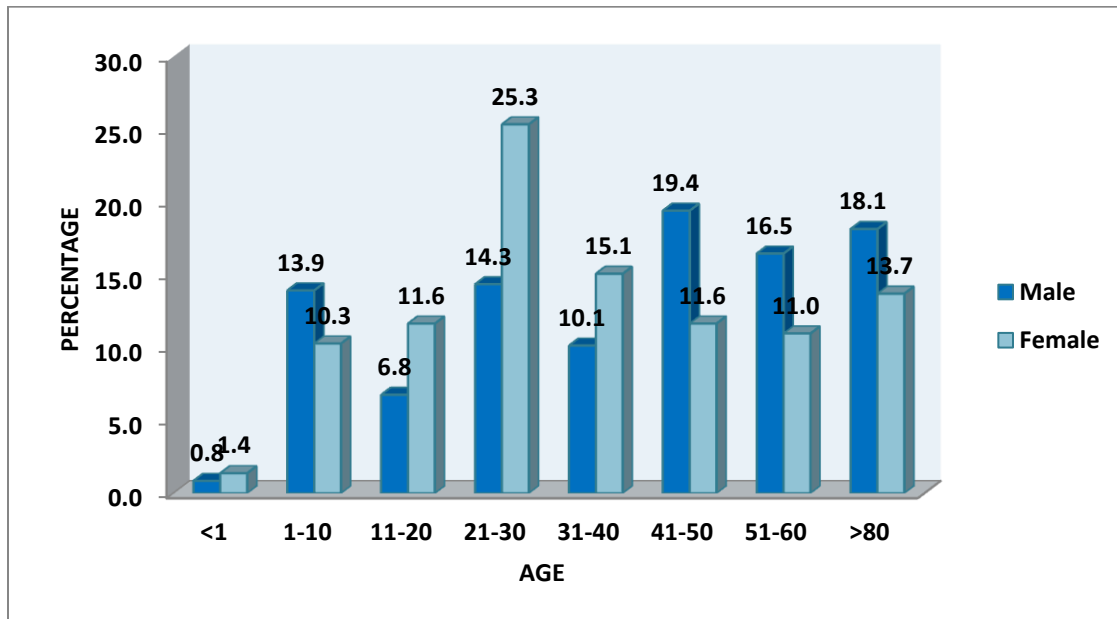


Table 5: Ward wise Distribution of *S. aureus*.

WARD	N	%
IPD	281	73.4
OPD	102	26.6
Total	383	100

Figure 4: Ward wise Distribution of *S. aureus*.

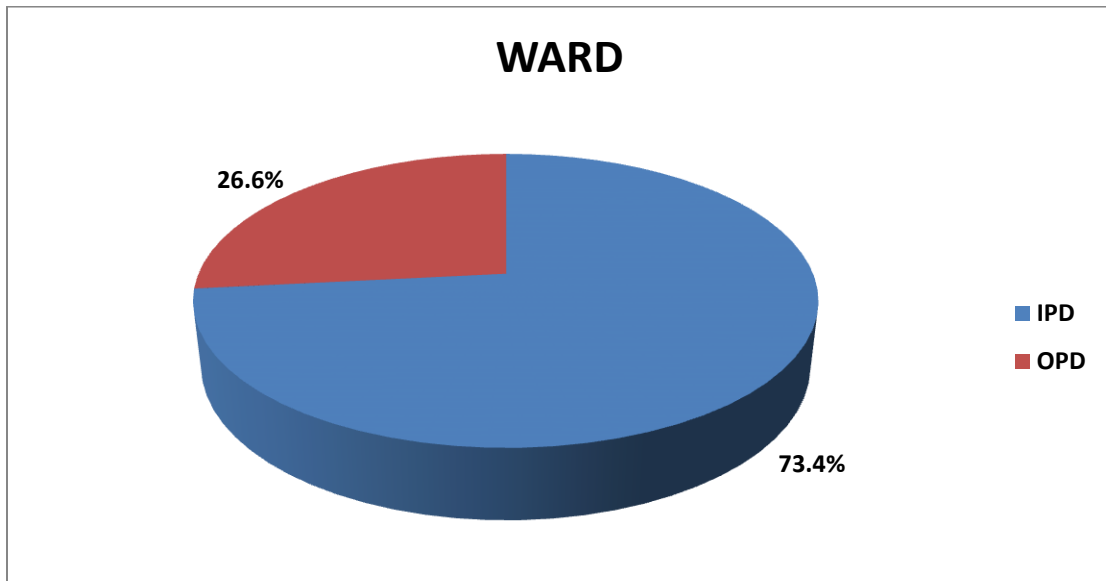


Table 6: Specimen wise distribution of *S. aureus*.

Specimen	N	%
Blood	8	2.1
CSF	3	0.8
Ear swab/discharge	30	7.8
Pus	304	79.4
Sputum	14	3.7
Throat swab	6	1.6
Urine	15	3.9
Vaginal swab	3	0.8
Total	383	100

Figure 5: Specimen wise distribution of *S. aureus*.

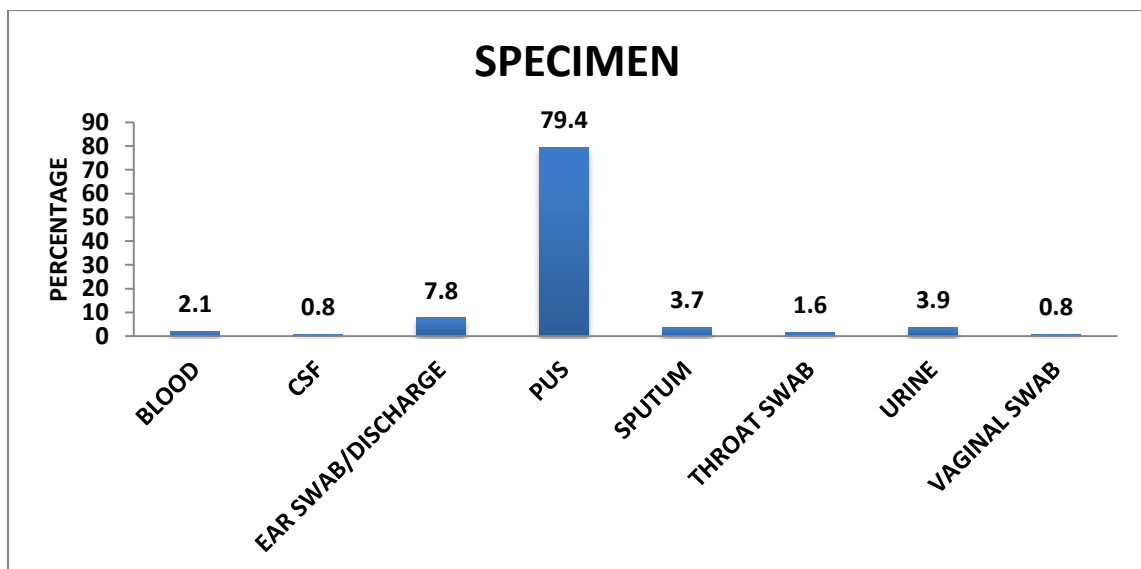


Table 7: Distribution of *S. aureus* according to department

Department	N	%
Ent	49	12.8
Medicine	29	7.6
Obg	22	5.7
Ortho	25	6.5
Pediatrics	11	2.9
Skin	25	6.5
Surgery	209	54.6
Urology	13	3.4
Total	383	100

Figure 6: Distribution of *S. aureus* according to department

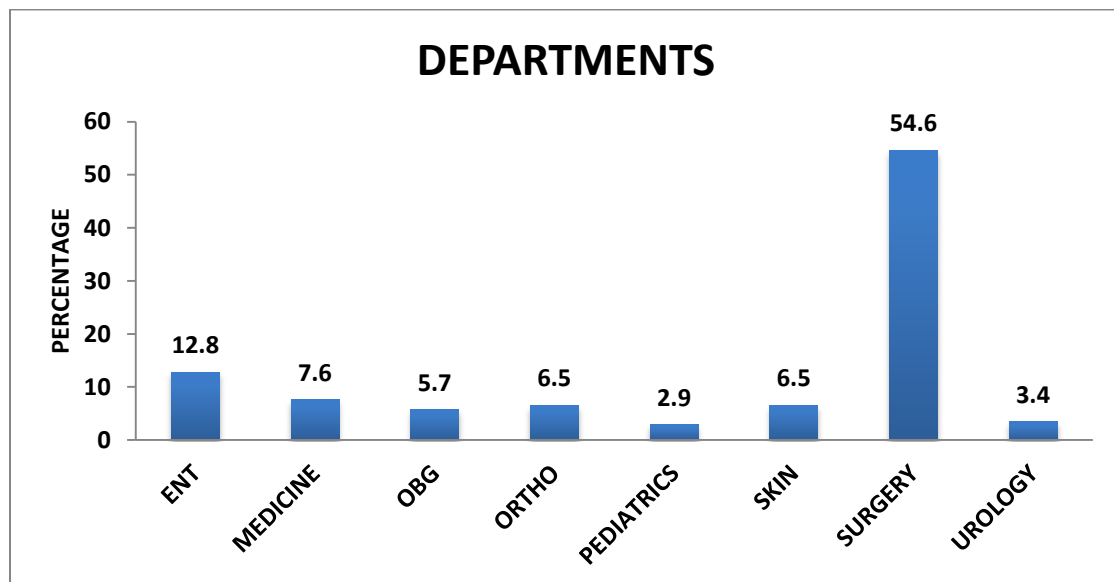


Table 8: Distribution of MRSA and MSSA

S. aureus	N	%
MSSA	197	51.4
MRSA	186	48.6
Total	383	100

Figure 7: Distribution of MRSA and MSSA

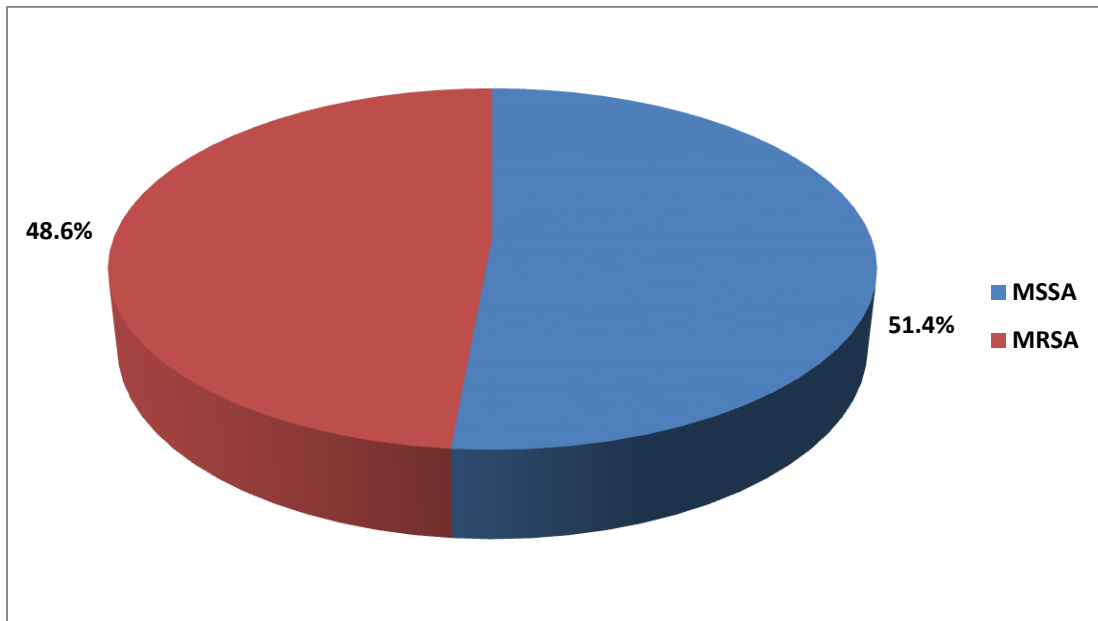


Table 9: Distribution of MRSA and MSSA according to age

AGE (YRS)	MSSA		MRSA		Total	p value
	N	%	N	%		
<1	4	2.0	0	0.0	4	0.273
1-10	24	12.2	24	12.9	48	
11-20	16	8.1	17	9.1	33	
21-30	35	17.8	36	19.4	71	
31-40	24	12.2	22	11.8	46	
41-50	35	17.8	28	15.1	63	
51-60	33	16.8	22	11.8	55	
>80	26	13.2	37	19.9	63	
Total	197	100.0	186	100.0	383	

Figure 8: Distribution of MRSA and MSSA according to age

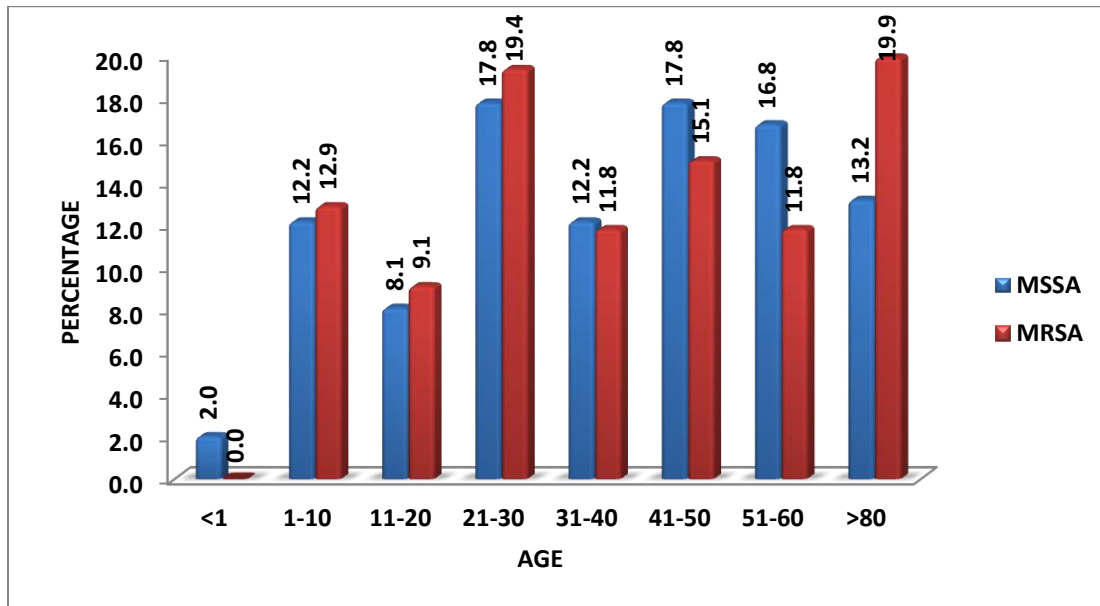


Table 10: Distribution of MRSA and MSSA according to sex

SEX	MSSA		MRSA		Total	p value
	N	%	N	%		
MALE	116	58.8	121	65.1	237	0.214
FEMALE	81	41.2	65	34.9	146	
Total	197	100	186	100	383	

Figure 9: Distribution of MRSA and MSSA according to sex

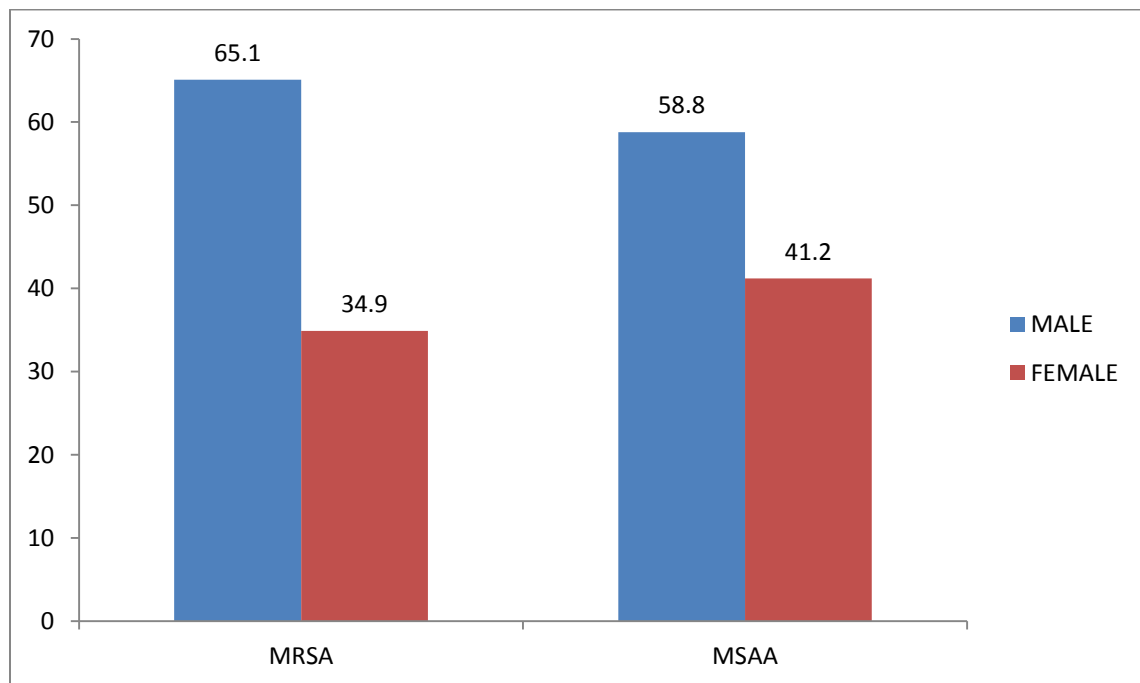


Table 11: Ward wise distribution of MRSA and MSSA .

WARD	MSSA		MRSA		Total	p value
	N	%	N	%		
IPD	144	73.1	137	73.6	281	0.901
OPD	53	26.9	49	26.4	102	
Total	197	100	186	100	383	

Figure 10: Ward wise distribution of MRSA and MSSA .

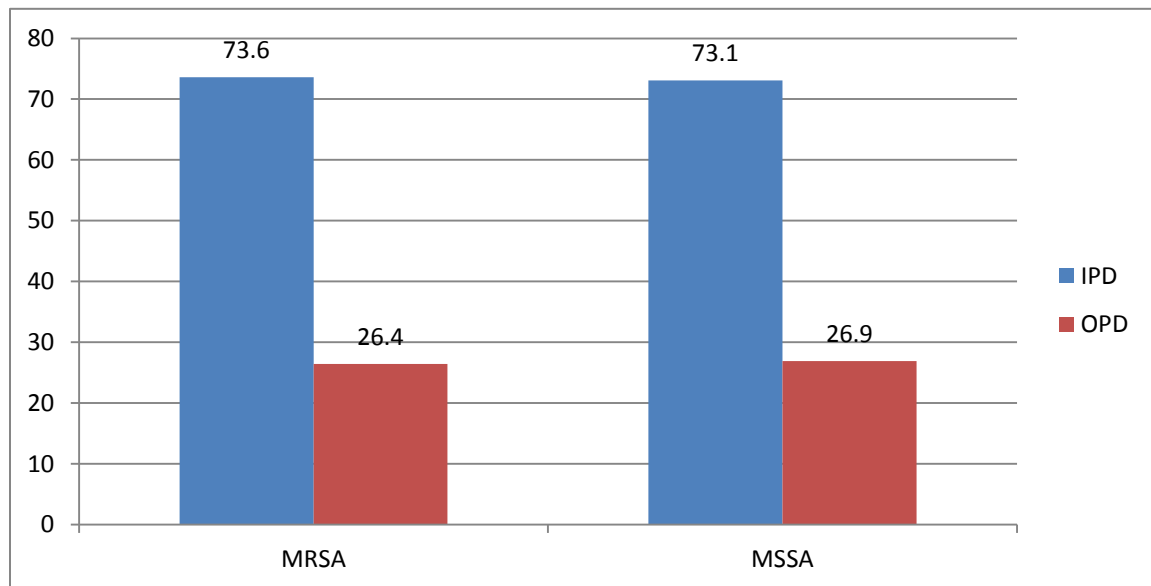


Table 12: Distribution of MRSA and MSSA according to department

Department	MSSA		MRSA		Total	p value
	N	%	N	%		
Ent	23	11.7	26	14.0	49	0.089
Medicine	18	9.1	11	5.9	29	
Obg	17	8.6	5	2.7	22	
Ortho	14	7.1	11	5.9	25	
Pediatrics	5	2.5	6	3.2	11	
Skin	13	6.6	12	6.5	25	
Surgery	104	52.8	105	56.5	209	
Urology	3	1.5	10	5.4	13	
Total	197	100.0	186	100.0	383	

Figure11: Distribution of MRSA and MSSA according to department

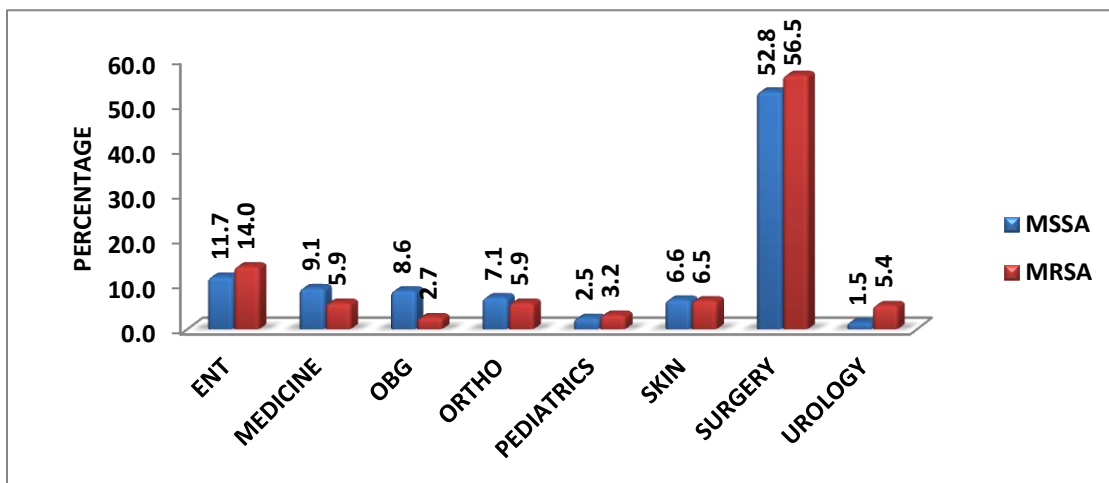


Table 13 :Specimen wise distribution of MRSA and MSSA .

SPECIMEN	MSSA		MRSA		Total	p value
	N	%	N	%		
BLOOD	2	1.0	6	3.2	8	0.329
CSF	3	1.5	0	0.0	3	
EAR SWAB/ DISCHARGE	13	6.6	17	9.1	30	
PUS	162	82.2	142	76.3	304	
SPUTUM	8	4.1	6	3.2	14	
THROAT SWAB	2	1.0	4	2.2	6	
URINE	5	2.5	10	5.4	15	
VAGINAL SWAB	2	1.0	1	0.5	3	
Total	197	100.0	186	100.0	383	

Figure 12 :Specimen wise distribution of MRSA and MSSA .

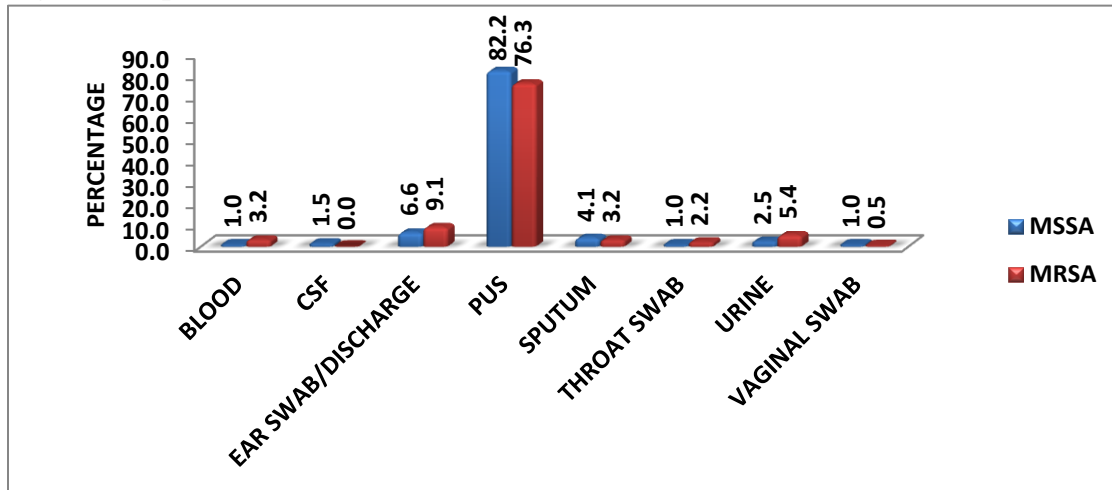


Table 14: Results of different methods for identification of MRSA

Total no of isolates=383
MRSA isolates detected by PCR (mec A gene) =186(gold std)
MRSA isolates detected by Oxacillin disc =148
MRSA isolates detected by cefoxitin disc =178

Table 15: Comparative results of phenotypic methods with PCR.

TEST METHODS	MRSA isolates detected	Sensitivity	Specificity	PPV	NPV	Accuracy
Oxacillin	148	79.6%	94.9%	93.7	83.1	87.5%
Cefoxitin	178	95.7%	100.0%	100.0	96.1	97.9%
PCR	186	100.0%	100.0%	100.0	100.0	100.0%

Figure 13: Comparative results of phenotypic methods with PCR

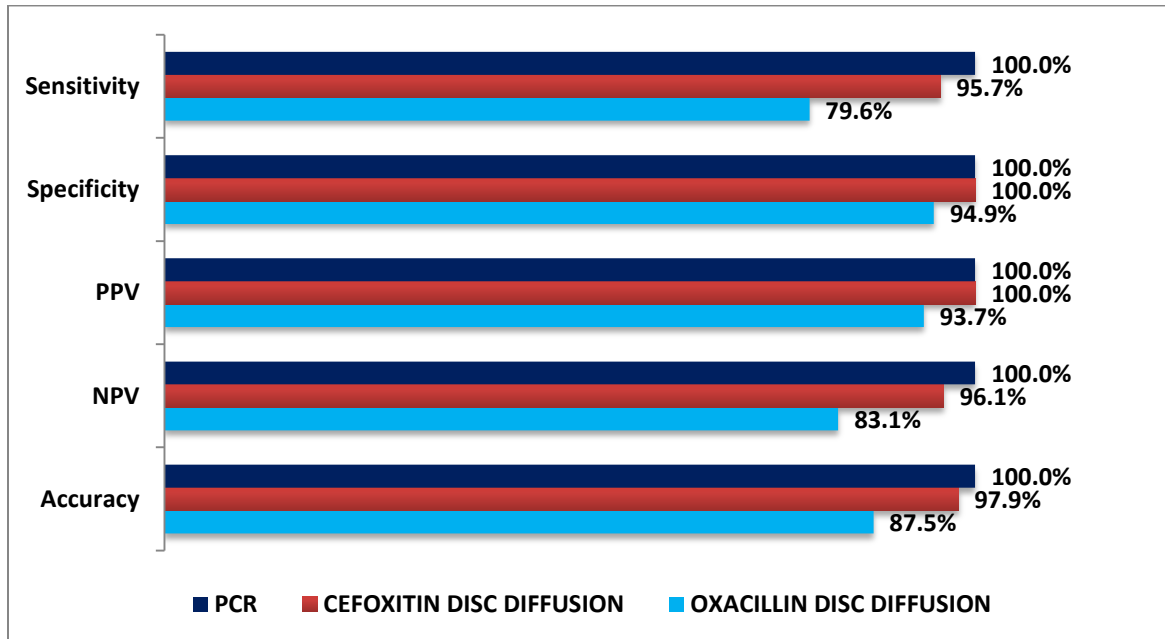


Table 16: Antibiotic susceptibility pattern among MSSA isolates

Antibiotic	MSSA (N=197)			
	SENSITIVE		RESISTANT	
	N	%	N	%
PENICILLIN-G	18	9.1	179	90.9
EYTHROMYCIN	87	44.2	110	55.8
TETRACYCLINE	176	89.3	21	10.7
CEPHALEXIN	109	55.3	88	44.7
CLOXACILLIN	91	48.9	95	51.1
PEFLOXACIN	50	25.4	147	74.6
PIPERACILLIN/TAZOBACTAM	147	79.0	39	21.0
CEFOPERAZONE /SULBACTAM	136	69.0	61	31.0
GENTAMICIN	151	76.6	46	23.4
CIPROFLOXACIN	47	25.3	139	74.7
AMOXICILLIN/CLAVULANIC ACID	69	35.0	128	65.0
CEFUROXIME	128	65.0	69	35.0
AZITHROMYCIN	118	59.9	79	40.1
VANCOMYCIN	163	87.6	23	12.4
LINEZOLID	172	92.5	14	7.5

Table 17: Antibiotic susceptibility pattern among MRSA isolates

Antibiotic	MRSA (N=186)			
	SENSITIVE		RESISTANT	
	N	%	N	%
PENICILLIN-G	7	3.8	179	96.2
EYTHROMYCIN	67	36.0	119	64.0
TETRACYCLINE	158	84.9	28	15.1
CEPHALEXIN	61	32.8	125	67.2
CLOXACILLIN	98	49.7	99	50.3
PEFLOXACIN	34	18.3	152	81.7
PIPERACILLIN/TAZOBACTAM	152	77.2	45	22.8
CEFOPERAZONE /SULBACTAM	122	65.6	64	34.4
GENTAMICIN	124	66.7	62	33.3
CIPROFLOXACIN	47	23.9	150	76.1
AMOXICILLIN/CLAVULANIC ACID	45	24.2	141	75.8
CEFUROXIME	109	58.6	77	41.4
AZITHROMYCIN	81	43.5	105	56.5
VANCOMYCIN	166	84.3	31	15.7
LINEZOLID	176	89.3	21	10.7

Table 18: Comparison of resistance pattern.

Antibiotic susceptibility pattern	MSSA (N=197)		MRSA (N=186)		p value
	R	%	R	%	
PENICILLIN-G	179	90.9	179	96.2	0.033*
EYTHROMYCIN	110	55.8	119	64.0	0.106
TETRACYCLINE	21	10.7	28	15.1	0.199
CEPHALEXIN	88	44.7	125	67.2	0.001*
CLOXACILLIN	95	48.2	99	53.2	0.883
PEFLOXACIN	147	74.6	152	81.7	0.114
PIPERACILLIN/TAZOBACTAM	39	19.8	45	24.2	0.652
CEFOPERAZONE /SULBACTAM	61	31.0	64	34.4	0.485
GENTAMICIN	46	23.4	62	33.3	0.032*
CIPROFLOXACIN	139	70.6	150	80.6	0.732
AMOXICILLIN/CLAVULANATE	128	65.0	141	75.8	0.031*
CEFUROXIME	69	35.0	77	41.4	0.215
AZITHROMYCIN	79	40.1	105	56.5	0.002*
VANCOMYCIN	23	11.7	31	16.7	0.339
LINEZOLID	14	7.1	21	11.3	0.284

Note: * significant at 5% level of significance (p<0.05)

Table 19: MRSA resistance pattern according to departments

	ENT		MEDICINE		OBG		UROLOGY		PEDIATRICS		SKIN		SURGERY		ORTHO	
	N (26)	%	N (11)	%	N (5)	%	N(10)	%	N(6)	%	N(12)	%	N(105)	%	N(11)	%
P	25	96.2	11	100.0	5	100.0	8	80.0	6	100.0	12	100.0	101	96.2	11	100.0
E	16	61.5	6	54.5	3	60.0	7	70.0	2	33.3	9	75.0	65	61.9	7	63.6
T	4	15.4	2	18.2	0	0.0	3	30.0	0	0.0	3	25.0	14	13.3	0	0.0
CEP	15	57.7	9	81.8	2	40.0	8	80.0	5	83.3	5	41.7	73	69.5	6	54.5
CLO	13	50.0	4	36.4	2	40.0	9	90.0	1	16.7	5	41.7	55	52.4	5	45.5
PEF	21	80.8	10	90.9	2	40.0	9	90.0	4	66.7	7	58.3	85	81.0	11	100.0
PT	5	19.2	2	18.2	0	0.0	4	40.0	2	33.3	2	16.7	20	19.0	2	18.2
CS	8	30.8	4	36.4	1	20.0	2	20.0	4	66.7	2	16.7	38	36.2	2	18.2
G	9	34.6	4	36.4	1	20.0	2	20.0	0	0.0	3	25.0	41	39.0	1	9.1
CIP	20	76.9	9	81.8	2	40.0	8	80.0	4	66.7	8	66.7	79	75.2	7	63.6
AC	18	69.2	9	81.8	4	80.0	8	80.0	5	83.3	9	75.0	75	71.4	6	54.5
CEF	7	26.9	8	72.7	2	40.0	5	50.0	2	33.3	4	33.3	43	41.0	4	36.4
AZ	11	42.3	6	54.5	3	60.0	8	80.0	3	50.0	9	75.0	55	52.4	6	54.5
V	4	15.4	1	9.1	0	0.0	3	30.0	1	16.7	3	25.0	10	9.5	1	9.1
L	1	3.8	0	0.0	0	0.0	3	30.0	1	16.7	2	16.7	6	5.7	1	9.1

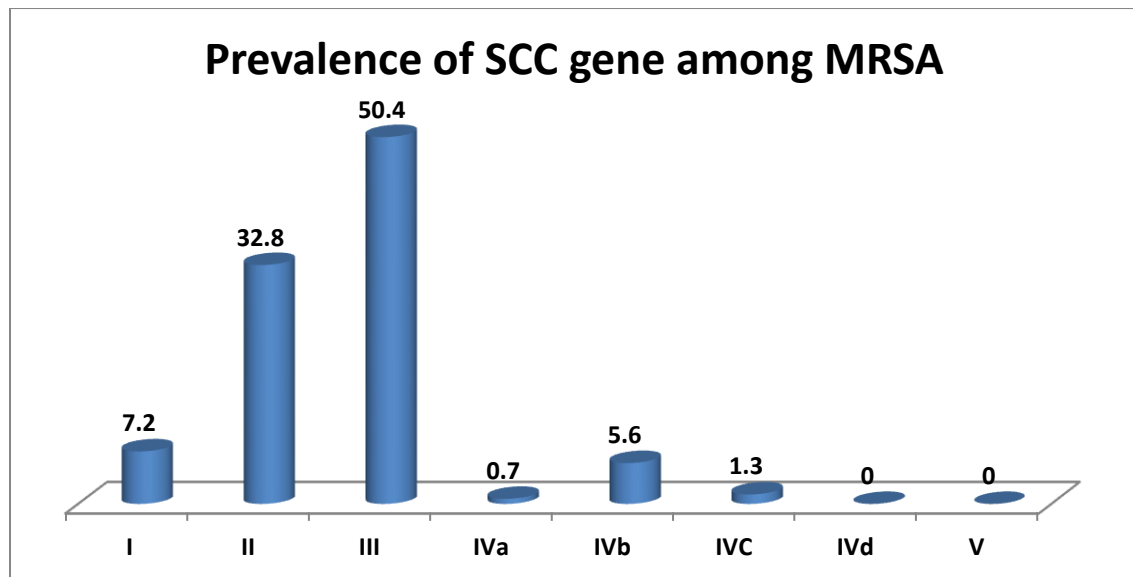
Table 20: MRSA resistance pattern according to departments by specimen

SPECIMEN	DEPARTMENT															
	ENT		MEDICINE		OBG		ORTHO		PEDIATRICS		SKIN		SURGERY		UROLOGY	
	N (26)	%	N (11)	%	N (5)	%	N (11)	%	N (6)	%	N (12)	%	N (105)	%	N (10)	%
BLOOD	0	0.0	1	9.1	0	0.0	0	0.0	1	16.7	0	0.0	4	3.8	0	0.0
CSF	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
EAR SWAB /DISCHARGE	16	61.5	0	0.0	0	0.0	0	0.0	1	16.7	0	0.0	0	0.0	0	0.0
PUS	8	30.8	5	45.5	3	60.0	10	90.9	1	16.7	11	91.7	98	93.3	6	60.0
SPUTUM	1	3.8	4	36.4	0	0.0	1	9.1	0	0.0	0	0.0	0	0.0	0	0.0
THROAT SWAB	1	3.8	0	0.0	0	0.0	0	0.0	3	50.0	0	0.0	0	0.0	0	0.0
URINE	0	0.0	1	9.1	1	20.0	0	0.0	0	0.0	1	8.3	3	2.9	4	40.0
VAGINAL SWAB	0	0.0	0	0.0	1	20.0	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0

Table 21: Prevalence of SCC gene among MRSA isolates

SCC type	Numbers	Prevalence in %
I	14	7.2
II	59	32.8
III	94	50.4
Iva	1	0.7
IVb	10	5.6
IVC	2	1.3
IVd	0	0
V	0	0

Figure 14: Prevalence of SCC gene among MRSA isolates



6. *Discussion*

Discussion:

MRSA is a major nosocomial pathogen causing significant morbidity and mortality. Predominant mode of transmission being person to person, important reservoirs of MRSA in hospital or institutions are, infected or colonized patients and transient carriage on the hands of health care personnel³⁰. MRSA had been recognized late and emerged as a problem in the 80's and in the 90's.³⁰

Table 22: MRSA Prevalence patterns in various studies.

Study	Prevalence
K Rajadurai pandi et al ³⁰ (2006)	31.1%
Bandara et al ⁵³ (2012)	47%
Virendra et al ⁵⁴ (2014)	61.4
Eshani et al ⁵⁵ (2014)	40%
Present Study (2018)	48.6%

As shown in the Table 22 , prevalence of MRSA in the present study was 48.6% which correlates with the study of Bandara et al⁵³ (47%). Study of Eshani et al⁵⁵ (40%) was close to the present study. However some western countries have reported decline in MRSA rates, specially in UK which was achieved due to nationwide intervention based on audit and quality improvement.

Table 23: Age Wise distribution of MRSA isolates in various studies.

Year	Study	Age in years	Percentage
2006	Huang et al ⁵⁶	21 – 30	31%
2015	Sumit et al ³⁵	21 – 30	30.90%
2015	Tahira et al ⁵⁷	21 – 30	50%
2018	Present study	21 – 30	19.4%

(Age wise distribution of MRSA isolates in the present study analysed in table 23). As shown in Table 23, in the present study, 19% of MRSA isolates were observed in the age group of 21 – 30 years, which correlates with the study of Sumit et al³⁵(30.9%) and Huang et al⁵⁷(31%) respectively. The highest prevalence rates of MRSA infections were observed in the young and elderly. The association between MRSA and younger age group, likely to reflect acquisition in the community.

Table 24: Sex-Wise distribution of MRSA isolates in various studies.

Study	Predominant sex in %
Goyal et al ¹⁸ (2013)	Male (58%)
Fomda et al ¹⁹ (2014)	Male (53%)
Sumit et al ³⁵ (2015)	Male (55%)
Present study (2018)	Male (65%)

(Sex wise distribution of MRSA isolates analysed in table 24). As shown in table 13, in the present study, MRSA was predominantly isolated from males(65%) which correlated with study of Sumit et al³⁵ (male 55%) and Fomda et al¹⁹ (male 53%).

Table 25 :Department wise distribution of MRSA isolates invarious studies.

Study	Ward	Percentage
Lahari et al ⁵⁸ (2009)	Surgery	47%
V. Pai et al ⁵⁹ (2010)	Surgery	71%
Virendra et al ⁵⁴ (2014)	Surgery	61.40%
Present study (2018)	Surgery	54.50%

In the current study more number of the organisms were from surgical patients and which correlates well with suppurative nature of Staphylococcal infections. Similar results were revealed by various studies as shown in table. The reasons for higher proportion of MRSA cases in surgery department may be related to the poor environmental cleaning, operation theatre surveillance and infection control measures of hospitals in Indian setup and also because of high usage of antibiotics as noted by Swanston et al². In the present study, MRSA isolates (76%) were from pus samples, which coincides with the study of Khadri et al⁶⁰ [Pus, 55%].

Table 26: Sensitivity and specificity of Cefoxitin Disc Diffusion method for detection of MRSA.

Authors	Sensitivity(%)	Specificity(%)
Karami et al ³⁶ (2011)	100	98.9
Priya et al ³⁷ (2011)	98.5	100
Zaidi et al ³⁸ (2013)	94	84.6
Present study (2018)	95	100

Detection of *mecA* gene is considered the gold standard for MRSA confirmation. In our study, the *mecA* gene PCR detected 186 isolates as MRSA and the 197 isolates as MSSA.

Recent studies including our s indicate that cefoxitin disc diffusion test is better than most of the phenotypic methods like oxacillin disc diffusion and oxacillin screen agar testing and is now an accepted method for the detection of MRSA by many reference groups including CLSI. The accurate and early determination of methicillin resistance is of key importance in the prognosis of infections caused by *S. aureus*.⁵ This higher sensitivity to cefoxitin can be explained by the increased expression of the *mecA*-encoded protein PBP2a, cefoxitin being an inducer of the *mecA* gene.⁵ Our study reveals that cefoxitin disc is better than oxacillin disc for the detection of methicillin resistance. Results of cefoxitin disc diffusion test is as good as PCR used for *mecA* gene, and thus the cefoxitin can be used for identification of MRSA and the test can be used as cost effective method when compared to PCR for detection of MRSA .

Antibiotic susceptibility pattern of the MRSA isolates:

Antibiotic susceptibility pattern revealed a high resistance to routinely used antibiotics. Resistance to quinolones i.e. ciprofloxacin and pefloxacin were high in this study. This is comparable to the study done by Sanjana et al⁶¹, in Nepal. Resistance to cephalexin (67%) was also much higher in this study. This is consistent with the study carried out by Sanjana et al.,⁶¹ who reported the similar resistant rate to cephalexin. Vidhani *et al.* found that there was a marked difference between sensitivity pattern of MRSA and MSSA isolates. Majumder et al.⁶² also revealed that resistance to various antibiotics with methicillin resistant strains was higher in comparison to methicillin-sensitive isolates. Factors responsible for drug resistance in MRSA are as follows. Antibiotics are available without prescription at drug stores or even at general stores and injudiciously used in communities, animal husbandries, and fisheries and use of allopathic drugs by traditional practitioners.²

Multidrug resistance among MRSA:

Multidrug resistance is defined as resistance of a MRSA strain towards three or more antibiotics at a given point of time. In the USA, some workers have reported multidrug resistance rates of 65.7% MRSA isolates. In Nigerian women, a total of 43 *S. aureus* out of 60 were found to be multidrug resistant. We found a high percentage of multidrug-resistant MRSA (76.8%) in our hospital. If we look into the Indian literature, it seems the burden of multi drug resistant-MRSA is increasing over time: for instance, 23.2% was reported by Majumder and colleagues⁶², 32% by Anupurba and colleagues⁶ and 63.6% by Rajadurai pandi and colleagues.³⁰ The lesson is clear: MRSA surveillance and strict drug policy are of paramount importance, or else the threat will increase.

SCCmec typing:

This is the first reports of the prevalent genotypes of MRSA in Vijayapur. This study shows that the prevalent MRSA types in Vijayapur are *SCCmec-III* and *SCCmec-II*. Of 186 MRSA isolates tested, 50% were SCCmec type III ,33% were SCCmec type II. These results are similar to those from most Asian countries⁶³⁻⁶⁶. In addition to the *mecA* gene, SCCmec type III contains genes encoding resistance to several non-b-lactam antibiotic classes, such as macrolides and tetracyclines. These resistance genes are found on transposons and plasmids integrated into the SCCmec element. Five different SCCmec types have been characterised in MRSA. Two distinct SCCmec types of MRSA strains have been identified in Asia. SCCmec type II is most common distributed in Japan and Korea whereas SCCmec type III is predominant in some other Asian countries such as Saudi Arabia, India, Sri Lanka, Singapore, China, Thailand⁶³⁻⁶⁷ (Chongtrakool et al. Ko et al. 1999). In Europe, varied SCCmec types have distributed and dominated in different countries. The SCCmec type I have been identified especially in Croatia, and Switzerland . The SCCmec type IV has been reported from Spain , Portugal, Germany. In the US, SCCmec type II and IV have been observed most common types.⁶⁸

7. Summary and Conclusion

Summary and Conclusion:

- Our study observed that the prevalence of MRSA as a whole did not vary significantly by gender but were more frequent among male patients,
- The predominant age group commonly affected was 21 – 30 years (19%).
- In the present study, majority of MRSA isolates were from pus samples (76%).
- Higher rate of MRSA isolates were from department of Surgery (56.5%).
- Linezolid (89% sensitive) was the most effective agent against MRSA isolates followed by tetracycline (86%) and Vancomycin (83%).
- Anti-biograms of MRSA isolates revealed high level of resistance (more than 75%) to penicillin, pefloxacin, gentamicin and amoxicillin/clavulanic acid.
- Results of cefoxitin disc diffusion test was as good as PCR for *mecA* gene, and thus the cefoxitin disk diffusion method is very suitable for detection of MRSA and the test can be an alternative to PCR for detection of MRSA in resource constraint settings.
- The current study revealed the prevalence of MRSA in the tertiary hospital in this part of India is very high (48.6%). Therefore, it is necessary to choose suitable antibiotics with respect to their antimicrobial profiles for treating the infections.
- We found a higher number of multidrug-resistant MRSA in our hospital. If we look into the Indian setting, it seems the burden of drug resistant-MRSA is increasing over time.

- This is the first reports of the prevalent genotypes of MRSA in our hospital settings in Vijayapur. This study shows that the prevalent MRSA types in Vijayapur are *SCCmec-III* and *SCCmec-II*.

Study limitations:

- Molecular diagnosis was restricted to MRSA cases .
- Complete gene sequencing.

Future Prospective: Previously unknown genotypes of the *SCCmec* have been identified. Future study of these genotypes may reveal various features that enable these isolates to succeed in this setting.

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9. Annexure

Papers presented in conferences:





MICROCON KC 2017

INDIAN ASSOCIATION OF MEDICAL MICROBIOLOGISTS (KC)
DEPARTMENT OF MICROBIOLOGY,
Vijayanagar Institute of Medical Sciences, Ballari



Certificate

This is to certify that

DR. BASAVARAJ C METRI

bearing Reg. No. 64274 registered with Karnataka Medical Council , Address BMPMC, Vijayapur has participated as Delegate/ ~~Chairperson~~ / Presented a Scientific Paper (~~Oral~~/Poster) in the XXI Annual Conference of IAMM (KC) conducted at VIMS, Ballari on 18th February 2017.

Karnataka Medical Council has granted 2 credit hours vide letter KMC/CME/929/2016 dated 03.12.2016

Zonal Chairman
Karnataka Medical Council
CME Accreditation Committee

Dr. Surekha Y A
Organising Secretary
Department of Microbiology
VIMS, Ballari

Dr. Krishna S
Organising Chairman
Department of Microbiology
VIMS, Ballari

Proforma

Patient details

Name : Age & sex :
Address : IP No. / OP No. :
Referred by : Lab No. :
Date of receipt :

Presenting complaints

Chills and rigors : Urgency :
Frequency : Fever :
Pain abdomen : Burning micturation:

History of present illness:

Past history and treatment

General physical examination

Pallor : Pulse :
Icterus : Temperature :
BP :

Systemic examination :

CVS RS
PA CNS

Investigation:

Blood routine : Hb TC DC ESR

Urine routine : Microscopy
 Sugar Protein

Microbiological study:

I. Preliminary tests:

Gram stainingCatalase test

Slide coagulase

II. Cultural study:

Mac Conkey's Agar:

Nutrient agar :

Blood Agar :

CLED agar :

III. Biochemical tests :

Tube coagulase

DNAase test

Mannitol fermentation

Bacitracin disc test

Nonobiosin disc test

ornithine decarboxylase

DNAase test

Lysostaphin test

Tellurite media

Gelatin liquefaction

Phosphatase test

Urease test

Nitrate reduction test

VP test

Sugar fermentation tests: Mannose, Mannitol, Trehalose, Lactose .

IV. Antibiogram :By Kirby-Bauer disc diffusion method.

Ampicillin

Pefloxacin

Penicillin

Amoxicillin-clavulinic acid

Pipercillin-Tazobactam

Cefoperazone-Salbactam

Cloxacillin

Cephalxin

Ciprofloxacin

Erythromycin

Azithromycin

Tetracycline

Gentamicin

Linezolid

Vancomycin

V. Detection of the MRSA

- Oxacillin disc diffusion method^{13,14}
- Cefoxitin disc diffusion test

VI. Confirmation of MRSA

- PCR amplification of the *mecA* gene.
- Typing of SCCmec gene

Ethical clearance certificate



B.L.D.E. UNIVERSITY

(Declared vide notification No. F.9-37/2007-U.3 (A) Dated. 29-2-2008 of the MHRD, Government of India under Section 3 of the UGC Act,1956)
The Constituent College

SHRI. B. M. PATIL MEDICAL COLLEGE, HOSPITAL AND RESEARCH CENTRE

IEC Ref No, no 01/2012/dt/10/12/12

INSTITUTIONAL ETHICAL CLEARANCE CERTIFICATE

The Ethical Committee of this University met on 21-11-2012 at 11 Am
to scrutinize the Synopsis / Research projects of Postgraduate student / Undergraduate
student / Faculty members of this University / college from ethical clearance point of view.
After scrutiny the following original / corrected & revised version synopsis of the Thesis /
Research project has been accorded Ethical Clearance.

Title Detection of Methicillin Resistant Staphylococcus Aureus
(MRSA) in Tubercy case hospital of North Karnataka.

Name of P. G. / U. G. Student / Faculty member Dr Basavaraj. C. Metri
Associate Professor Deptg Microbiology

Name of Guide _____

Dr. Sharada Metgud
Chairperson, I.E.C
BLDE University,
BIJAPUR – 586 103

Dr. G. V. Kulkarni
Mr.G.V.Kulkarni
Secretary, I.E.C
BLDE University,
BIJAPUR – 586 103.

Institutional Ethical Committee
BLDEU's Shri B.M. Patil
Medical College, BIJAPUR-586103.

Following documents were placed before Ethical Committee for Scrutinization:

- Copy of Synopsis / Research project
- Copy of informed consent form
- Any other relevant document's

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Original Article

DRUG RESISTANCE PATTERNS OF CLINICAL ISOLATES OF *STAPHYLOCOCCUS AUREUS* IN TERTIARY CARE CENTER OF SOUTH INDIA

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ABSTRACT

Objectives: *Staphylococcus aureus* were initially described in 1961 and emerged in the last decade as one of the most important nosocomial pathogens. The current study was undertaken to provide data for empirical selection of appropriate antibiotics for the treatment of diseases caused by *S. aureus*.

Methods: Various clinical samples like pus, urine, stool, sputum, blood and other body fluids of patients were selected for study from June 2012 to June 2013. *Staphylococcus aureus* were identified by various biochemical tests and antimicrobial susceptibility testing of the isolates were performed by Kirby Bauer disc diffusion method. Detection of the MRSA was done by Oxacillin disc diffusion method.

Results: A total of 137 isolates of *S. aureus* were obtained over duration of 12 months. These included isolates from the sample of pus, urine, sputum, body fluids. Out of 137 *S. aureus* strains isolated, 62 (45.3%) were identified as MRSA and 75 (54.7%) were identified as MSSA based on oxacillin disk diffusion method. Anti-biograms revealed the high level of resistance among MRSA isolates when compared to MSSA isolates. The most effective agent against MRSA isolates was linezolid (96.8% sensitive), followed by tetracycline (90.9%) and piperacillin/tazobactam (80.6%).

Conclusion: The prevalence of MRSA in our hospital was high. Therefore to reduce the incidence of infections due to MRSA, we suggest implementation of the strict antibiotic policy guidelines and continuous monitoring of antibiotic susceptibility patterns of such pathogens.

Keywords: Antibiotic susceptibility pattern, Drug resistance, Methicillin resistant *Staphylococcus aureus* (MRSA), antibiotic resistance, *Staphylococcus aureus*.

INTRODUCTION

Staphylococcus aureus was first described by Sir Alexander Ogston in 1882. This centuries-old pathogen still causes significant morbidity and mortality despite huge advances in medical care. Indeed, infections due to *S. aureus* continue to grow in number and complexity as a consequence, ironically, of advances in patient care and of its ability to adapt to a changing environment [1, 2].

Methicillin resistant *Staphylococcus aureus* (MRSA) strains emerged after the introduction of methicillin into the clinical practice [3]. Methicillin resistant *Staphylococcus aureus* (MRSA) strains were initially described in 1961 and emerged in the last decade as one of the most important nosocomial pathogens. Infected and colonized patients provide the primary reservoir and transmission is mainly through hospital staff [4].

The risk factors which contribute to MRSA are excessive antibiotic usage, prolonged hospitalization, intravascular catheterization and hospitalisation in an intensive care unit. With the increased incidence of MRSA, the effectiveness of penicillin and cephalosporins is questioned. In fact many strains of MRSA exhibit resistance to beta-lactams and aminoglycosides [4]. The worst feature of MRSA has been simultaneous drug resistance to many of the antibiotics, chronic carrier stage among health care workers and greater resistance of the strains [5]. The current study was undertaken to provide data for empirical selection of appropriate antibiotics for the treatment of diseases caused by *S. aureus*.

MATERIALS AND METHODS

Source of data

The study was conducted in the Department of Microbiology, Shri B. M Patil Medical College Hospital, Bijapur. *S. aureus* isolated from all the clinical samples that came to the microbiology department formed the material for study.

Ethical clearance and consent

The study was conducted after obtaining the ethical clearance from institutional ethical committee.

Method of collection of data: (including sampling procedure)

Various clinical samples like pus, urine, stool, sputum, blood and other body fluids of patients attending Shri B M Patil Medical College and Hospital were selected for study for a period of one year from June 2012 to June 2013.

Statistical analysis

Data was analyzed by

- 1) Diagrammatic representation
- 2) Proper statistical tests like chi square test etc.

Inclusion criterion

Samples which yielded *S. aureus* were included in the study.

Exclusion criterion

Samples which did not yield *S. aureus* were excluded from the study.

Specimens were screened by preliminary Gram's stain and then inoculated on 10% sheep blood agar and MacConkey's agar. *S. aureus* was identified by conventional techniques [6]. Antimicrobial susceptibility testing of the isolates was performed by Kirby Bauer disc diffusion method using following discs: penicillin-G (10 unit); cloxacillin (30µg); cephalosin (30µg); cefuroxime(30 µg); tetracycline (30µg);erythromycin (15µg); gentamycin (10µg); ciprofloxacin (5µg); pefloxacin (5µg); Cefoperazone/sulbactam(75 µg/30 µg) piperacillin/tazobactam(100µg/10 µg); amoxicillin/ clavulanic acid (20 µg/10 µg); azithromycin(15µg); linezolid (15µg). Finally, the data were recorded and analyzed at the completion of

SCREENING OF *STAPHYLOCOCCUS AUREUS* AND COAGULASE NEGATIVE *STAPHYLOCOCCUS* FROM URINE SAMPLES

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ABSTRACT

Objectives: The resistance profile of isolated Gram-positive organisms such as *Staphylococcus aureus* were left undone despite the increasing prevalent rate of this organism in urinary tract infections (UTI) and its role in antibiotic resistance. Therefore, the current study was carried out to identify the antibiotic resistance pattern of the *S. aureus* and coagulase negative *Staphylococcus* (CONS).

Methods: The study was carried out in the Department of Microbiology, Shri BM Patil Medical College, Bijapur, India over a period of 3 years from January 2010 to December 2012. Urine specimens from both outpatients and inpatients of our hospital having one or more urinary symptoms, such as burning during micturition, fever, pyuria, frequency of urine, dysuria, hematuria, flank pain, suprapubic discomfort, etc., were processed.

Results: Out of total staphylococcal isolates, 55% were *S. aureus* and 45% were CONS. Out of total isolates of *Staphylococci* 60.5% were from inpatient department and 39.5% were from out patient department. Linezolid (9.52%) piperacillin/tazobactam (14.3%) cefoperazone/sulbactam (28.6%) showed least resistance against *S. aureus* and penicillin-G (90.5%), cloxacillin (71.4%), ciprofloxacin (71.4%) showed highest resistance against *S. aureus*. CONS isolates showed similar resistance profile, but when compared with *S. aureus*, CONS were more sensitive to the all antibiotic used.

Conclusion: This study observed that *Staphylococcus* is the one of the most common etiologic agent of UTI in our hospital. The drug of choice that could be considered in the treatment of UTI caused by staphylococcus in our setting are linezolid, piperacillin/tazobactam, cefoperazone/sulbactam. *Staphylococcus* was found to be highly resistant to penicillin-G, cloxacillin, and ciprofloxacin.

Keywords: Antimicrobial resistance, Drug resistance, *Staphylococcus aureus*, Coagulase negative staphylococcus, Urinary tract infections.

INTRODUCTION

Staphylococcus aureus is one of the most important pathogens affecting humans, has acquired resistance to various antibiotics and is a leading cause of hospital and community acquired infections [1]. *S. aureus*, which was first isolated by Alexander Ogden in 1880s, is known to cause post-operative wound infections. The mortality rate of the individuals, due to *S. aureus* infections was around 80% before the introduction of penicillin. The first penicillin resistant *S. aureus* was isolated from a clinical environment in 1942. The problem of penicillin resistance was later circumvented by the introduction of methicillin. In 1961, methicillin resistant *S. aureus* made an appearance, probably due to the acquisition of the *mecA* gene, leaving vancomycin as the drug of last resort to treat it [2].

Urinary tract infections (UTI) are one of the most common bacterial infections in humans both in the community and hospital setting [3]. UTI is a heterogeneous disease, which can be divided into several types of infections, such as acute, uncomplicated bacterial pyelonephritis, complicated UTI, recurrent cystitis and asymptomatic bacteriuria. The urinary tract is generally a hostile environment for bacteria and except for the distal urethra it is usually sterile. Infection results when the bacteria virulence factor overcomes the numerous host defense mechanism [4].

The common pathogens of UTI include enteric Gram-negative bacteria with *Escherichia coli* being the most predominant, coagulase negative *Staphylococcus saprophyticus* (CONS) accounting for 10-20% while *Proteus mirabilis*, *Klebsiella* and *Enterococcus* account for <5%. However, recent studies have reported the increasing prevalence of *S. aureus* in UTIs [4-9].

This changing spectrum of microorganisms involved in UTI necessitates the need for continuous and regular antimicrobial resistance surveillance in these organisms in order to guide empirical therapy in UTI. Most studies on UTI have concentrated on the antimicrobial resistance profile of Gram-negative enterobacteria, especially *E. coli* which is known to be the most prevalent UTI causative organism while the resistance profile of isolated Gram-positive organisms such as *S. aureus*, were left undone despite the increasing prevalent rate of this organism in UTI and its role in antibiotic resistance [9]. Therefore, the current study was done to identify the antibiotic resistance pattern of the *S. aureus* and CONS.

METHODS

The study was carried out in the Department of Microbiology over a period of 3 years from January 2010 to December 2012.

Ethical clearance and consent

As it was a retrospective study, ethical clearance and consent were not obtained.

Patient evaluation

Urine specimens from both outpatients and inpatients of our hospital having one or more urinary symptoms, such as burning during micturition, fever, pyuria, frequency of urine, dysuria, hematuria, flank pain, suprapubic discomfort, etc., were processed.

Inclusion criterion

Urine samples which yielded *Staphylococcus* were included in the study.

301	MONAPPA	60	IPD	MALE	1-13219	9-May	SUR		R	R	Y	R	R	S	R	R	R	R	R	R	R	R	R	S	S		
302	shirama	50	IPD	MALE	26613	20-Aug	KOTENNA SUR	CHYLOCOEL	S	S	N	R	R	S	S	S	S	S	S	S	S	S	R	R	S	S	
303	RAFIQUE	60	IPD	MALE	26582	16-Aug	M S BIRAI MED	COUGH	S	S	N	R	R	S	R	R	R	S	S	S	R	R	R	R	S	S	
304	KASHBAI	35	IPD	FEMALE	26705	16-Aug	KOTENNA SUR	ABCESS	S	S	N	R	R	S	R	R	R	S	S	R	R	R	R	S	S		
305	ANITA	20	IPD	FEMALE	26783	18-Aug	CBG	WOUND GAPING	S	S	N	R	S	S	R	S	R	S	S	S	R	R	R	S	S		
306	KENCHAPPA		OPD	MALE	124505	17-Aug	TEJASWII SUR	CELLULITIS	S	S	N	R	R	S	R	S	S	S	S	S	R	R	R	S	S		
307	GURUBAS	55	IPD	MALE	26709	16-Aug	KOTENNA SUR	NECRO FASOTIS	S	S	N	R	R	R	R	R	S	R	R	S	S	R	R	R	S	S	
308	MANGOC	68	IPD	MALE	25495	17-Aug	M B PATIL SUR	FISTULA	S	S	N	R	R	R	R	R	S	R	R	R	S	R	S	R	S	S	
309	PUNDAPP	55	IPD	MALE	26808	18-Aug	TEJASWII SUR	DUODINAL PERFOR	S	N	R	R	S	R	R	S	S	R	S	S	R	R	R	R	R	R	
310	GANAPAT	65	IPD	MALE	26986	19-Aug	SUR	ULCER	R	R	Y	R	R	R	S	R	R	S	R	R	S	R	S	R	S	S	
311	SIDDAPP	45	IPD	MALE	26988	19-Aug	SUR	GLUTIAL ABCESS	R	R	Y	R	S	S	R	S	S	S	R	R	R	R	R	S	S	S	
312	LAXMI	60	OPD	FEMALE	26841	22-Aug	KOTENNA SUR	DIABETIC FOOT ULCS	S	N	R	R	R	R	R	S	R	R	R	S	R	R	R	S	S	S	
313	ASTHA	10	OPD	FEMALE	299280	21-Aug	ENT	OTOMYOSIS	S	S	N	S	S	S	S	S	S	S	S	S	S	S	R	S	S	S	
314	MAHADEVI	50	OPD	FEMALE	308615	21-Aug	CBG	UTI	S	S	N	S	S	S	S	S	S	S	S	S	S	S	S	S	S	S	
315	MADAPP	82	OPD	MALE	309199	21-Aug	KARADI	ENT	EAR DISCHARGE	R	R	Y	R	R	S	S	S	R	S	S	S	S	R	S	R	S	S
316	SUBHAS	65	IPD	MALE	27296	21-Aug	S B PATIL URO	CALCULUS	R	R	Y	R	R	S	R	R	R	R	S	S	R	R	R	R	S	S	
317	SHASHIV	28	OPD	FEMALE	308327	20-Aug	CBG	WHITE DISCHARGE	R	R	Y	R	R	S	R	S	R	S	S	S	S	S	R	R	S	S	
318	RAMESH	35	IPD	MALE	26985	20-Aug	HEMANTH SUR		R	R	Y	R	R	S	S	S	R	S	R	S	R	R	S	R	S	S	
319	MANUHA	35	IPD	MALE	20957	20-Aug	SUR	ULCER	R	R	Y	R	R	S	R	S	R	R	S	S	R	R	S	R	S	S	
320	MAHANNA	35	IPD	FEMALE	26994	20-Aug	HEMANTH		S	S	N	R	S	S	R	S	S	R	S	S	R	R	S	S	S	S	
321	RAHA	65	OPD	MALE	307117	20-Aug	ENT	CSDM	S	R	Y	R	S	S	R	S	S	S	S	S	S	S	S	S	S	S	
322	SHANTA	55	OPD	FEMALE	307395	20-Aug	JAUU	CBG	VEGINAL DISCHARGE	S	N	R	R	S	R	R	R	S	S	S	R	R	S	R	S	S	
323	KIRAN	16	IPD	MALE	19258	5-Jul	METAM	SUR	ULCER	S	S	N	R	S	S	R	S	S	S	R	R	S	S	S	S	S	
324	UMESH	6	IPD	MALE	19275	4-Jul	METAM	SUR	NECRO FASOTIS	S	S	N	R	S	S	S	S	R	S	S	S	R	S	S	S	S	
325	ROHT	5	IPD	MALE	20895	3-Jul	METAM	SUR	ABCESS	S	S	N	R	S	S	S	S	R	S	R	S	R	S	S	R	S	S
326	PARASHI	2	OPD	MALE	240335	3-Jul	SKIN	PSORIASIS	S	S	N	R	R	S	S	S	R	S	S	R	R	S	S	R	S	S	
327	AIVA	44	OPD	FEMALE	255660	14-Jul	BIDRI RC MED		R	R	Y	R	S	S	S	S	R	S	S	R	S	S	R	S	S	S	
328	SHIVAPPA	35	IPD	MALE	22200	13-Jul	L S PATIL MED		R	R	Y	R	R	S	R	S	R	R	S	S	R	S	R	R	S	S	
329	RACHAVI	45	IPD	FEMALE	23201	20-Jul	SKIN	BULLOUS PEMPHIGS	S	N	R	R	S	R	S	R	R	R	S	R	S	R	R	S	R	S	S
330	PRAJIVAL		OPD	MALE	263173	20-Jul	TEJASWII SUR	SWELLING	R	R	Y	R	R	S	R	S	R	S	S	R	R	R	R	R	S	S	
331	LAXMI	25	IPD	FEMALE	23161	20-Jul	BARADOL	BREAST LUMP	R	R	Y	R	R	S	R	R	R	S	S	S	S	S	R	S	S	S	
332	ASHA	33	OPD	FEMALE	263396	20-Jul	CBG	VEGINAL DISCHARGE	R	R	Y	R	S	S	S	S	S	S	S	S	R	S	S	S	S	S	
333	KANAKAN	9	IPD	FEMALE	21626	16-Jul	NANDI	ORTHO	SWELLING	R	R	Y	R	R	S	S	S	R	S	S	S	R	S	S	R	S	S
334	SHARANA	65	IPD	FEMALE	22209	15-Jul	NANDI	ORTHO	SWELLING	R	R	Y	R	S	S	S	S	R	S	S	S	R	S	S	S	S	S
335	SHIVABAI	38	OPD	FEMALE	258340	16-Jul	CBG	DISCHARGE	S	S	N	S	R	R	R	S	R	S	R	R	R	S	R	R	S	S	S

