Comparison of Cefoxitin Disc Diffusion Test with Polymerase Chain Reaction(PCR) to Detect *mecA* Gene for Detection of Methicillin Resistant *Staphylococcus aureus.*

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ENDORSEMENT BY THE HEAD OF DEPARTMENT, PRINCIPAL/ HEAD OF THE INSTITUTION

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LIST OF ABBREVIATIONS:

PCR	-	Polymerase Chain Reaction
MRSA	-	Methicillin resistant *Staphylococcus aureus*
CDD	-	Cefoxitin disc diffusion test
S. aureus	-	*Staphylococcus aureus*
SCC	-	Staphylococcal Cassette Chromosome
DNA	-	Deoxyribonucleic Acid
CoNS	-	Coagulase-negative *Staphylococci*
CA-MRSA	-	Community-associated MRSA
HA-MRSA	-	Health care-associated MRSA
CDC	-	Centre for Disease Control
EUCAST	-	European Committee for Antimicrobial Susceptibility Testing
CLSI	-	Clinical and Laboratory Standards Institute
MHA	-	Mueller-Hinton Agar
NaCl	-	Sodium chloride
MIC	-	Minimum inhibitory concentration
E test	-	Epsilometer test
INSAR	-	Indian Network for Surveillance of Antimicrobial Resistance group, India
PVL	-	Panton- Valentine- Leukocidin
PBP	-	Penicillin- binding proteins

ABSTRACT

Background

Methicillin-resistant *Staphylococcus aureus* is a persistent and ever growing problem for health-care institutions. The hospital-acquired methicillin-resistant *Staphylococcus aureus* is a threat to appropriate, efficient and beneficial therapy of the patients. Again the worldwide emergence of community-acquired MRSA is a threat to individuals both in the community and in the hospital environment as these strains are more virulent than the hospital-acquired MRSA strains. Furthermore community-acquired MRSA have started to replace hospital-acquired MRSA in health-care settings.

Objectives:

1. To compare Cefoxitin disc diffusion test with PCR to detect *mecA* gene for detection of methicillin-resistant *Staphylococcus aureus,*
2. To detect prevalence of methicillin- resistant *Staphylococcus aureus*.

Materials and methods:

All *Staphylococcus aureus* isolated from different clinical samples received in Microbiology Department of J.N.M.C Belgaum, from January 2013 to December 2013 were included in this study. The *Staphylococcus aureus* isolates were reconfirmed by gram staining, by the characteristic growth on blood agar and nutrient agar and by putting up biochemical reactions. Routine antibiotic sensitivity testing to the antibiotics- ampicillin, amoxyclav, ciprofloxacin, clindamycin, gentamycin and erythromycin carried out. Cefoxitin (30 microgram) disc diffusion test done for all

the isolates. PCR carried out for 60 of the isolates which were selected by systematic sampling.

Results:

Out of the 372 isolates, 160 were methicillin-resistant by Cefoxitin disc diffusion test. 60 of the isolates were taken based on systematic sampling. By PCR, of these 60 isolates 48 were *mecA* positive (methicillin-resistant) and 12 were *mecA* negative (methicillin-sensitive). Whereas by Cefoxitin disc diffusion, 42 were MRSA and 18 were MSSA. Prevalence of MRSA was 43% on screening by Cefoxitin disc diffusion test.

Conclusion:

Accurate detection of methicillin-resistant *Staphylococcus aureus* is essential for proper treatment of patients and also to monitor their response to the various antibiotics. The present study provides an evidence that Cefoxitin disc diffusion test can be used effectively on a routine basis for screening of methicillin-resistant *Staphylococcus aureus* as it has a specificity of 100%. But the role of PCR is undisputable in detection of *mecA* gene for MRSA as Cefoxitin disc diffusion test had a sensitivity of 87.5%.

Key words: *mecA gene, MRSA, cefoxitin.*

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INTRODUCTION

Staphylococcus aureus is a potentially pathogenic bacterium causing a broad spectrum of diseases ranging from minor and self-limiting skin infections to invasive and life-threatening diseases. It can adapt to the selective pressure of antibiotics rapidly and this has led to the emergence of methicillin resistant *Staphylococcus aureus* (MRSA).[1]

The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) heralded an era of uncertainity in health-care as antibiotic resistance converged to create a major crisis. There has been a dramatic rise in prevalence of antibiotic-resistant strains (MRSA) in both hospital and community. Mortality and length of hospital stay were mostly attributable to *Staphylococcus aureus* infections. Methicillin sensitive *Staphylococcus aureus* (MSSA) infections led to more than two-fold increase in mortality. Methicillin-resistance also contributed to additional 80% excess mortality at day 30 after infection.[2]

Antibiotic resistance, including MRSA, threats in United States is increasing at high proportions. More than two million people are sickened with antibiotic-resistant infections, and more than 20,000 people die annually as a result in the United States alone.[3] Methicillin resistant *Staphylococcus aureus* (MRSA) is endemic in India also and is a dangerous pathogen for hospital acquired infections.

The 2.1 kb *mecA* gene is located on a mobile genetic element called the Staphylococcal Cassette Chromosome *mec* (*SCCmec*). The resistance of *Staphylococcus aureus* to methicillin, a semi-synthetic penicillinase resistant penicillin is caused by the presence of *mecA* gene which encodes the 78kDa

penicillin-binding protein (PBP)2a. Beta lactam antibiotics cannot bind to the PBP2a and so synthesis of the peptidoglycan layer and the cell-wall of the organism are able to continue.[1]

Strains of MRSA that possessing *mecA* gene are either heterogeneous or homogeneous in their expression of resistance. The heterogeneous expression occasionally results in false interpretations of borderline minimal inhibitory concentrations as susceptible. So accurate diagnosis is needed.[4] The *mecA* gene detection tests based on PCR correctly identify even the most heterogeneous of strains. Detection of *mecA* gene or penicillin binding proteins (PBP2a), is considered the gold standard for MRSA confirmation. It should be considered the gold-standard for methicillin resistance.[5]

Most clinical laboratories rely on disc diffusion testing for the detection of methicillin resistance in *Staphylococcus aureus*. Out of various disc diffusion methods, cefoxitin disc diffusion test is recommended for detection of methicillin resistance in *Staphylococcus aureus* (MRSA). Cefoxitin, a cephamycin antibiotic is a potent inducer of *mecA* regulatory system and is shown to be more accurate for detection of *mecA* gene mediated methicillin resistance in *Staphylococcus aureus*. Cefoxitin disc diffusion zones are much easier to read than those of oxacillin. It is due to the hazy oxacillin zones formed which are misinterpreted as susceptible to oxacillin.[6]

PCR is the most well-developed molecular technique up to now. It has a wide range of clinical applications including specific or broad-spectrum pathogen detection, evaluation of emerging novel infections, surveillance, early detection of bio-threat agents and antimicrobial resistance profiling.[7] But PCR based detection

of MRSA is expensive and is not readily available in routine laboratories in developing countries like India.[8]

Thus the study is undertaken comparing the cefoxitin disc diffusion test with PCR to detect *mecA* gene for detection of methicillin-resistant *Staphylococcus aureus* aiming to accurately, correctly and efficiently diagnose methicillin - resistant *Staphylococcus aureus* and thereby appropriate and efficient and beneficial therapy of the patients.

OBJECTIVES

1. To compare Cefoxitin disc diffusion test with Polymerase Chain Reaction (PCR) to detect *mecA* gene for detection of methicillin-resistant *Staphylococcus aureus,*

2. To detect prevalence of methicillin- resistant *Staphylococcus aureus.*

REVIEW OF LITERATURE

Mortality rate of patients was 80% till penicillin was introduced in the 1940s for treatment of staphylococcal infections. Again as early as 1942 strains of *Staphylococcus aureus* resistant to penicillin were detected in hospitals. Within two decades, 80% of both hospital and community-acquired *Staphylococcus aureus* isolates were penicillin resistant.[1,9]

Methicillin, a semisynthetic penicillinase resistant penicillin was introduced in 1960 for treatment of penicillinase producing strains of *Staphylococcus aureus* and methicillin resistant strains of *Staphylococcus aureus* were identified in 1961 in U.K.[9,10]

Methicillin resistant *Staphylococcus aureus* (MRSA) is a specific strain of the *Staphylococcus aureus* bacterium that has developed resistance to all penicillins. It includes resistance to methicillin and other narrow-spectrum β-lactamase resistant penicillin antibiotics.[11]

MRSA infections occur in people who had been in health care set-ups and then it is known as health care-associated MRSA (HA-MRSA). It is associated with invasive procedures like surgeries, insertion of intravenous tubing or implantation of artificial joints. HA-MRSA can be defined as the MRSA isolate which is associated if the entry criteria of hospitalization for more than72 hours before culture of the sample taken was met and if in the year before the present admission to hospital, the patient had undergone any of the following: hospitalization, surgery, had been living in a long-term care facility and hemodialysis or peritoneal dialysis or at presently had indwelling catheters or other percutaneous devices.[12]

There is another type of MRSA infection occurring in the wider community, among healthy people. This form is the community-associated MRSA (CA-MRSA). It usually begins as a painful skin boil and spreads by skin-to-skin contact. Populations which are at risk are high school children, wrestlers, child-care workers and people living in crowded conditions.

In 2000, Centre for Disease Control, USA (CDC) created a case definition for a CA-MRSA infection: any MRSA infection diagnosed for an outpatient or within 48 hours of hospitalization if the patient lacks the following health care-associated MRSA risk factors: hemodialysis, surgery, residence in a long-term care facility or hospitalization during the previous year, the presence of an indwelling catheter or a percutaneous device at the time of culture, or previous isolation of MRSA from the patient.

Other MRSA infections were considered to be HA-MRSA. [12]

About 2 billion people carry some form of *Staphylococcus aureus* strains worldwide. Among them, 53 million carry MRSA. In the United States alone, 95 million carry *Staphylococcus aureus* strains in their noses and among them 2.5 million carry MRSA.[11]

The nose is the major site of *Staphylococcus aureus* from where the organism can spread to other parts of the body.[13] Therefore, nasal carriage of *Staphylococcus aureus* is very common. A nasal carrier contaminates his or her hands by nose picking or any contact with nose. Thereby, transmitting the organism in the course of their daily activities. Skin contact with skin is the most significant mode of transmission. So hand washing is of utmost importance in preventing the spread of

MRSA infection. Direct contact transmission involves contact of body surfaces and physical transfer of *S. aureus* to the host from an infected or colonized person.[14]

Between 1960 and 1963 a moderate increase in the number of methicillin resistant strains on the order of 4% was noted.[15] Homogenous populations replaced progressively to heterogeneous populations of MRSA.[16] Staphylococcal enterotoxin A was first produced in vitro in 1963.[17]

The first identified virulence factors of *Staphylococci* were leucocidins.[18]

The methicillin-resistant strains brought about in vitro destruction of methicillin and oxacillin, but the rate of hydrolysis was slow. This was attributed to high concentrations of Staphylococcal penicillinase, and not to another specific enzyme.[19]

Methicillin resistance is a multifactorial process, one element of which is specified by a plasmid.[20]

The epidemiology started changing with strains of methicillin-resistant *Staphylococcus aureus* emerging in the community and their prevalence in the community increasing substantially.[21]

The successful development of meticillin antibiotics in the 1960s was quickly followed by emergence and worldwide dissemination of methicillin-resistant *Staphylococcus aureus* in 1970. Worldwide epidemics of *S. aureus* diseases have been recognized over the years.[22,23]

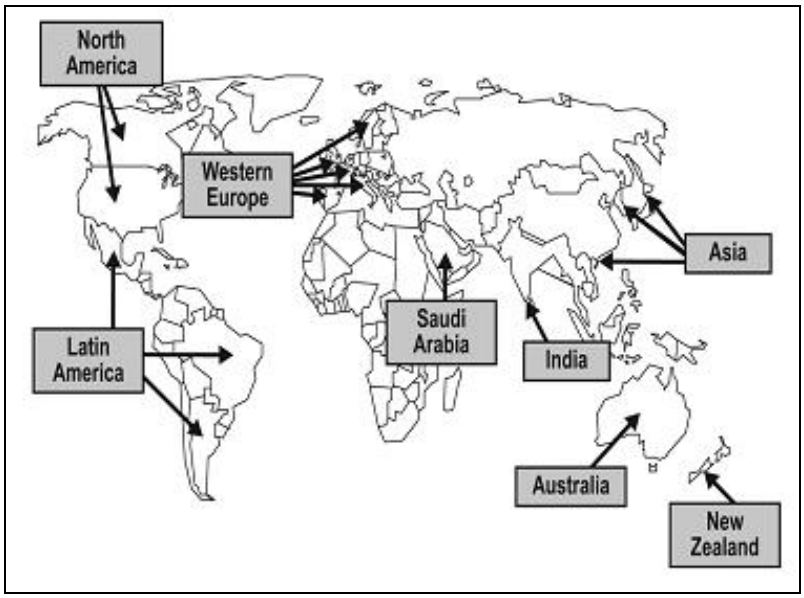


Fig1: [Global Outbreak of Community-associated Methicillin-Resistant *Staphylococcus aureus* infection, 1997-2000 (Adopted from Boucher HM and Corey GR.CID 2008;46:S344-349)[24]]

Todd et al. (1978) first described the Toxic Shock Syndrome (TSS). It is a staphylococcal illness characterized by acute onset of high fever above 104°F, diffuse erythematous rash, desquamation of the skin one to two weeks after onset , especially on the palms and soles and hyperemia of mucous membranes, hypotension, and involvement of multiple organ systems as evidenced by diarrhoea, thrombocytopenia, cardiopulmonary dysfunction or a variety of other symptoms.[25,26]

The toxin responsible for TSS, known as the toxic shock syndrome toxin (tsst-1) was first identified by Schlievert et al 1981 and the criteria for defining TSS cases were established in 1981.[27,28] TSST-1 was found to be a potent super antigen eliciting a variety of cytokines that along with tumour necrosis factor, contribute a variety of illness. It has also been described in association with many types of *Staphylococcus aureus* infections some of which are quite minor. It is produced

exclusively by *Staphylococcus aureus* and about 20% of natural isolates produce it. TSST-1, formerly designated enterotoxin F, was the first toxin shown to be involved in TSS. It is accepted as the major toxin associated with this illness, whether menstrual or non-menstrual, accounting for about 75% of all cases.

Staphylococcus aureus is the most prevalent organism causing intravascular device associated bacteremia.[29] Blood stream infections are defined as infections in which no other primary site can be discerned.[30]

On analysing the natural population dynamics and how expansion of pathogenic clones of *Staphylococcus aureus* occur, evidence was found that essentially any *Staphylococcus aureus* genotype present in humans can transform into a life-threatening human pathogen. But their certain clones are more virulent than the others.[31]

Several epidemiological studies were carried out which found increased morbidity and mortality from HA-MRSA compared with those from MSSA.[32]

Outbreaks of MRSA have been reported in variety of settings including hospitals, long term care facilities, outpatient clinics, as well as in the community. Community MRSA strains have several distinguishing characteristics enabling them to more readily colonize and infect otherwise healthy hosts.[33,34,35,36]

Asghar and Ahmed (2014) reported a prevalence of 55.3% MRSA in Saudi Arabia.[37]

Kuehnert reported for 2001–2002 in USA, MRSA prevalence estimated to be 32.4%.[38]

Methicillin resistant *Staphylococcus aureus* (MRSA) is endemic in India and is a dangerous pathogen for hospital acquired infections. Reports of MRSA incidence found as 24 % in 1996 in Vellore[39] and Lucknow[40] 40.21% in 2007 are there. MRSA prevalence increased from 12% in 1992 to 80.83% in 1999.[41] Verma et al[41] also commented it to be of same order in Mumbai, Delhi and Bangalore.

Anupurba et al[42] (2003) reported the incidence of MRSA in eastern Uttar Pradesh to be 54.85%.

In India, a pilot surveillance programme for detection of MRSA was conducted using conventional laboratory methods led to the detection of MRSA, overall prevalence was found to be 32%, 27 % from Mumbai, 42.5 % from Delhi and 47 % from Bangalore.[43]

Another Indian pilot surveillance programme (2013) for detection of MRSA was conducted. This programme led to the detection of MRSA prevalence 41 %.[44]

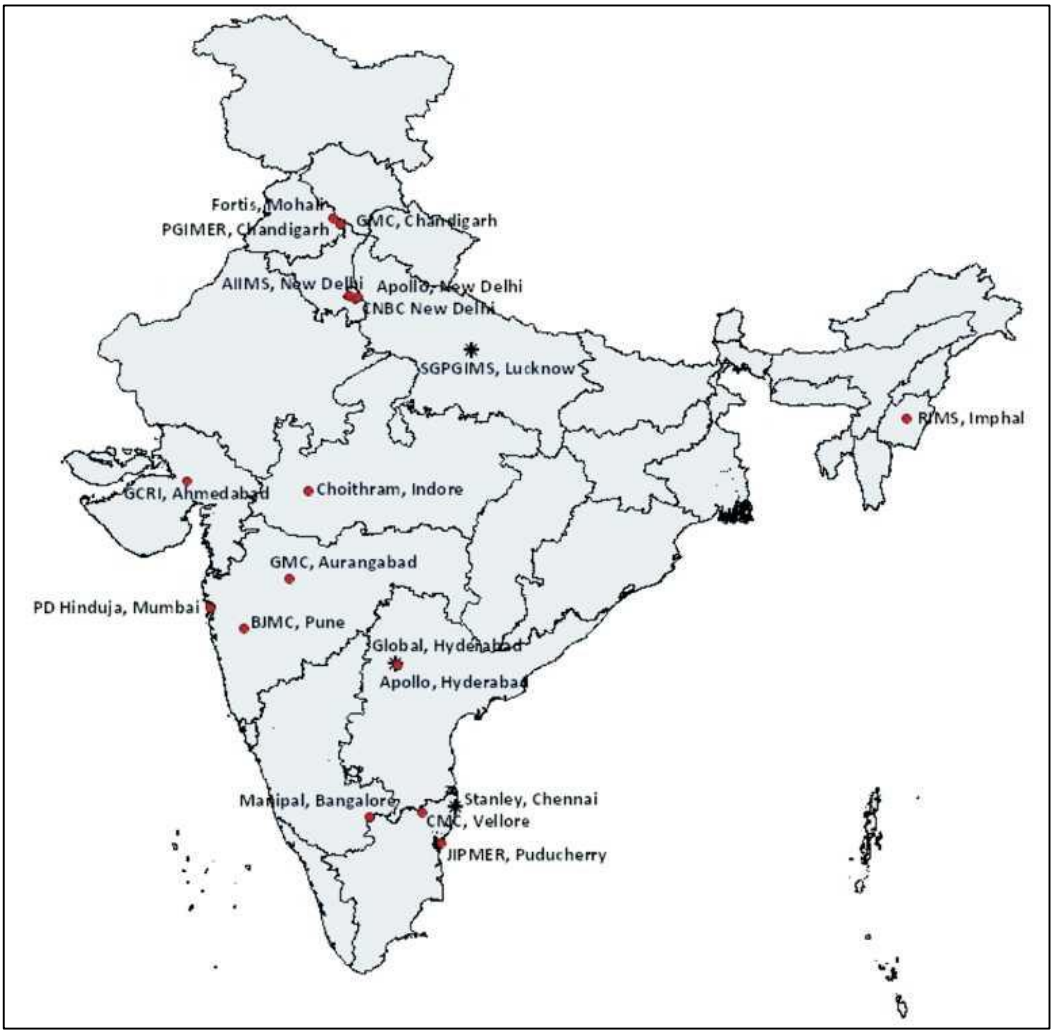

Fortis, Mohali
PGIMER, Chandigarh
GMC, Chandigarh
AIIMS, New Delhi
Apollo, New Delhi
ENBC New Delhi
SGPGIMS, Lucknow
RIMS, Imphal
GCRI, Ahmedabad
Choithram, Indore
GMC, Aurangabad
PD Hinduja, Mumbai
BJMC, Pune
Global, Hyderabad
Apollo, Hyderabad
Manipal, Bangalore
Stanley, Chennai
CMC, Vellore
JIPMER, Puducherry

Fig 2. Map showing members of Indian Network for Surveillance of Antibacterial resistance (INSAR) 2013

(Places marked with red dots are INSAR members whose data are included in the study. Places marked *are INSAR members whose data are not included in the study.)

[Adopted from Ind J Med Res 2013;137:363-369]

Khan et al (2014) reported a prevalence of 34.8% of MRSA in Bastar region of Chattisgarh, India.[45]

In Chennai, in South India, a tertiary referral hospital carried out a study on MRSA prevalence and it was found to be 31%.[46]

Alvarez-Uria et al studied the rural population and observed that the proportion of CA- MRSA infections was 64.7% and the proportion of HA-MRSA infections was 70.7%. The proportion of HA-MRSA was found not to be significantly higher than that of CA-MRSA. The proportion of children below 5 years with CA-MRSA infections was 73.7% .[47]

13 tertiary care centers from different European countries provided data showing that mortality and length of hospital stay were attributable to *Staphylococcus aureus*. MSSA infections increased mortality greater than two-folds. To it methicillin resistance added an additional 80% excess mortality at day 30 after infection. This

scenario emphasize the fact that invasive *Staphylococcus aureus* infections cannot be ignored by the clinicians. They also underline the additional burden imposed by methicillin resistance which aggravates the clinical outcome and increases overall the caseload of patients with *Staphylococcus aureus* infection.

In September 2013, the Centers for Disease Control, USA (CDC) released a report stating that antibiotic resistance threats in United States is increasing at alarming proportions. More than two million people are affected with antibiotic-resistant infections and more than 20,000 people die annually as a result in the United States alone.[48]

In India a significant part of nosocomial infections are now caused by MRSA and the percentage of resistant strains are ever increasing. Hence for timely decisions regarding prompt therapy, accurate and rapid isolation of MRSA is needed.

The Genetic determinants of Methicillin Resistance:

Staphylococcal cassette chromosome *mec* (*SCCmec*), is a mobile genetic element of *Staphylococcus* bacterial species. This genetic sequence includes the 2.1kb *mecA* gene coding for resistance to the antibiotic methicillin. The resistance of *Staphylococcus aureus* to methicillin, a semi-synthetic penicillinase resistant penicillin is caused by the presence of *mecA* gene which encodes the 78kDa penicillin-binding protein (PBP)2a. Beta lactam antibiotics cannot bind to the PBP2a and so synthesis of the peptidoglycan layer and the cell-wall of the organism are able to continue.[1]

SCC mec elements shared the following characteristics:

(i) presence of *mecA* in a *mec* gene complex,

(ii) presence of a *ccr* gene in a *ccr* gene complex,

(iii) integration at a specific site in the *staphylococcal* chromosome, referred to as the integration site sequence (*ISS*) for *SCC,* which serves as a target for *ccr*-mediated recombination and

(iv) the presence of flanking direct repeat (DR) sequences containing the *ISS*.[49]

Eight *SCCmec* types have been described for *S.aureus*. The first three *SCCmec* elements were designted as types I, II and III and the other types as IV to VIII. An additional system for naming the novel *SCCmec* elements which is based on the type of *ccr* and class of *mec* present has also been proposed. According to this system, Type I (1B) *SCCmec* indicates a *SCCmec* having a type 1 *ccr* and a class B *mec* gene complex. The other known *SCCmec* types would be designated type II (2A), type III (3A), type IV (2B), type V (5C2), type VI (4B), type VII (5C1), and type VIII (4A). *SCCmec* types should be designated by roman numerals in an order in which they are reported, followed by the *ccr* gene complex and the *mec* gene complex together in parentheses.[49]

Cefoxitin disc diffusion susceptibility testing is widely used for *Staphylococcus aureus* and coagulase-negative *staphylococci* (CONS) isolated from human beings. It has better results than oxacillin disc diffusion testing.[50] Cefoxitin disc diffusion testing does not require alteration of incubation conditions and additional supplementary media.[51] Their zones of growth inhibition are clearly demarcated and easier to interpret.[52] Cefoxitin is also a potent inducer of the *mecA* regulatory system.[53]

HA-MRSA strains carry a relatively large Staphylococcal chromosomal cassette *mec* (*SCCmec*) belonging to type I, II, or III. These cassettes contain the *mecA* gene and they are often resistant to many classes of non-β-lactam antimicrobials. HA-MRSA strains seldom carry the genes for the Panton-Valentine leukocidin (PVL). But CA-MRSA isolates carry smaller *SCCmec* elements, most commonly *SCCmec* type IV or type V. These smaller elements carry the *mecA* gene and are resistant to fewer non-β-lactam classes of antimicrobials and frequently carry PVL genes.[12]

Pathogens are able to spread, establish ecological reservoirs, colonize and cause disease and can acquire resistance genes and adjust resistance to increasing concentrations of antimicrobial agents. Resistance genes can have a very broad host range. Mobile genetic elements involve in the spread of resistance and virulence in *Staphylococci* are genomic islands, bacteriophages, pathogenicity islands, chromosomal cassettes, plasmids, insertion sequences and transposons.[54]

PCR-based methods are being used increasingly by reference laboratories as the 'gold standard' for determining methicillin resistance in S*taphylococci*. This is due to the fact that MIC tests are subject to differences in size of inoculum, time of incubation, pH of the medium, salt concentration of the medium and so on.[55]

Detection and characterisation of microrganisms in diagnostic microbiology have been revolutionized by molecular methods. They have become part of routine laboratory investigations. Polymerase chain reaction (PCR) techniques have led the way into the present era by helping in rapid detection of microorganisms that were earlier difficult to detect by traditional investigation methods.[56]

Molecular methods can detect antimicrobial resistance genes. Use of multiplex PCR, real-time PCR and also the automated methods, the molecular methods are becoming a important part of laboratory investigations and their costs are decreasing. In near future their roles will further increase.[56]

Molecular methods are being incorporated into the clinical microbiology laboratory. They have the advantages of rapid turnaround time and high sensitivity and specificity. But must rigorous validation and quality control is essential in the molecular laboratories.[56]

PCR is the most well developed molecular technique up to now. It has a wide range of potential, clinical applications, including specific or broad spectrum pathogen detection, evaluation of emerging novel infections, surveillance, early detection of bio-threat agents and antimicrobial resistance profiling. PCR-based methods may also be cost effective relative to traditional testing procedures.[57]

Real-time PCR has been shown to be extremely useful for studies in the field of clinical microbiology. Most of the assays developed allow an increased frequency as well as enhanced speed of pathogen detection as compared to conventional culture techniques.[58]

Patrinos and Ansorge (2005) opined that in the coming years, molecular diagnostics will continue to be of clinical importance to public health worldwide. Molecular genetic testing will facilitate the detection and characterization of disease, as well as monitoring of drug response, and will assist in the identification of genetic modifiers and disease susceptibility.[59]

A comparison of PCR detection of *mecA* with Cefoxitin disc diffusion test:

Antibiotic susceptibility testing of methicillin resistance in *Staphylococcus aureus* is problematic due to the expression of heterogeneous resistance by many clinical isolates.[60] The few isolates that contain *mecA* appear to be phenotypically susceptible, but can show resistance if they are exposed to the action of *anti*-staphylococcal penicillins. Also standard susceptibility testing requires longer time in comparison to the time required for assays for *mecA* or PBP 2a.[61]

But, cefoxitin, which is a cephamycin, is a more potent inducer of the *mecA* regulatory system than are the penicillins. Reports exists about the results of cefoxitin disc diffusion (DD) tests correlating better with the presence of *mecA* than the results of disc diffusion tests using oxacillin.[5, 53]

Boutiba-Ben Boubaker et al carried out a study evaluating use of Cefoxitin disc diffusion test in routine detection of MRSA compared with PCR and oxacillin disc diffusion tests. It had 96.5% sensitivity and 100% specificity.[62]

Another study showed that Cefoxitin disc diffusion Test has sensitivity of 94.4% to detect MRSA when compared with PCR and other phenotypic method.[63]

Again, a study showed that the sensitivity of Cefoxitin disc diffusion test is 96.5% in detecting MRSA.[64]

Evaluating various methods Datta et al (2011) found that the cefoxitin disc diffusion test diffusion test had a sensitivity of 98.5%, whereas the oxacillin disc had a sensitivity of 91.4%. Specificity for the cefoxitin disc was 100%, whereas that for the oxacillin disc was 99.2%.[65]

PCR assay has advantages over it in that:

i. it can be performed rapidly,

ii. it can be performed without radioisotopes and

iii. a much smaller amount of DNA is sufficient for the detection of the MRSA-PBP gene compared with that needed for DNA probe analysis.[56]

The PCR assay was found to be a sensitive and reliable procedure for the rapid diagnosis of MRSA infection. This was proved in cases in which the conventional MIC assay failed to detect MRSA. Vannuffel et al used a comprehensive multiplex PCR assay simultaneously identifying three genetic markers that characterize the species, the antibiotic resistance mechanism and a consensus sequence used as the control. This led to the opinion that multiplex PCR approach is beneficial adjunct to standard microbiological methods for rapid and specific identifications of pathogens and resistance patterns.[66]

PCR carried out to detect methicillin-resistant *Staphylococci* reduces the time for identification from 24 to 48 hours to less than 3 hours.[67]

Hagen et al found that PCR assay proved to be a sensitive and reliable tool for the rapid identification of MRSA from isolated colonies or clinical specimens. It can be integrated into the workflow of a diagnostic laboratory. Though PCR technology is more expensive than the conventional methods, the benefit of saving time regarding expenses is comparable. Patients colonized with MRSA are often kept in isolation till their cultures become MRSA negative. In such cases, a quick PCR assay helps to reduce isolation time and costs.[68]

Phenotypic expression of methicillin-resistance is usually heterogeneous and methicillin-resistance is influenced by culture conditions such as temperature,

medium, pH and NaCl content in medium. These factors complicate the detection of methicillin-resistance, especially for strains with low level resistance. The PCR methods have higher sensitivity and specificity and there is no need to take precautions regarding the physical and chemical conditions as it is done in case of culture methods.[69]

Mulligan et al found that the principal mode of transmission of MRSA is patient-to-patient transfer of the MRSA strain by transiently colonized hands of the hospital personnel. Hospital personnel acquire them from direct patient contact or by handling the contaminated material. Air borne transmission is also an important mode of transmission in patients who had undergone tracheostomy in Intensive Care Units.[70]

The study by Kumar, Sukla and Varshney observed that highest percentage of nasal carriage of MRSA among healthcare workers was doctors and less percentage in laboratory technicians. They opined that screening should be made an essential protocol to assess the transmission of drug resistant strains of S*taphylococc*i from the community to hospital set-up and hospital to vice-versa.[71]

The study by Saravanan, Nana and Tesfaye provided a comprehensive information of epidemiology as well as burden of MRSA infection among Septicemia Suspected paediatric age group in Hosur, South India. During the study, they identified a stable increase in the overall incidence of *S. aureus* infection and that of MRSA infection.[72]

***Staphylococcus aureus*:**

Staphylococci are gram-positive bacteria occurring in grape–like clusters. Koch differentiated gram-positive *cocci* in 1878. He recognized that different diseases correlated with the presence of clusters of gram-positive cocci. In 1882 Ogston gave the name to the clustered micrococci "*staphylococci*," from the Greek word "*staphyle*", meaning a bunch of grapes. Rosenbach in 1884 described the two types of pigmented colony of *staphylococci*. He proposed the nomenclature: *Staphylococcus aureus* (yellow) and *Staphylococcus albus* (white). The species *Staphylococcus albus* is now named as *Staphylococcus epidermidis*. Coagulase testing later provided a better classification of *staphylococci* than pigment production wherein a positive coagulase test confirmed the identity of *S. aureus* and this correlated much better with pathogenicity.[73]

Classification of *Staphylococci* and identification of *S. aureus:*

Identification of *Staphylococcus aureus* begins with presumptive identification of gram-positive cocci in clusters in the gram-stained smear that signal a positive result. Direct microscopy by itself cannot adequately differentiate between different species of *staphylococci, enterococci* and *streptococci*. Final identification awaits subculture and overnight incubation. Conventionally, the identification of bacteria in the clinical microbiology laboratory is done by the isolation of the organism and by gram staining, culture, and biochemical characteristics, which have been regarded till date as the gold standard procedure for bacterial identification.

Staphylococcus aureus secretes free plasma coagulase which is a virulence factor and also an important criterion for distinguishing it from Coagulase-Negative *Staphylococci.*[74]

Mannitol fermentation test, coagulase tests, agglutination test also help in identifying *S. aureus* from other *staphylococci*.[75]

The slide and tube coagulase tests are more reliable as they give more reliable results than the latex agglutination and microtube coagulase tests. Tube coagulase test still remains a test of choice for identification *S.aureus* due to its high sensitivity and specificity.[76]

A generally accepted criterion for the identification of *S. aureus* is a positive coagulase test. Coagulase is an enzyme that converts fibrinogen into fibrin, i.e. causes blood clotting. *Staphylococcus aureus* produces both free coagulase and coagulase bound on cell surfaces while Coagulase-Negative *Staphylococci* does not express the protein. The tube coagulase test is considered the gold standard for distinguishing a coagulase-positive species from the generally less virulent Coagulase-Negative *Staphylococci*. To date, six species of coagulase-positive *staphylococci* have been identified in addition to *Staphylococcus aureus*. These include *S.intermedius, S. schleiferi subsp.coagulans,S. hyicus, S. lutrae, S. delphini and S. pseudointermedius*.[77] Coagulase-positive species other than *S. aureus* are predominantly animal pathogens.[78] The first generation of latex agglutination tests for rapid identification of *S. aureus* colonies detected bound coagulase and protein A on bacterial cell surfaces.[79] A new generation of highly sensitive commercial slide agglutination tests for identification of *S. aureus* was based on monoclonal antibodies against multiple antigens, including the capsular polysaccharides.[80] These tests still suffer from false-positive results due to some Coagulase-Negative *Staphylococcus* strains known to share the same antigens with *S. aureus*.[81]

Simple PCR assays for reliable identification of *S. aureus* colonies have been around for two decades. [82]

S. aureus carriage:

About 20% of the population are persistent carriers, while approximately 30% carry the organism only intermittently.[83] Children have higher persistent carriage rates compared to adults.[84] Other sites of colonization include the perineum, vagina, throat, axillae/groin and intertriginous skin folds.[85] The carriage of *S. aureus* is usually highest in hospitalized patients. The organism is also found on clothing, bed linen and other fomites of human environment. *S. aureus* can spread to other individuals in hospitals and in the community from healthy carriers among patients and personnel as well as from infected persons. The most important route of transmission is direct contact via the hands of hospital staff. It can also spread by contamination from the environment or by aerial dissemination especially from skin and upper respiratory tract.[86]

Virulence mechanisms:

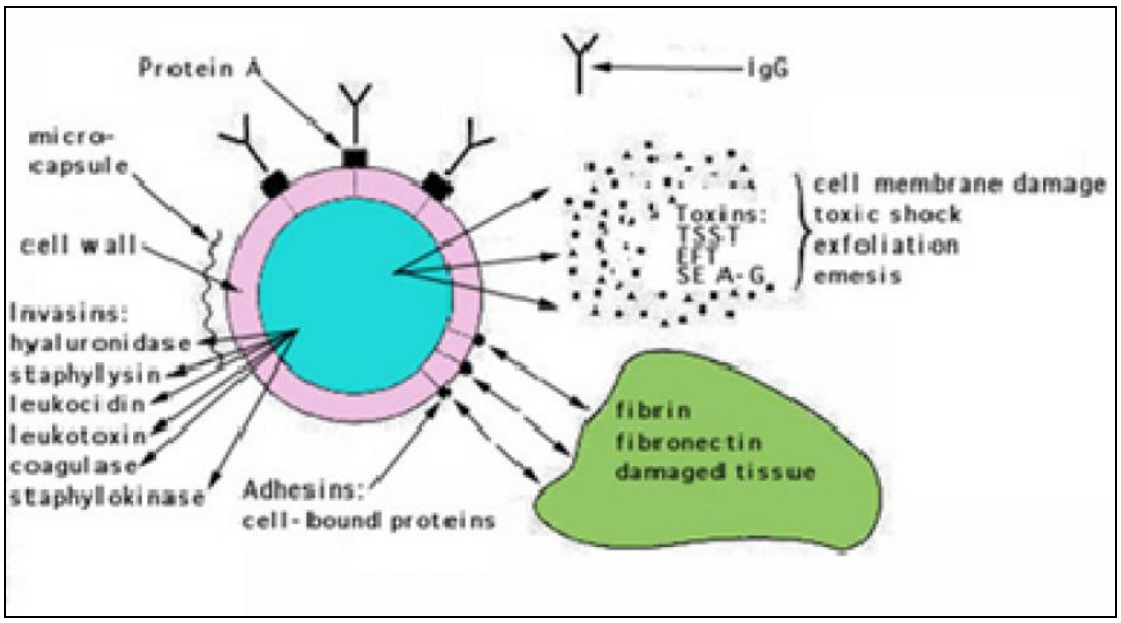


Fig 3: [Virulence Determinants of *Staphylococcus aureus*]

Virulence mechanisms of *Staphylococcus aureus* can cause various types of infections like abscess formation, evasion of host immune responses at many levels and induction of the sepsis syndrome.[87] For example protein A binds immunoglobulins on *S. aureus* surfaces and is thought to act as an immunologic disguise.[88] Nearly all strains secrete a variety of proteins to the surrounding media that convert host tissues to nutrients for the invading bacterium.[89]

Staphylococcal superantigens causes food poisoning and nonmenstrual Toxic Shock Syndrome.[90]

Pathogenesis:

The pathogenesis of *S. aureus* infections is divided into five stages[91]: colonization, local infection, systemic dissemination, metastatic infection and, finally, toxic shock. Healthy skin presents an effective barrier, but through small abrasions a local skin infection may develop. Unless limited by the host immune defences, the infection advances into a local abscess and eventually gains access to the blood stream. Through blood, the organism can spread to any distant organ causing endocarditis, osteomyelitis, deep abscesses, etc. Septic shock may ensue as a result of systemic effects of circulating toxins.

Emergence of methicillin resistance and resistance mechanisms:

The first reports of methicillin resistance in *S. aureus* appeared soon after the introduction of penicillinase-stable penicillins.[92] Methicillin resistance in *staphylococci* is caused by the *mecA* gene which encodes an altered penicillin-binding protein 2a (PBP2a) with a low affinity for beta-lactam antibiotics such as penicillin,

anti-staphylococcal penicillins including methicillin, and cephalosporins.[93] MRSA a regenerated when MSSA acquire the *mecA* gene.

The Staphylococcal cassette chromosome *mec (SCCmec*) is a family of large mobile genetic elements that include the *mecA* gene.[49] The *mecA* MRSA strains are thought to have emerged by means of horizontally transferred *SCCmec* from Coagulase Negative *Staphylococci* (CoNS). Transfer of DNA containing the *mecA* code from *S. epidermidis* to *S. aureus* has been witnessed in vivo.[94]

Epidemiology:

MRSA clones have spread around the world reaching an endemic status in most of the developed countries. It is not known whether this is due to differentiation from only one specific clone or introduction of *SCCmecA* into numerous clones.[95] Until lately, MRSA clones have mainly been of hospital or health care-associated (HA) origin. MRSA infections in persons with no previous history with healthcare contact were first reported in the early 1990s from Australia, in the late 1990s from the USA, and in the early 2000s from Europe.[96] Initially, the strains were termed community-acquired MRSA, but presently, the most common term used is community-associated (CA) MRSA and Hospital-associated (HA) MRSA to avoid the ambiguity as to whether the strain has been acquired in the community or in hospital. Indeed, HA-MRSA and CA-MRSA strains cannot be reliably differentiated based on epidemiological data alone, indicating a need for a genotypic definition. In recent years, MRSA has also been isolated from livestock (livestock-associated MRSA, LA-MRSA).[97] Today, pandemic MRSA includes dissemination of HA-MRSA clones from the 1960s, CA-MRSA clones from the 1990s, and LA-MRSA clones from the 2000s. [96,98,99,100]

Risk factors, modes of transmission:

HA-MRSA:

Patients undergoing extensive operative procedures, prosthetic implant surgeries or burn patients, patients in intensive care or trauma units are prone to HA-MRSA. Damaged skin and insertion of intravascular catheters increase the risk for colonization.[101] Transmission of HA-MRSA occurs most commonly through the hands of hospital workers from MRSA-colonized or infected patients to other patients and from contaminated inanimate environment or sometimes through aerial dissemination.

HA-MRSA strains are almost always acquired during healthcare contact, but the onset of infection may be in hospital or in the community. The variety and clinical presentation of infections caused by HA-MRSA correspond to those caused by MSSA during hospital stay. These infections include e.g., wound and other skin and soft-tissue infections, bacteremia and endocarditis, pneumonia, bone and joint infections, prosthetic device-associated infections and central nervous system infections.[102]

CA-MRSA:

CA-MRSA by definition affects patients with no prior contact with healthcare settings. An epidemiological definition of CA-MRSA proposed by the CDC is as follows: MRSA must be identified in the outpatient setting or less than 48 hours after hospital admission in an individual with no medical history of MRSA infection or colonization, admission to a healthcare facility, dialysis, surgery, or insertion of indwelling devices in the past year.[103] The absence of conventional risk factors for MRSA in patients colonized with CA-MRSA suggests that some of these strains may

spread more easily than customary HA-MRSA strains. Like all *S. aureus* strains, CA-MRSA is transmitted by direct contact with infected or colonized individuals, or through a MRSA-contaminated environment.[104] CA-MRSA can be acquired through activities in which direct body contact is common. The CDC has proposed 5 factors (or 5 Cs) associated with CA-MRSA transmission, i.e. 1) Crowding, 2) skin-to-skin contact, 3) Compromised skin integrity, 4) Contaminated items and surfaces, and 5) lack of Cleanliness. These factors are common in populations with increased numbers of infections or colonization caused by CA-MRSA.[1] It is important to differentiate between CA-MRSA and HA-MRSA in order to provide optimal clinical treatment and infection control measures, as well as to reliably monitor the epidemiological MRSA situation worldwide.

	HA-MRSA	**CA-MRSA**
Genetic	Various *SCC*	PVL, *SCC* IV
Area affected	Blood stream, surgical site, implant site.	Skin, lungs
Person affected	Immunocompromised, resident in long-term facilities, recent surgery, recent hospitalization, dialysis.	Young, otherwise healthy, no recent hospitalistion: Anyone.
Tansmission	Skin to skin contact, contaminated equipment, poor hygiene.	Skin to skin contact, cuts or scrapes, crowded area, poor hygiene.
Recombination treatment	Debridement, antibiotic, education.	Incision and drainage, antibiotic, educating, hygiene.

Prevention	Good hygiene, infection control, staff education,careful antibiotic administration, follow up.	Good hygiene, proper wound care, no-touch technique, education, no item sharing**.**
Screening/ diagnosis	Test not required; skin or nasal swab taken; PCR for rapid testing; screen high risk patients; CLSI recommended testing	Testing not recommended; testing wound drainage; testing colonization culture not recommended.

Laboratory diagnosis of MRSA:

Identification of MRSA from pure cultured colonies:

Genotypic methods:

The gold standard: detection of the *mecA* gene. An intact *mecA* gene is essential for the expression of clinically significant methicillin resistance in *S. aureus*. There are several auxiliary genes, found in both susceptible and resistant *S. aureus* strains, with effects on the level of methicillin resistance.[105] As a result, *S. aureus* strains possessing the *mecA* gene have variable resistance phenotypes from borderline to highly resistant. The heterogeneous nature of methicillin resistance limits the accuracy of all phenotypic methods; consequently, a genotypic approach, detection of the *mecA* gene, is accepted as the gold standard of the MRSA diagnostics.[99]

Phenotypic methods:

Disc diffusion tests:

After identification of *Staphylococcus aureus* from a pure culture, phenotypic methods can be used for the testing of methicillin resistance. The disc diffusion method is the most widely used technique for susceptibility testing in clinical laboratories since it is inexpensive and technically simple. Interpretation of the test results has been well standardized. Both the US Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) periodically review the test method for optimal performance.

Isolated colonies from a pure culture are used to prepare an inoculum which is then inoculated on a Mueller-Hinton agar (MHA) plate. After 16 to 24 hours of incubation visible zones of growth inhibition are formed around the discs. Zone diameters are interpreted using the CLSI/EUCAST criteria and the results are reported qualitatively as sensitive, intermediate, or resistant.

Expression of the *mecA* gene is affected by environmental factors such as temperature, osmolarity and certain antimicrobial agents. Therefore, culture conditions are modified to enhance the expression of methicillin resistance. Recent studies suggest that disc diffusion techniques using cefoxitin are superior to oxacillin-based methods. Cefoxitin has been recognized as a potent inducer of the *mecA* regulatory system. Studies have shown improved accuracy of the cefoxitin disc diffusion test when performed at 30°C, although high accuracies in standard 37°C have been reported as well.[106] The EUCAST/CLSI standards currently promote usage of cefoxitin instead of oxacillin for MRSA diagnostics using the disc diffusion

method . For a 30 µg cefoxitin disc a single breakpoint is recommended: an inhibition zone diameter ≤ 21 mm (CLSI) / < 22 mm (EUCAST) indicates MRSA. MHA plates without additional salt and incubation at 35°C for 18 hours should be used.

Antimicrobial gradient method:

An Etest is the most commonly used antimicrobial gradient diffusion method. It employs a test strip impregnated with a dried antibiotic concentration gradient marked on the surface of a scale. Multiple strips can be placed on the surface of an agar plate that has been inoculated with a standard bacterial suspension. Again, a pure culture is needed before the Etest can be employed. After an overnight incubation the MIC can be determined by reading the scale on the strip at the intersection of the bacterial growth.[107]

Methicillin and oxacillin Etests can be used as a simple way to detect MRSA. According to CLSI, the MIC breakpoint of oxacillin resistance for *S. aureus* is ≥ 4 microg/mL.[108] A cefoxitin MIC value of ≥ 4 microg/mL (CLSI 2011) indicates MRSA. MHA plates supplemented with 4% NaCl and incubated at +35°C for 24 hours are recommended.[109]

Broth dilution:

The tube-dilution method was one of the earliest antimicrobial susceptibility testing methods. This involved preparing multiple dilutions of an antibiotic in a liquid growth medium. Separate test tubes for each dilution were all inoculated with standard bacterial suspension derived from the pure culture of the original clinical sample. After an overnight incubation the tubes were examined for turbidity caused

by bacterial growth. The lowest concentration of the antibiotic that prevented growth was reported as the MIC value.[106]

MATERIALS AND METHODS

The present study was conducted at the Department of Microbiology, Jawaharlal Nehru Medical College,Belgaum.

Source of data:

All *Staphylococcus aureus* isolated from different clinical samples received in Microbiology Department of J.N.M.C, Belgaum, from January 2013 to December 2013 were included in this study.

Sample size- n = Z_{α}^{2}Sensitivity [100-Sensitivity] ÷(d^2 x prevalence)

Z_{α}= 1.96

Sensitivity= 95% (ref.10,63)

$\alpha = 0.05$, 95% confidence

Absolute error= d= 1%

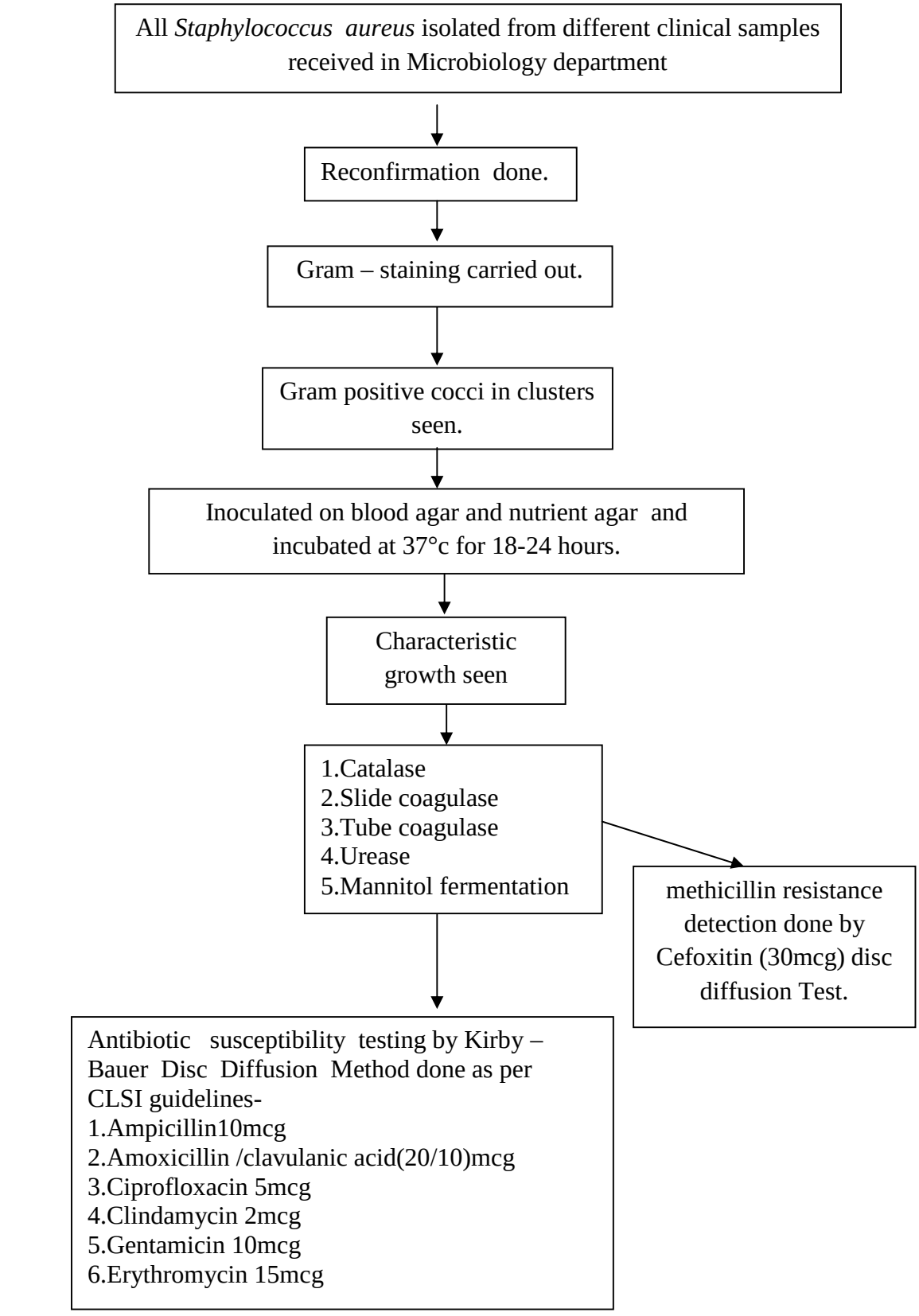
Prevalence= 31% (ref.46)

n= sample size

n= $(1.96)^2 \times 95\ [100 - 95] \div \{(1)^2 \times\ 31\}$=58.8=60,

Inclusion criteria- *Staphylococcus aureus* isolates.

The following is the plan of investigation-



Gram stain-

A thin smear was made on a glass slide from a single colony and fixed by flaming over the Bunsen burner. After fixing, gention violet was poured over the smear, care was taken to completely cover the smear. It was allowed to stand for 1minute and then washed with tap water. Then Gram's iodine was poured over the slide and kept for 1 minute, washed with water and was decolorized by 95% alcohol till no coloured solvent flowed down the slide. The slide was then washed with tap water and counter-stained with safranine for 1 minute. Again it was washed with water and dried and was observed under the oil immersion objective.

Quality control-

Positive control- *Staphylococcus aureus* ATCC 25923.

Negative control- *Escherichia coli ATCC* 25922.

Coagulase test:

This was done to differentiate between the *Staphylococcus species.* Both slide coagulase test and tube coagulase test was done.

Slide coagulase test:

Principle:

Staphylococcal coagulase is a protein that has prothrombin like activity which can convert fibrinogen to fibrin. A visible clot will result. Side coagulase test detects bound coagulase which is attached to bacterial cell wall and not present in culture filtrate. Fibrin strands are formed between bacterial cells when suspended in plasma (fibrinogen), causing them to clump into visible aggregates.

Procedure:

A drop of normal saline is taken on a clean glass slide. In it a portion of the isolated colony is emulsified. A drop of plasma is placed next to the earlier drop and mixed to get a smooth suspension. The slide is rocked gently for 5 to 10 seconds.

Interpretation:

Coarse clumping of cocci visible to naked eye within 10 seconds is positive.

Negative when absence of clumping and slow reaction was seen after 10 seconds.

Quality control-

Positive control- *Staphylococcus aureus*

Negative control-*Staphylococcus epidermidis*

Tube coagulase test-

Detects free coagulase, a thrombin like substance present in culture filtrate. Free coagulase reacts with serum substance (coagulase reacting factor) to form a complex that, in turn, reacts with fibrinogen to produce the fibrin clot.

Procedure-

Three test tubes were taken and labeled “test”, “negative control” and “positive control”. Each tube was filled with 0.5 ml of 1 in 10 diluted plasma. To the tube labelled test, 0.1 ml of overnight broth culture of test isolate was added. To the tube labelled positive control, 0.1 ml of overnight broth culture of known *S.aureus* strain was added and to the tube labelled negative control, 0.1 ml of sterile broth was added. All the tubes were incubated at 37◦C and observed up to 1,2 and 4 hours by tilting the tube at 90◦C.

Interpretation:

Positive when any degree of visible clot formation or stiff gel formation or if clots were seen floating in the medium.

Negative when no visible clots were seen or showed only a flocculent or ropy precipitate.

Quality control-

Positive control-*Staphylococcus aureus*

Negative control-*Staphylococcus epidermidis*

Catalase test-

Principle- The enzyme catalase mediates the breakdown of hydrogen peroxide into water and oxygen. The presence of the enzyme is evident in Bacterial isolates when a small inoculum is introduced into 3% H_2O_2. Rapid effervescence of oxygen bubbles occur.

Procedure-

Presence of catalase was demonstrated by test tube method. With a sterile glass rod a portion of the colony is taken and inserted in to 3% H_2O_2. Effervescence produced immediately gives positive result.

Interpretation-

Effervescence produced indicates positive reaction.

No gas bubbles produced is a negative reaction.

Quality control-

Positive control-*Staphylococcus aureus*

Negative control- *Streptococcus pyogenes*

Urease test-

Principle-This was done to determine the ability of bacteria to decompose urea in ammonia. Here Christensen's urease agar was used.

Procedure- Christensen's urease agar was heavily inoculated over the entire surface with peptone water culture and incubated at 37◦C.Then it is examined after overnight incubation.

Interpretation-

Positive: when the indicator turns to purple pink.

Negative: no change in colour.

Quality control:

Positive control-*Proteus species,*

Negative control-*Escherichia coli*

Antibiogram-

Antibiotic sensitivity was tested by Kirby-Bauer's disc diffusion method. Mueller- Hinton agar plate was used. One–two colonies from the culture plate were inoculated into 2 ml of peptone water and incubated at 37◦C for 2 hours. Turbidity was compared to that of 0.5 Mcfarland's standard ($1.5x10^8$ CFU/ml). A cotton swab was immersed, rotated in this inoculum, the swab was then pressed to the sides of the tube so on to remove excess inoculum. The swab was then used to inoculate the plate

of Mueller-Hinton agar, in three different directions to ensure an even and complete distribution of the inoculum over the entire plate. The antibiotic discs were applied within 15 minutes of inoculation of plate and the plate inverted and incubated for 18-24 hours at 37◦C.

Commercially obtained Himedia discs were used. The strength of discs used and their zone size interpretative standards were according to guidelines by CLSI standards.

The drugs used for sensitivity testing were:

- Ampicillin10mcg
- Amoxicillin /clavulanic acid(20/10)mcg
- Ciprofloxacin 5mcg
- Clindamycin 2mcg
- Gentamicin 10mcg
- Erythromycin 15mcg.

Cefoxitin (30mcg) Disc Diffusion Test to detect methicillin resistance-

i. A 0.5 McFarland standard suspension of isolates was made,
ii. Lawn culture was done on MHA plate,
iii. Plates incubated at 33-35°c for 16-18 hours,
iv. Zone diameters measured,
v. As per CLSI 2011 guidelines-

   Inhibition zone diameter of ≤21 mm=resistant

   Inhibition zone diameter of ≥ 22 mm= sensitive

vi. Positive control= methicillin resistant *S.aureus* ATCC 43300
vii. Negative control= methicillin sensitive *S.aureus* ATCC 25923.

PCR for detection of *mecA* gene in *S. aureus* isolates-

DNA Extraction:

- It was carried out by DNA extraction kit from Invitrogen By ***life* technologies.**
- First 1-2 colonies of the *Staphyococcus aureus* isolate was inoculated in nutrient broth and incubated for 2 hours.
- At the end of 2 hours, the broth was centrifuged and a cell pellet containing $\leq 2 \times 10^{9}$ cells were harvested. This was equivalent to 0.5 Mcfarland ($1.5x10^{8}$CFU/ml).
- Two water baths were set up at 37◦C and 55◦C, respectively.
- To 200µL of Lysozyme Digestion Buffer, 8 microlitre of fresh Lysozyme was added.
- The cell pellet was resuspended in 180 µL of Lysozyme Digestion Buffer and lysozyme preparation.
- Then mixed well by brief vortexing.
- After that incubated at 37◦C for 30 minutes in the water bath.
- 20 microL of Proteinase K was added.
- Was mixed well by brief vortexing.
- Then 200 microL PureLinkR Genomic Lysis/Binding Buffer was added and mixed well by brief vortexing.
- Again, incubated at 55°C for 30 minutes in another water bath.
- 200 µL 96–100% ethanol was added to the lysate.
- Was mixed well by vortexing for 5 seconds to yield a homogenous solution.

Binding DNA:

- PureLinkR Spin Column was removed in a collection tube from the package (They were provided in the DNA extraction kit).
- Then 640 microL of the lysate prepared with PureLinkR Genomic Lysis/Binding Buffer and ethanol was added to the PureLinkR Spin Column.
- Then centrifuged at 10,000 x g for 1 minute at room temperature.
- The collection tube was discarded and the spin column was placed into a clean PureLinkR Collection Tube supplied with the kit.

Washing DNA:

- 15 ml of ethanol was added to Wash Buffer 1 provided with the kit.
- 500 microL of this preparation was added to the spin column from the earlier step.
- The column was centrifuged at room temperature at 10,000 x g for 1 minute.
- The collection tube was discarded and the spin column was placed into a clean PureLinkR collection tube supplied with the kit.
- Again, 17.5ml of ethanol was added to Wash Buffer 2 provided with the kit.
- 500 microL of this preparation was added to the spin column from the previous step.
- The column was centrifuged at 10,000 x g for 3 minutes at room temperature. The collection tube was discarded.

Eluting DNA:

- The spin column was placed in a sterile 1.5-mL microcentrifuge tube.
- 200 µL of PureLinkR Genomic Elution Buffer provided with the kit was added to the spin column.
- Then kept standing at room temperature for 1 minute.
- The column was again centrifuged at 10,000 x g for 1 minute at room temperature.
- The microcentrifuge tube after centrifugation contains purified genomic DNA.

Storing of DNA:

The purified DNA was stored at –20°C.

To confirm whether DNA extraction was successful as well as the quality of DNA, agarose gel electrophoresis with the extracted DNA sample was carried out.

Purity of DNA was also checked by the use of Eppendorf Biophotometer.

PCR was carried out by the following protocol:

PCR reaction mixture:

- 47 microL of PCR supermix,
- 1 microL of extracted DNA,
- 1 microL of forward primer,
- 1 microL of reverse primer.

↓

Amplification was carried out in Eppendorf Mastercycler gradient thermal cycler.

The steps were:

- Initial denaturation at 94◦c for 1minute,
- Denaturation at 94◦c for 45 seconds,
- Annealing at 54◦c for 45 seconds,
- Extension at 72◦c for 1minute and
- Final extension at 72◦c for 2minutes, with a total of 30 cycles.

↓

Then 10 microlitre of amplified sample was taken and analysed by agarose gel electrophoresis using 2% agarose gel.

The PCR supermix consisted of (Invitrogen By *life* technologies):

- 22 mM Tris-HCL (pH8.4)
- 55 mM KCL
- 1.65 mM $MgCL_2$
- 220 micro MdGTP
- 220 micro MdATP
- 220 micro MdTTP
- 22U recombinant Taq DNA Polymerase/ml
- Stabilizers.

Primers used were {Bioserve Biotechnologies(India)Pvt.Ltd.}:

(5 /-GTA GAA ATG ACT GAA CGT CCG ATA A-3/_)

(5 /-CCA ATT CCA CAT TGT TTC GGT CTA A-3/)

Primers	**Sequence**	**Amplicon size**	**Ref.**
Forward	(5/-GTA GAA ATG ACT GAA CGT CCG ATA A-3/_)	310bp	**Jonas et al**[64].
Reverse	(5 /-CCA ATT CCA CAT TGT TTC GGT CTA A-3/)		

PHOTO 1: Gram Stain of *Staphylococcus aureus*

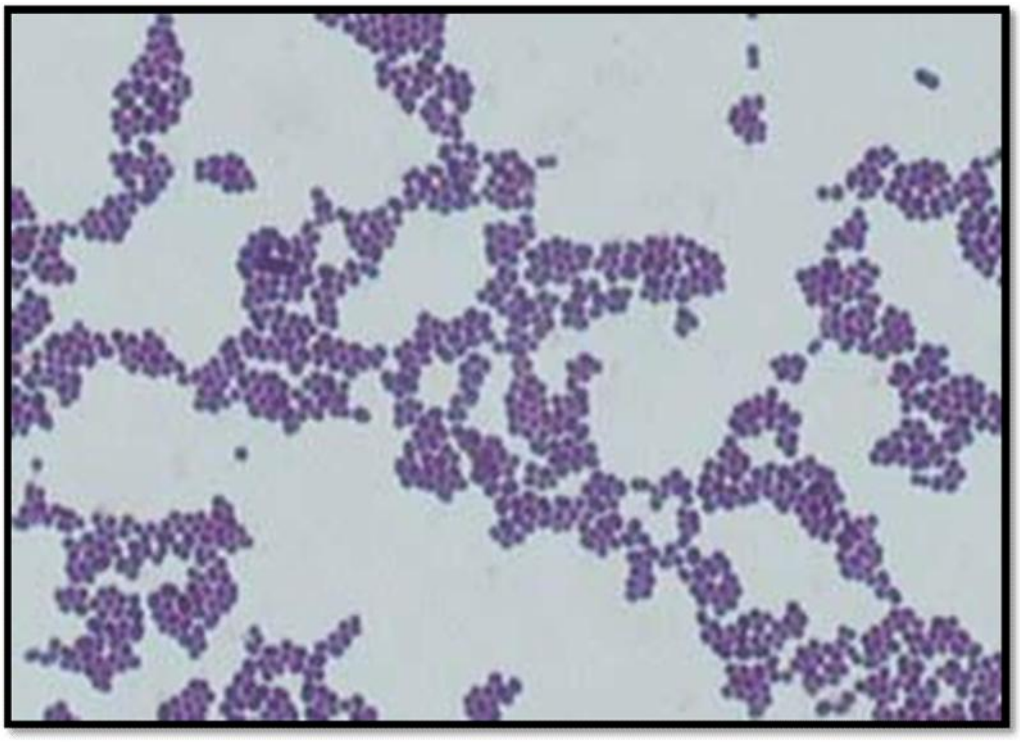


PHOTO 2: Nutrient agar showing golden yellow colonies of *Staphylococcus aureus*.

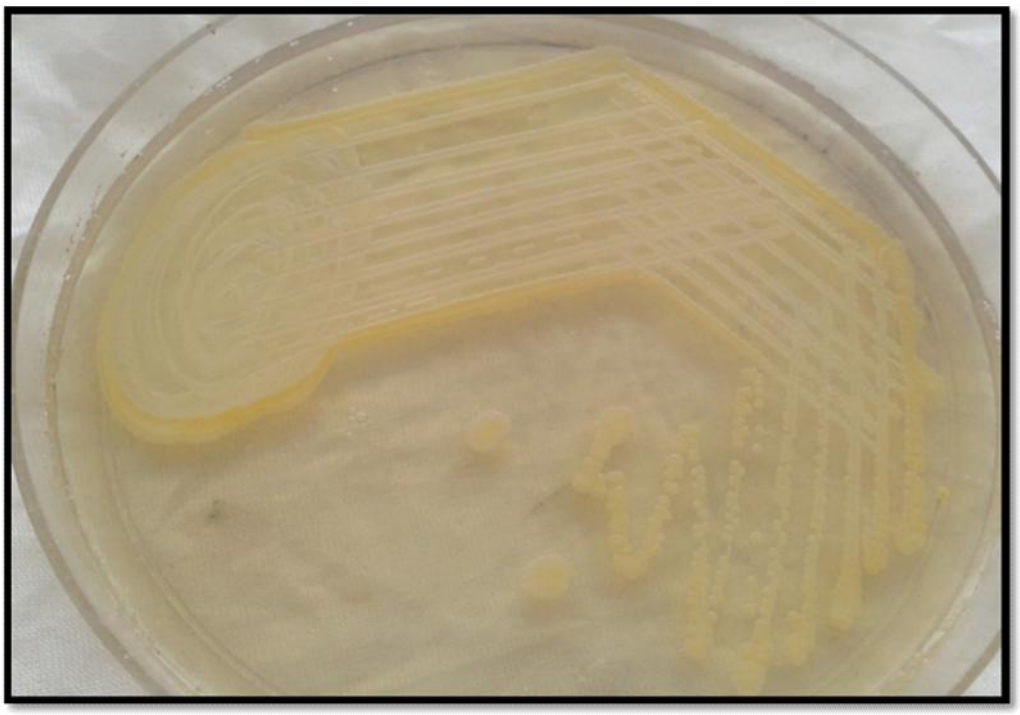


PHOTO 3: Blood agar showing beta haemolysis by *Staphylococcus aureus.*

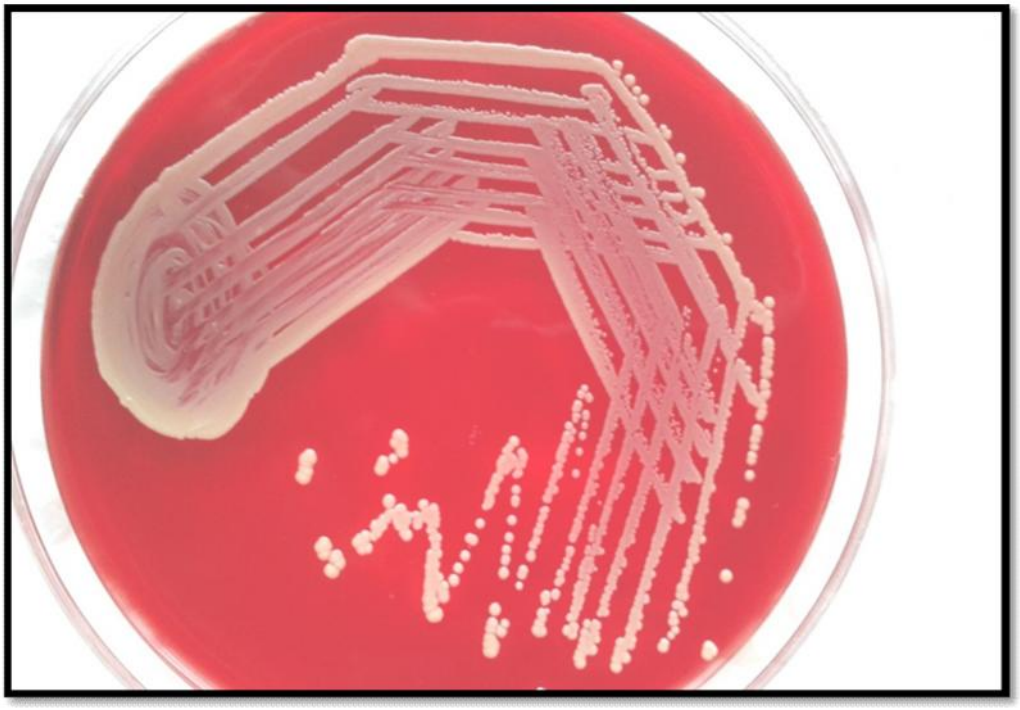


PHOTO 4: Biochemical reactions of *Staphylococcus aureus.*

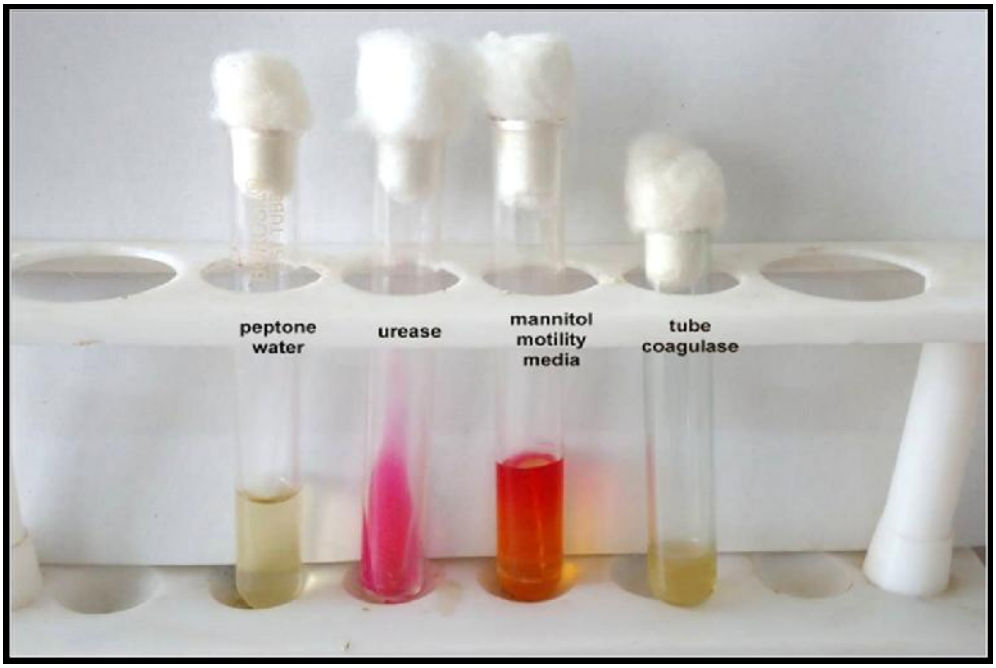


PHOTO 5: Cefoxitin disc diffusion test –showing sensitive zone =methicillin-sensitive.

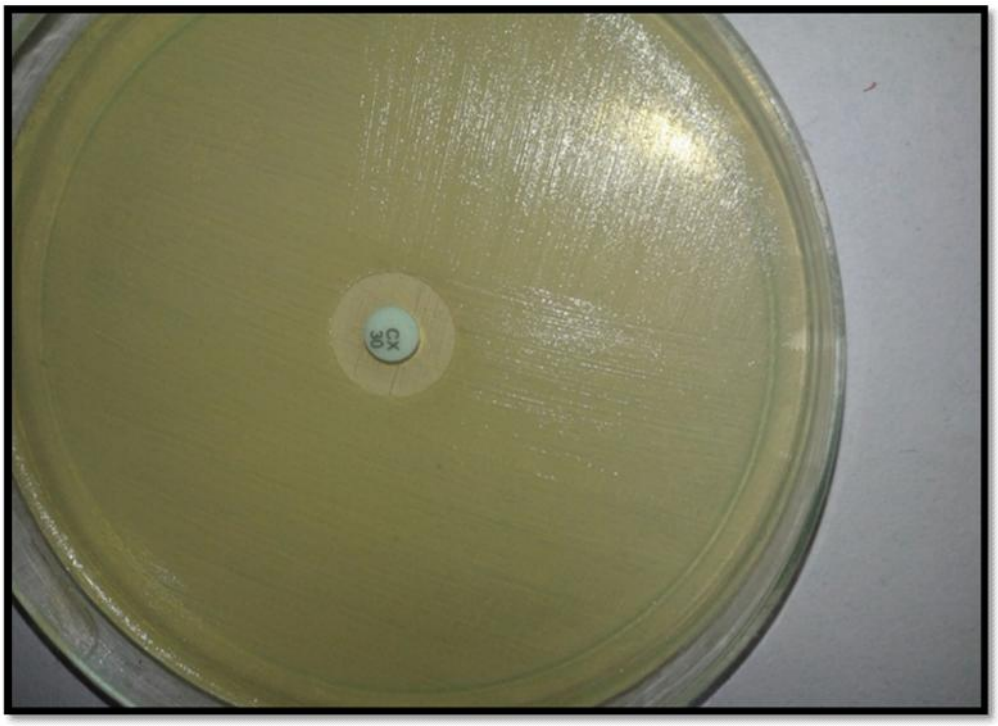


PHOTO 6: Cefoxitin disc diffusion test – showing no zone= methicillin -resistant.

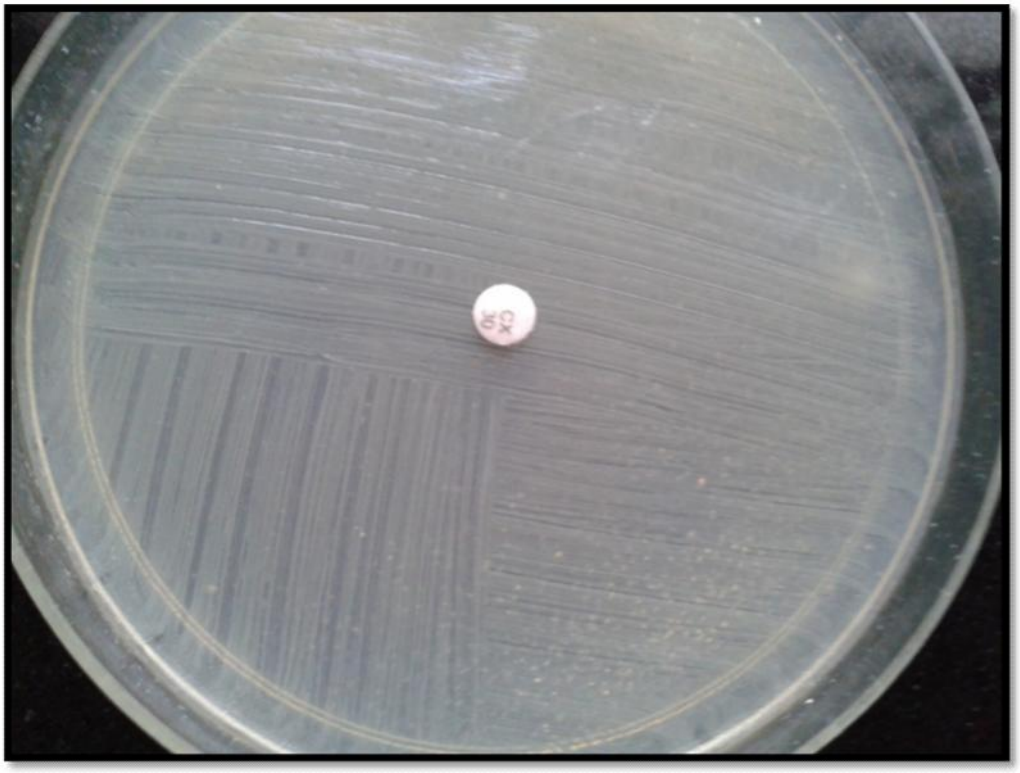


PHOTO 7: Eppendorf Mastercycler gradient thermal cycler.

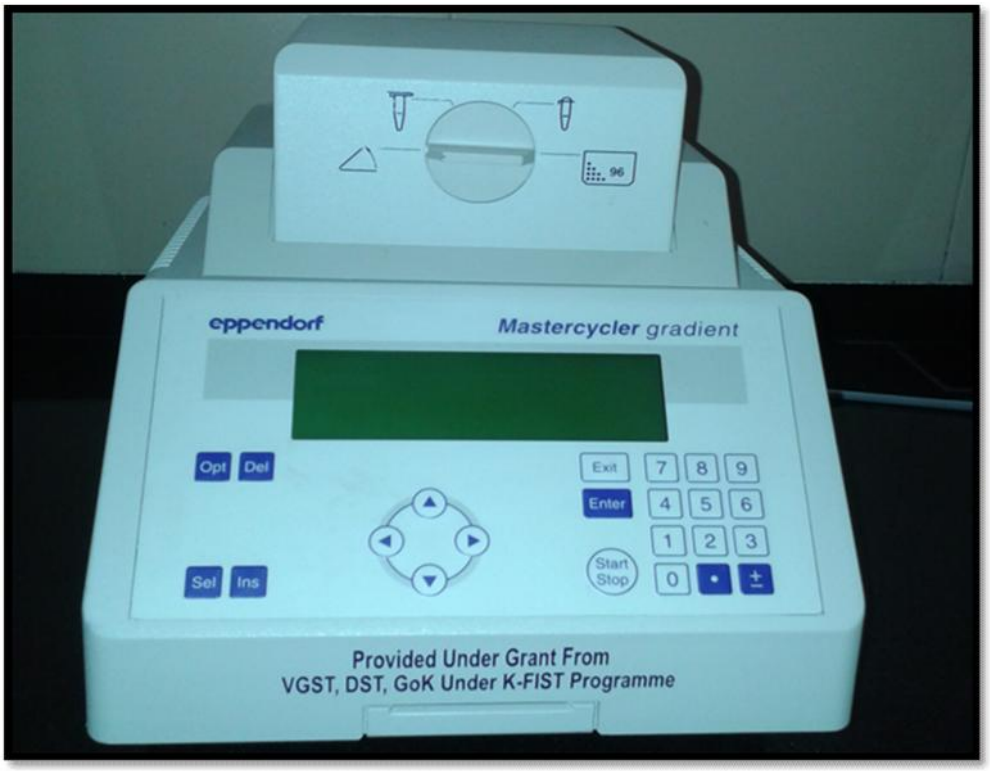
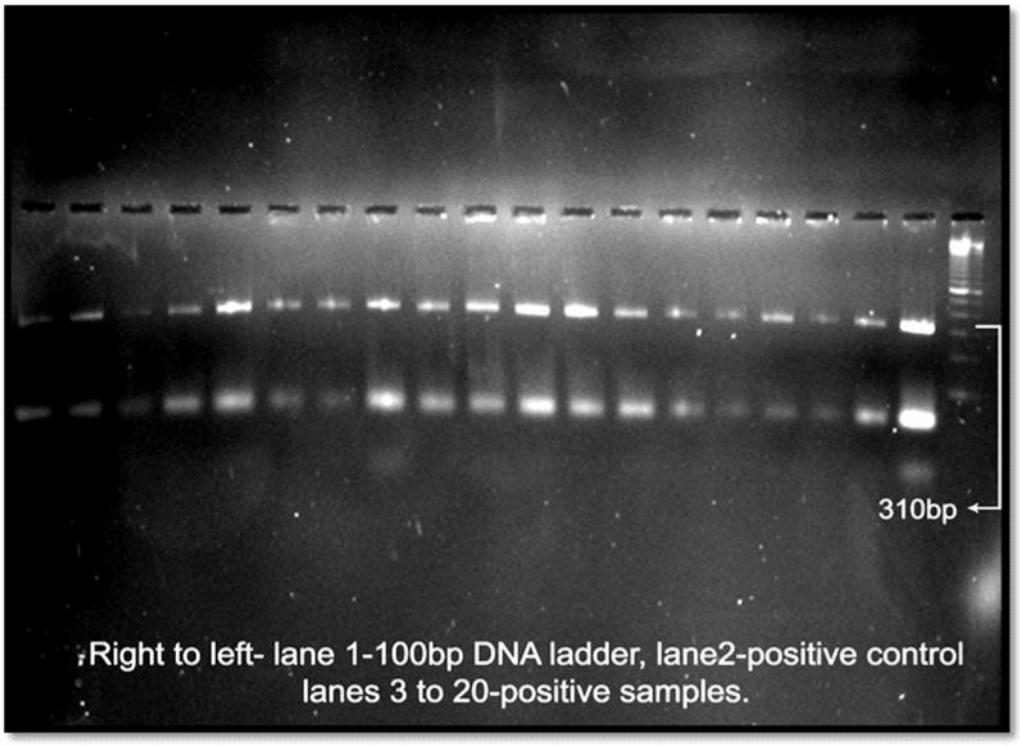
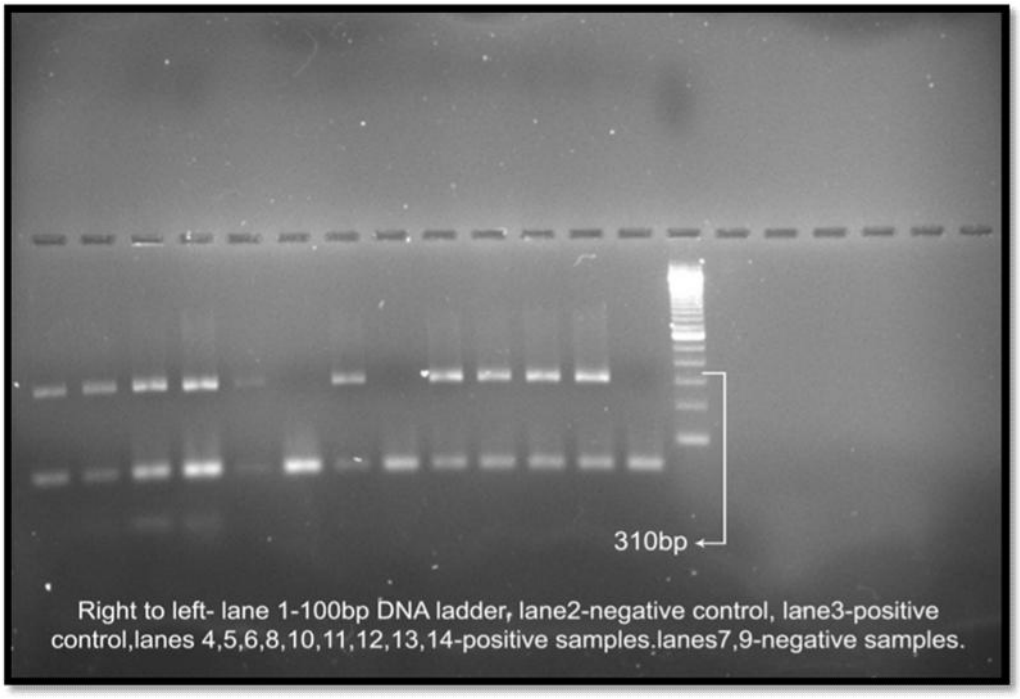


PHOTO 8 : Syngene Agarose Gel Documenttion system used to read and record the results of agarose electrophoresis.

PHOTOS 9 and 10 :Result of PCR for *mecA* gene[310 bp (base pair) product]





RESULTS

- In the present study, a total of 372 *Staphylococcus aureus* strains were isolated from different clinical samples. The clinical samples were pus samples (from wounds, eye infections and surgical site infections and abscesses), blood, urine, sputum, pleural fluid, synovial fluid, nasal crust, central and long line tips, catheter tips and skin biopsy material. The *S. aureus* isolates isolated from them were tested for methicillin- resistance by Cefoxitin disc diffusion test.

- 60 isolates out of the 372 were taken based on systematic sampling as our sample size was 60.

- Cefoxitin disc diffusion test and PCR for *mecA* gene, both, were carried out for these 60 *Staphylococcus aureus* isolates.

- Out of the 372 *S. aureus* isolates, 160 were methicillin-resistant. They were isolated from pus, blood, urine, catheter tip, pleural fluid and synovial fluid.

- **Percentage of MRSA isolates obtained from different clinical samples**:

  In the present study, percentage of MRSA isolates in different samples were 88.125% from pus (from wounds, eye infections, surgical site infections and abscesses) samples, 5.625 % from blood, 3.75% from urine, 1.25% from catheter tips, 0.625% each from pleural fluid and synovial fluid.

- **Distribution of MRSA according to sex:** Percentage of MRSA isolates found in the male and female population varied. It was 55.6% in males and 44.37% in females.

- **Distribution of MRSA according to different age groups** : Percentage of MRSA among the age groups 0-18 years was 19.375%, 19-39 years was 36.875% and 40 years and above was 43.75%.
- **Comparison of Cefoxitin disc diffusion test with PCR**: Cefoxitin disc diffusion test had sensitivity- 87.5%, specificity-100%, positive predictive value-100% and negative predictive value-66.7% when compared with the gold standard –PCR for *mecA* gene.
- 6 *S. aureus* isolates were tested false negative by cefoxitin disc diffusion test when compared with the gold standard test, PCR.
- **Prevalence**: Prevalence of MRSA was found to be 43% and that of MSSA 57% when tested by Cefoxitin disc diffusion test.
- **Antibiotic susceptibility pattern of *S. aureus* isolates to different antibiotics:** Among the 372 *S. aureus*, 176 (47.3%) of them were resistant to Amoxyclav, 137(36.8%) were resistant to Ampicillin, 99(26.6%) were resistant to Ciprofloxacin, 58(15.6%) were resistant to Erythromycin, 48(12.9%) were resistant to Gentamycin and 31(8.3%) were resistant to Clindamycin.
- **Antibiotic susceptibility pattern of the methicillin resistant *S. aureus* isolates:** Among the 160 MRSA strains, 144(90%) were resistant to Amoxyclav, 130 (81.25%) were resistant to Ampicillin, 79 (49.375%) were resistant to Ciprofloxacin, 47(29.375%) were resistant to Erythromycin, 37 (23.125%) were resistant to Gentamycin and 28(17.5%) were resistant to Clindamycin.

Table1: *S.aureus* strains from different clinical samples.

Samples	Number of *S. aureus* isolates
Pus(from wounds, eye infections, surgical site infections and abscesses)	325
Blood	15
Urine	14
Sputum	8
Catheter tip	3
Central line tip	1
Pleural fluid	1
Skin biopsy	1
Synovial fluid	2
Nasal crust	1
Long line tip	1
Total	372

Table2: MRSA strains from different clinical samples.

Samples	Number of methicillin resistant *S. aureus* isolates detected by Cefoxitin disc diffusion test	Percentage (%) of MRSA isolates
Pus	141	88.125
Blood	9	5.625
Urine	6	3.75
Catheter tip	2	1.25
Pleural fluid	1	0.625
Synovial fluid	1	0.625

Table 3: Distribution of MRSA among male and female population.

Sex	MRSA isolates
	No. (%)
Male	89 (55.6)
Female	71 (44.37)

Table 4: Distribution of MRSA among different age groups.

Age group	MRSA isolates
	No. (%)
0-18years	31(19.375)
19-39years	59(36.875)
40years and above	70(43.75)

Table 5: MRSA strains detected by Cefoxitin disc diffusion (CDD) and its Sensitivity, Specificity, PPV and NPV.

Test method	MRSA	Sensitivity	Specificity	PPV	NPV
Cefoxitin disc diffusion test(CDD)	42	87.5%	100%	100%	66.7%

Table6: Comparison of Cefoxitin disc diffusion(CDD) with Polymerase Chain Reaction(PCR).

Test method	Cefoxitin Disc Diffusion Test	PCR
No. of resistant *S. aureus* isolates	42	48
No. of sensitive *S. aureus* isolates	18	12
Total	60	60

Table 7: Prevalence of MRSA and MSSA by Cefoxitin disc diffusion test (CDD).

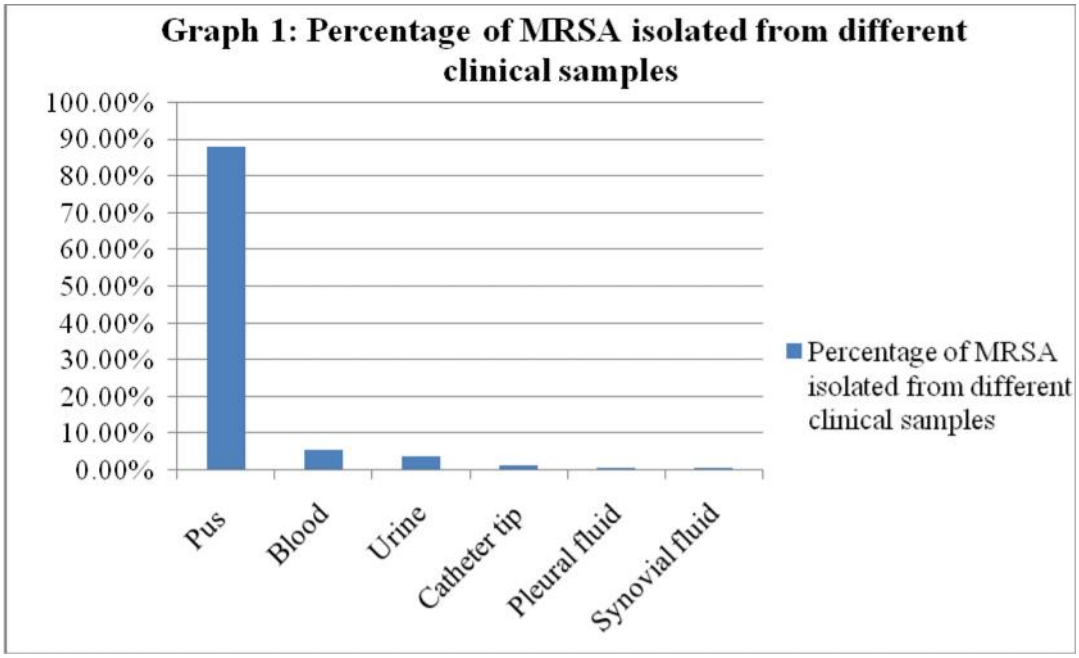
Type of *S. aureus* isolates	Prevalence
MRSA	43%
MSSA	57%

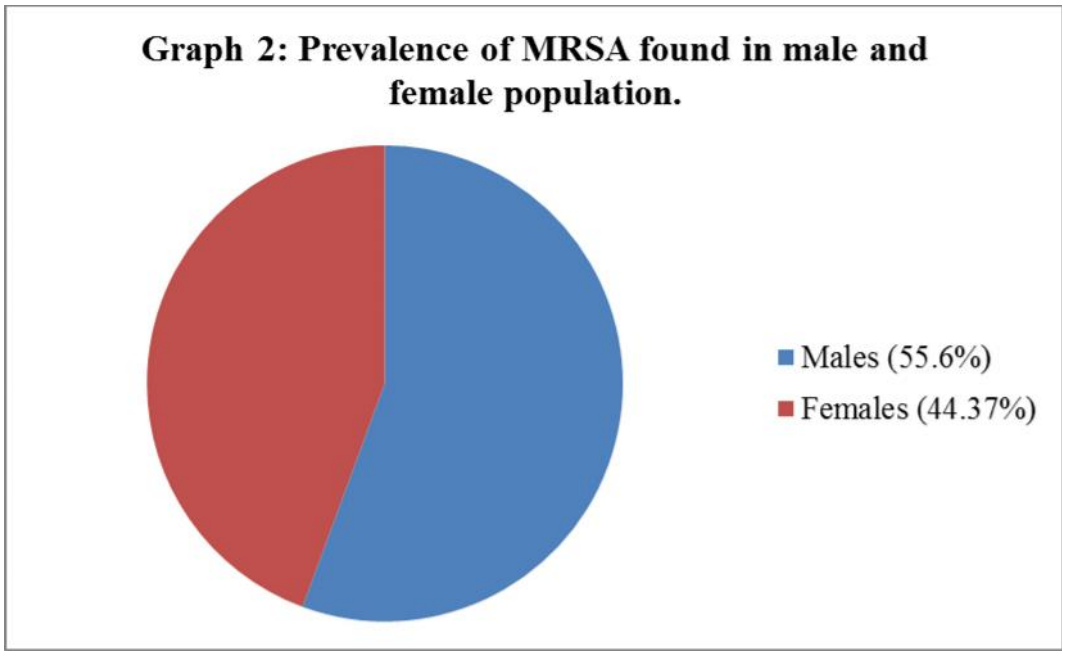
Table 8: Antibiotic susceptibility pattern of *S. aureus* strains to different antibiotics (n=372)

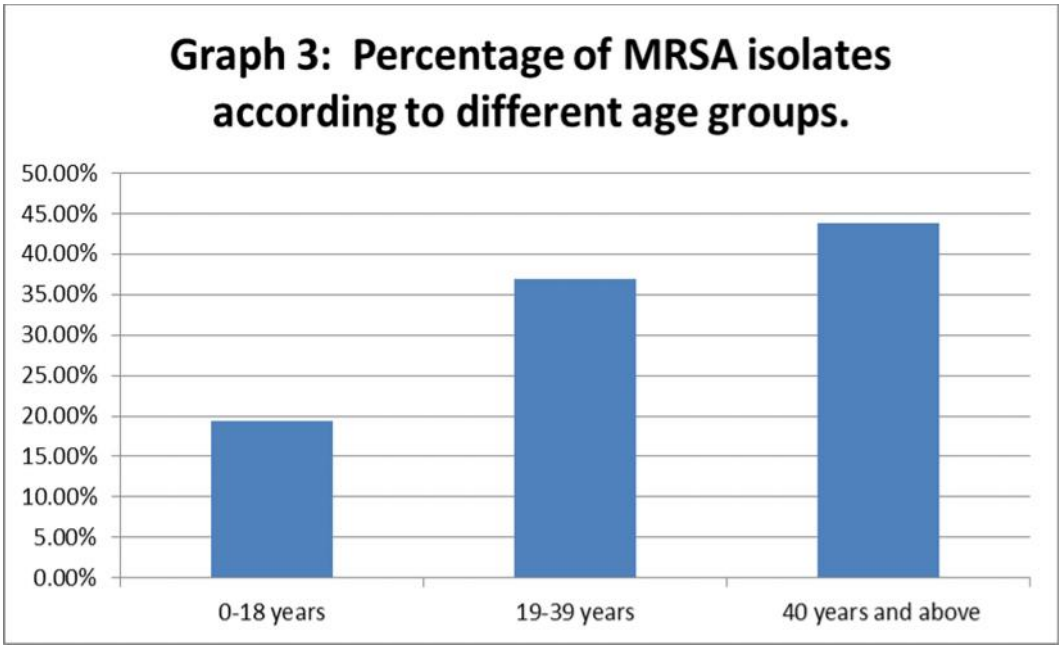
Antibiotics	Resistant	Sensitive
	No. (%)	No. (%)
Ampicillin(10mcg)	137(36.8)	235(63.1)
Amoxyclav(20/10mcg)	176(47.3)	196(52.6)
Ciprofloxacin (5mcg)	99(26.6)	273(73.387)
Clindamycin(2mcg)	31(8.3)	341(91.6)
Gentamycin(10mcg)	48(12.9)	324(87.09)
Erythromycin(15mcg)	58(15.6)	314(84.4)

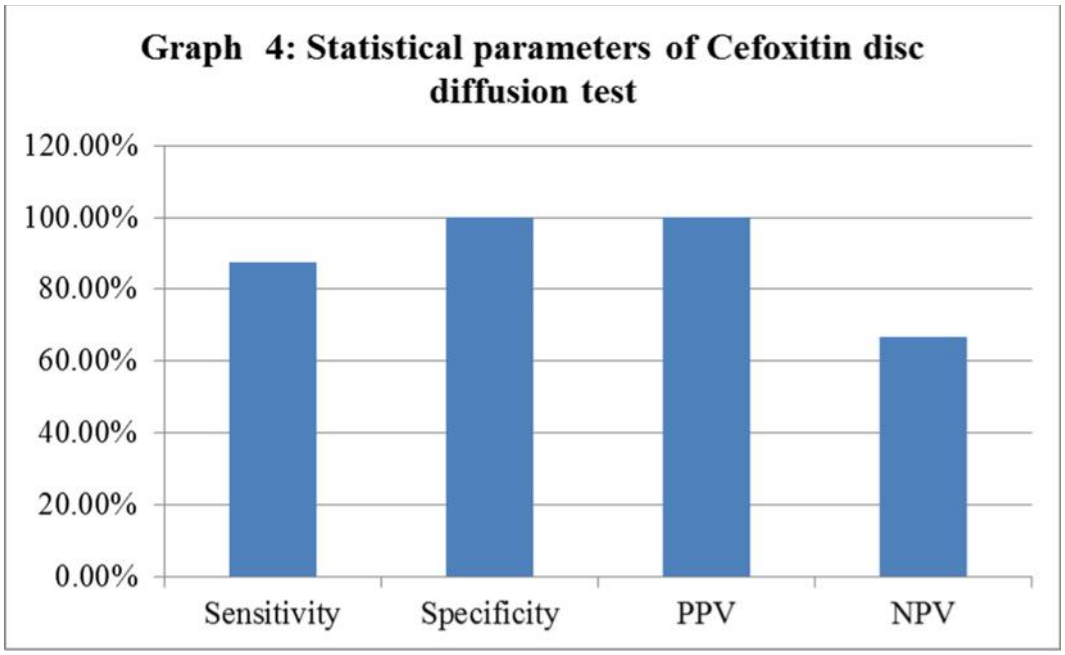
Table 9: Antibiotic susceptibility pattern of the MRSA isolates to different Antibiotics (n=160).

Antibiotics	Resistant	Sensitive
	No. (%)	No. (%)
Ampicillin(10mcg)	130(81.25)	30(18.75)
Amoxyclav(20/10mcg)	144(90)	16(10)
Ciprofloxacin(5mcg)	79(49.375)	81(50.625)
Clindamycin(2mcg)	28(17.5)	132(82.50)
Gentamycin(10mcg)	37(23.125)	123(76.875)
Erythromycin(15mcg)	47(29.375)	113(70.625)

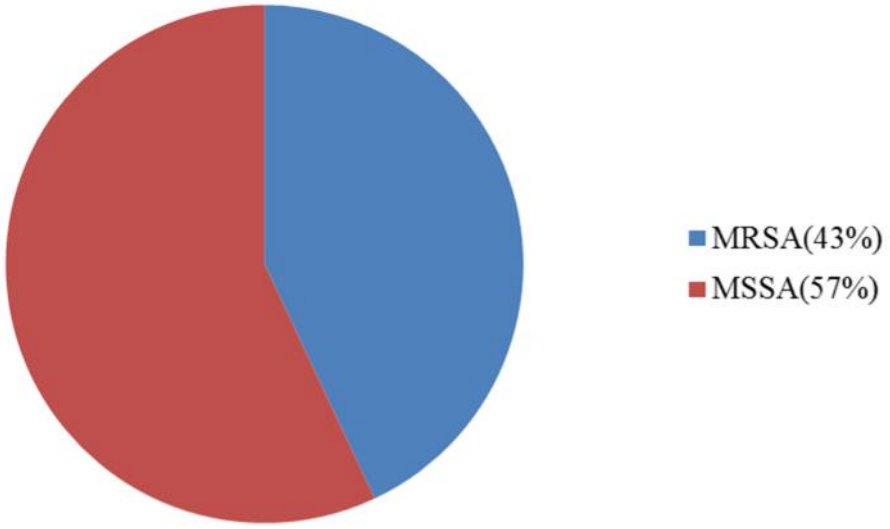

Graph 1: Percentage of MRSA isolated from different clinical samples
100.00%
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
Pus
Blood
Urine
Catheter tip
Pleural fluid
Synovial fluid
Percentage of MRSA isolated from different clinical samples


Graph 2: Prevalence of MRSA found in male and female population.
Males (55.6%)
Females (44.37%)

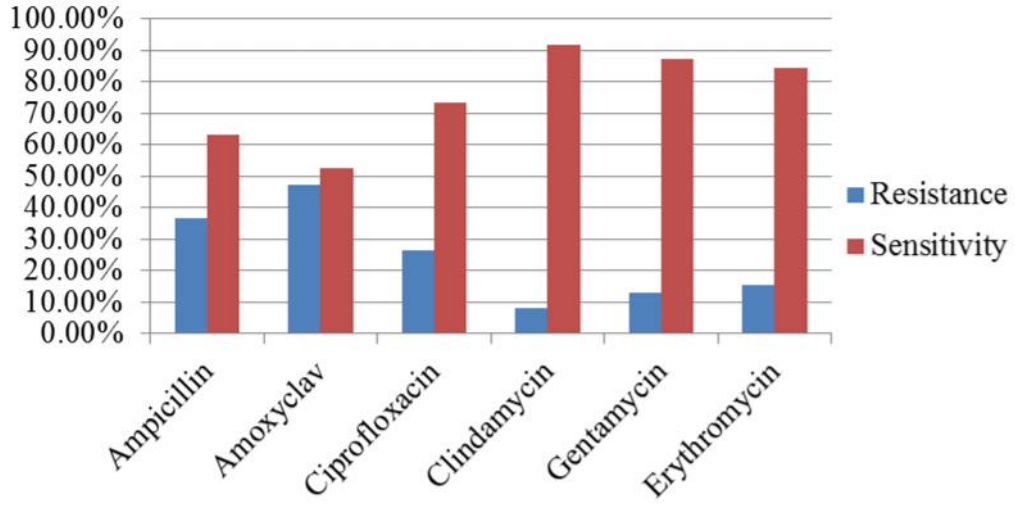

Graph 3: Percentage of MRSA isolates according to different age groups.
50.00%
45.00%
40.00%
35.00%
30.00%
25.00%
20.00%
15.00%
10.00%
5.00%
0.00%
0-18 years
19-39 years
40 years and above

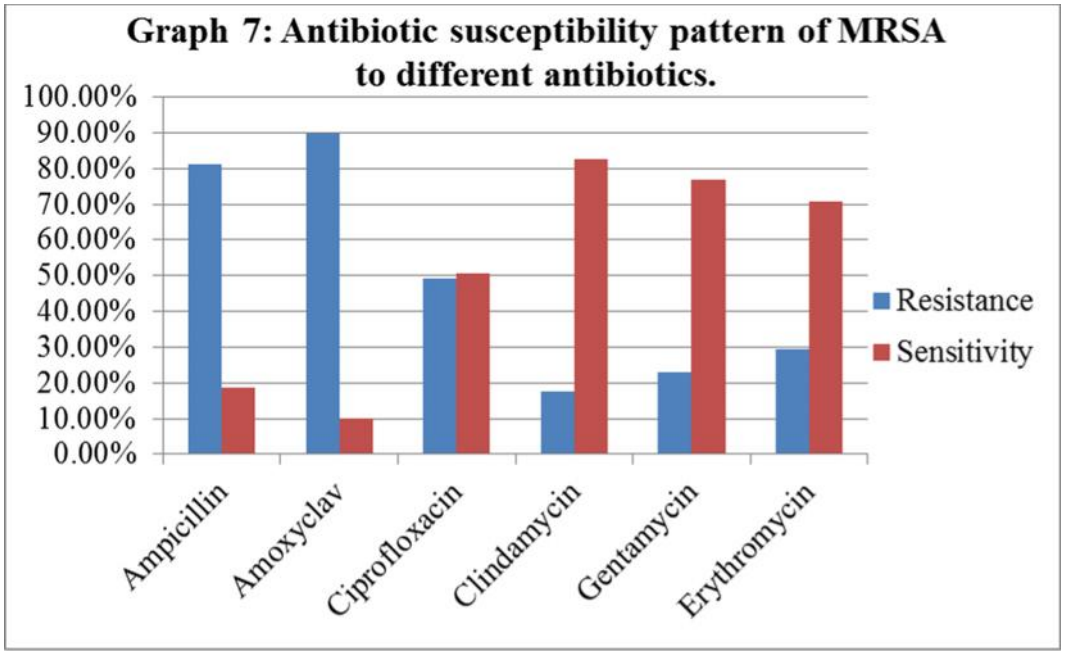

Graph 4: Statistical parameters of Cefoxitin disc diffusion test
120.00%
100.00%
80.00%
60.00%
40.00%
20.00%
0.00%
Sensitivity
Specificity
PPV
NPV

Graph 5: Prevalence of MRSA and MSSA



Graph 6: Antibiotic susceptibility pattern of *S. aureus* to different antibiotics.




Graph 7: Antibiotic susceptibility pattern of MRSA to different antibiotics.
100.00%
90.00%
80.00%
70.00%
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
Ampicillin
Amoxyclav
Ciprofloxacin
Clindamycin
Gentamycin
Erythromycin
Resistance
Sensitivity

DISCUSSION

The emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) heralded an era of uncertainity in health-care as antibiotic resistance converged to create a major crisis. There has been a dramatic rise in prevalence of antibiotic-resistant strains (MRSA) in both hospital and community. Mortality and length of hospital stay were mostly attributable to *Staphylococcus aureus* infections.[2]

Detection of the *mecA* gene is the most reliable method of detecting methicillin resistance in Staphylococcal isolates. All laboratories cannot include molecular biological techniques routinely. So phenotypic methods that can detect MRSA in clinical samples obtained from patients in a rapid and accurate manner may be used to implement the appropriate antibiotic treatment.

In the present study, a total of 372 *Staphylococcus aureus* isolates were isolated from different clinical samples. The clinical samples were pus samples (from wounds, eye infections and surgical site infections and abscesses), blood, urine, sputum, pleural fluid, synovial fluid, nasal crust, central and long line tips, catheter tips and skin biopsy materials. The *S. aureus* isolates isolated from them were tested for methicillin- resistance by Cefoxitin disc diffusion test.

Out of the 372 *S. aureus* isolates, 160 were methicillin-resistant. They were isolated from pus, blood, urine, catheter tip, pleural fluid and synovial fluid.

In the present study, percentage of MRSA isolates in different samples were 88.125% from pus (from wounds, eye infections, surgical site infections and

abscesses) samples, 5.625 % from blood, 3.75% from urine, 1.25% from catheter tips, 0.625% each from pleural fluid and synovial fluid.

In a similar study, Hassan et al had isolated 28% MRSA isolates from pus samples, 31% from blood, 10% from wound swabs and body fluids, 7% from sputum, 4% from bed sore swabs and 4% from catheter tips, 3% from tissue exudates and 3% from throat swabs.[110]

Ahmad et al carried out a study where % MRSA isolates in different samples- pus/wound swabs 37.2%, sputum 27.3%, aspirates 18.7%, ear swabs 15.4%, blood 7.7%, eye swabs 11.1% and no MRSA isolates were found in urine.[111]

High % of MRSA isolates were obtained from pus samples. These were followed mostly by blood samples yielding high percentage of MRSA isolates. In the present study, among the pus samples, highest percentage of MRSA isolates were isolated from wound swabs, followed by pus from abscesses, swabs from surgical site infections and eye swabs. Similar results were found in another study by Mukhiya et al where maximum (64.7%) isolation of MRSA were from pus samples.[112]

In some studies urine samples also yielded higher % of MRSA isolates. This was seen in a study conducted by Kulkarni et al where % of MRSA isolates detected in urine were 82.38%, pus 64.67%,blood 62.69% and sputum 80.55%.[113]

In a study conducted by Pillai et al, out of the 62 MRSA samples, 79% were pus samples, 16.12% were urine samples, 3.23% were blood samples, and 1.6% was an umbilical swab sample. Here both pus and urine samples yielded high % of MRSA isolates.[114]

In the present study, prevalence of MRSA was 55.6 % among male population and 44.37% among female population.

Similarly the males were more likely to get MRSA compared to females in the study conducted by Ojulong et al.[115] They had found that out of 17 *S. aureus* isolates which were positive for MSRA, 13 (76.5%) were from male patients and 4 (23.5%) were from females. 188 patients undergoing elective and emergency surgery developing surgical site infection after surgery were selected.

Khanal et al also found higher prevalence of MRSA among males (75.0%) than females (63.4%) from skin infection cases at a hospital in Nepal.[116]

Again in a study conducted by Shakya et al, out of 14 *S. aureus* isolates, 8 (57.1%) showed methicillin-resistance. The prevalence rate of MRSA among total population was 7.1%. 3 (37.5%) were from male subjects and 5 (62.5%) were from female subjects.[117]

In a tertiary surgical and trauma hospital, prevalence of MRSA was 31.8% and 30.4% of female and male patients, respectively. Buzaid et al had carried out this study.[118]

In this present study, percentage of MRSA isolates found among the age groups 0-18 years was 19.375 %, 19-39years was 36.875 % and 40years and above was 43.75 %.

Similar pattern of MRSA prevalence was seen in a study carried out by Buzaid et al.[118] In this study prevalence of MRSA increased with age. It was found to be 26.8% in the age group of less than 1- 19 years, 30.9% in 20-49 years and 36.1% in > 50 years.

Again, Hassan et al found that prevalence of MRSA was highest among 30-39 years age group (39%) followed by 18-29 years age group 21% and then 18% in 50-59 yrs which was followed by 10% in less than 18 yrs and 10% in 40-49yrs. There were no MRSA cases in above 60yrs.[110]

Similarities were also seen in the findings in the study conducted by Shrestha et al.[119] The study was carried out in two different populations in two tertiary care hospitals and found that higher occurrence of nosocomial infection was observed in the 15 to 45 years age group. In one population, MSSA was found to be prevalent in <1 year and 1.1 to 14 years age groups. MRSA and MSSA prevalence were equal in 14 to 45 years age group and MRSA was more prevalent in the remaining age groups 46 to 60 years, and >61years. This higher percentage of MRSA isolates found in the age group 40 years and above was similar to the findings in the present study. Again in the other population, the occurrence of MRSA was higher in 14 to 45 years age group. MSSA infection was prevalent in all groups except in the <1 year group.

Madani had reported that MRSA was prevalent in all age groups.[120] Half (45.9%) of the patients were either in the age groups <1year or> 60 years .

Ahmad S et al found prevalence of MRSA to be 20.8% , 26.5% and 25.0 % of S. aureus from patients <1-19, 20-49 and >50 years of age.[111]

Radhakrishna et al again found that in <25 yrs age group 84%, 25-35yrs was 80%, 35 -45yrs was 28% and 45 -55 yrs was 8%.[121]

In the present study, comparison of accuracy of Cefoxitin disc diffusion test to detect methicillin resistance with PCR was carried out. Here PCR was the gold standard.

60 *Stahylococcus aureus* isolates out of the 372 were taken based on systematic sampling as our sample size was 60. Both Cefoxitin disc diffusion test and PCR were carried out for these 60 *Staphylococcus aureus* isolates.

Observations for the phenotypic method were in accordance with CLSI 2011.[109] Specificity of Cefoxitin disc diffusion test was 100%. Boutiba-Ben Boubaker et al carried out a study where Cefoxitin disc diffusion test had 100% specificity similar to this study.[62] It was proved to be a useful test to be carried out routinely for MRSA. Anand et al also had found 100% specificity with Cefoxitin disc diffusion test.[4] Swenson et al also had found 100% specificity of Cefoxitin disc diffusion test. [53]

Mathews and Thomas et al had found similar results.[122] In these studies Cefoxitin Disc Diffusion test also had 100% specificity like the present study conducted.

Here, sensitivity of Cefoxitin Disc Diffusion test was 87.5. Similar sensitivity of Cefoxitin Disc Diffusion test was found in a study carried out by C. Nonhoff et al.[123]

Cefoxitin Disc Diffusion test was carried out at the temperature range of 33◦C to 35◦C. This was as per CLSI 2011 guidelines.[109] Skov et al carried out study where it was seen that Cefoxitin Disc Diffusion test was influenced by incubation temperature and the temperature should not exceed 35◦C for the reliable detection of MRSA.[51] Strains of *S. aureus* showing hetero-resistance grow more slowly than methicillin-susceptible populations and may be missed at temperatures above 35ºC.[106]

In the present study, PCR was carried out for 60 *S. aureus* isolates. Out of them 48 were MRSA (*mecA* positive) and 12 were MSSA (*mecA* negative). But in case of Cefoxitin disc diffusion test 42 were MRSA and 18 were MSSA. PCR was taken as the gold standard and so on comparing the results of both the procedures a false negative result of 6 isolates by Cefoxitin disc diffusion test was found. In a similar study carried out by Kaczmarek et al, Cefoxitin disc diffusion test falsely identified 2 strains as MSSA and there were no false resistant results.[124]

In another study by MeryemIraz et al, Cefoxitin disc diffusion test showed 3 false negative and 2 false positive results. It showed a sensitivity of 96.5%, specificity of 98.4%, positive predictive value of 97.6% and negative predictive value of 97.6%.[125]

Bhutia et al[126] in another study found that among 51 *mecA* positive isolates, 41 isolates were identified as MRSA by cefoxitin disc diffusion test and it also detected of 66 *mecA* negative isolates, 48 isolates as MSSA. The sensitivity, specificity, positive and negative predictive values for cefoxitin disc diffusion test were 86.27%, 83.33%, 80% and 88.70%, respectively.

Hence, heterogeneous nature of methicillin resistance shown by *S. aureus* limits the accuracy and reliability of phenotypic methods. Here PCR scores more. It can correctly detect even those strains expressing very low level of methicillin resistance, which can be falsely interpreted as susceptible by phenotypic methods such as Cefoxitin disc diffusion test.

Again in the present study prevalence was 43% by Cefoxitin disc diffusion test. A pilot surveillance programme for detection of MRSA was conducted

using conventional laboratory methods led to the detection of MRSA, overall prevalence was found to be 32%, 27 % from Mumbai, 42.5 % from Delhi and 47 percent from Bangalore.[43] Another Indian pilot surveillance programme (2013) for detection of MRSA was conducted. This programme led to the detection of MRSA prevalence 41 %.[44]

Antibiotic sensitivity pattern among the MRSA isolates in the present study showed highest resistance to Amoxyclav (90%) and then to Ampicillin (81.25%). Highest sensitivity was to Clindamycin (82.5%) and then to Gentamycin (76.875%) and to Erythromycin (70.625%).

Clindamycin had sensitivity of 82.50% and ciprofloxacin had that of 50.625% in the present study. Similarly Kumar and Joseph et al had found that the sensitivity of MRSA to Clindamycin was 78%. [127] INSAR group carried out a study where Ciprofloxacin sensitivity was 20.7% in 2008 and in 2009 it had resistance of 79.3% among the MRSA isolates.[44]

Similar results were also found in a studies by Tiwari et al and Sureka et al where 87.4% and 96.09% of MRSA strains were resistant to Ampicillin.[128,129] Also in a study by Rajaduraipandi et al similar sensitivity pattern to Gentamycin was found.[130]

CONCLUSION

- Rapid and prompt detection of methicillin-resistant *Staphylococcus aureus* is essential for proper treatment of patients and also to monitor their response to the various antibiotics. The present study provides an evidence that Cefoxitin disc diffusion test can be used effectively on a routine basis for screening of methicillin resistant *Staphylococcus aureus* as it has a specificity of 100%. But the role of PCR is undisputable in confirming the presence of *mecA* gene for MRSA.
- Cefoxitin disc diffusion had a sensitivity of 87.5%. It gave false negative results of 6 isolates. PCR detected 48 as *mecA* positive and 12 as *mecA* negative. Whereas, Cefoxitin disc diffusion test gave 42 MRSA and 18 as MSSA. This was due to the hetero-resistance exhibited by the MRSA isolates.
- Cefoxitin disc diffusion test had a sensitivity of 87.5%, specificity of 100%, positive predictive value of 100% and negative predictive value of 66.75%.
- Out of 372 *S.aureus* isolates, 160 were MRSA. Prevalence by Cefoxitin disc diffusion test was 43%. This shows proper monitoring of MRSA is needed in every health set-up.
- Higher percentage of MRSA was isolated from pus samples.
- It was also seen that higher percentage of MRSA was found in males.
- In the age group of 40 years and above it percentage of MRSA isolates was more.
- Higher resistance was seen to Amoxyclav and Ampicillin among the MRSA isolates.
- Sensitivity was greater to clindamycin among them.

SUMMARY

The present study was conducted in the Department of Microbiology, J.N.M.C, K.L.E University, Belgaum, from January 2013 to December 2013.

All *Staphylococcus aureus* isolated from different clinical samples received in Microbiology Department of J.N.M.C Belgaum, from January 2013 to December 2013 were included in this study.

- A total of 372 *Staphylococcus aureus* isolates were collected.
- Cefoxitin disc diffusion test was carried out for all of them.
- Highest percentage of MRSA isolates were from pus samples.
- 60 isolates out of the 372 were taken based on systematic sampling. This was done as sample size was 60.
- These 60 isolates were tested by Cefoxitin disc diffusion test as well as PCR for *mecA* gene.
- Cefoxitin disc diffusion test had sensitivity of 87.5%, specificity of 100%, PPV of 100% and NPV of 66.7%.
- Prevalence of MRSA by Cefoxitin disc diffusion test was 43%.
- Antibiotic sensitivity pattern among the MRSA isolates showed highest resistance to Amoxyclav (90%) and then to Ampicillin (81.25%). Highest sensitivity was to Clindamycin (82.5%) and then to Gentamycin (76.875%) and to Erythromycin (70.625%).
- Five of the MRSA isolates were resistant to all the antibiotics tested for.
- Prevalence of MRSA in 0-18 yrs 19.375%, 19-40 yrs 36.875%, 40yrs and above 43.75%.

Again, prevalence of MRSA in male 55.6 % and in female population 44.37%.

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ANNEXURE-I

PROFORMA

CASE NO.-

NAME OF PATIENT-

AGE-

SEX-

ADDRESS-

IP/OP NO.-

UNIT/WARD-

DATE OF SAMPLE COLLECTION-

TYPE OF SAMPLE-

CHIEF COMPLAINTS-

HISTORY OF PRESENT ILLNESS-

HISTORY OF PAST ILLNESS-

TREATMENT HISTORY-

CLINICAL DIAGNOSIS-

OTHER INVESTIGATIONS-

MICROBIOLOGICAL INVESTIGATIONS-

LAB. No.-

SOURCE OF DATA-

All *Staphylococcus aureus* isolated from different clinical samples received in Microbiology department.

RECONFIRMATION-

1.GRAM STAINING-

2.BLOOD AGAR-

3.NUTRIENT AGAR-

4.CATALASE –

5.SLIDE COAGULASE -

6.TUBE COAGULASE-

7.UREASE-

8.MANNITOL FERMENTATION-

9.ANTIBIOGRAM-

10. CEFOXITIN (30mcg) DISC DIFFUSION TEST-

11.PCR FOR DETECTION OF *mecA* GENE-

ANNEXURE-II

KEYS FOR MASTERCHART-

P = Positive

R = Resistant

S = Sensitive

M = Male

F = Female

D_{10} = Day 10

Amp = Ampicillin

Amc = Amoxyclav

Cip = Ciprofloxacin

Cd = Clindamycin

Gen = Gentamycin

E = Erythromycin

GPC = Gram-positive cocci

SHADED AREAS= Cases Taken For Comparison Between PCR And Cefoxitin Disc Diffusiuon Test

Case No.	In / Out patient number	Age	Sex	Type of sample	Investigations							Antibiogram						
					Lab Number	Gram stain	Catalase	Slide Coagulase	Tube coagulase	Urease	Mannitol fermentation	Amp	Amc	Cip	Cd	Gen	E	Cefoxitin Disc Diffusion Test
1	508166	50yrs	F	Pus	73/74	GPC in clusters	P	P	P	P	P	S	S	R	S	S	S	S
2	508561	30yrs	M	Pus	82/83	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
3	508173	65yrs	M	Pus	227	GPC in clusters	P	P	P	P	P	R	R	S	S	S	S	R
4	508567	336	F	Pus	336	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
5	504842	19yrs	M	Pus	354	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
6	**508980**	**11yrs**	**M**	**Pus**	**470**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**R**	**R**	**S**	**S**	**S**	**S**	**R**
7	508174	-	-	Pus	474	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
8	-	27yrs	M	Pus	485	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	R
9	509100	47yrs	M	Pus	492	GPC in clusters	P	P	P	P	P	R	R	S	S	S	S	R
10	2375995	65yrs	F	Pus	494	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	R
11	505778	30yrs	M	Pus	496	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	R
12	**509632**	**24yrs**	**F**	**Pus**	**811**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
13	509284	60yrs	M	Pus	909	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
14	510318	21yrs	M	Pus	1343	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
15	2386722	26yrs	F	Pus	1344	GPC in clusters	P	P	P	P	P	R	R	R	S	S	R	R
16	509362	66yrs	M	Blood	1511	GPC in clusters	P	P	P	P	P	R	R	R	R	R	R	R
17	508796	35yrs	M	Pus	1573	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
18	**510618**	**14yrs**	**F**	**Catheter tip**	**1579**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**R**	**R**	**R**	**S**	**R**	**R**	**R**
19	502375	40yrs	F	Pus	1589	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
20	511186	23yrs	M	Pus	1696	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
21	505778	33yrs	M	Pus	1935	GPC in clusters	P	P	P	P	P	S	S	S	S	S	R	S
22	511385	-	F	Pus	1948	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
23	511329	31yrs	M	Pus	2071	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
24	**511628**	**55yrs**	**M**	**Pus**	**2836**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**R**	**R**	**S**	**S**	**S**	**S**	**R**
25	511500	-	F	Pus	2838	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
26	510764	30yrs	M	Pus	2855	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
27	511674	17yrs	M	Pus	2966	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
28	511500	-	F	Pus	2976	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
29	2405070	32yrs	M	Pus	3001	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
30	**512486**	**yr6mth**	**F**	**Pus**	**3005**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
31	512905	14yrs	F	Pus	3031	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
32	512980	65yrs	F	Pus	3056	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
33	512900	40yrs	M	Pus	3070	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
34	513031	20yrs	M	Pus	3100	GPC in clusters	P	P	P	P	P	S	R	S	S	S	S	S
35	513721	9yrs	M	Pus	3118	GPC in clusters	P	P	P	P	P	R	R	S	S	S	S	R
36	**513125**	**30yrs**	**F**	**Pus**	**3121**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**S**	**S**	**S**	**S**	**S**	**R**	**S**
37	512976	25yrs	M	Pus	3171	GPC in clusters	P	P	P	P	P	R	R	S	S	R	R	R
38	514280	50yrs	M	Pus	3173	GPC in clusters	P	P	P	P	P	R	R	R	S	S	R	R
39	508315	46yrs	F	Pus	3189	GPC in clusters	P	P	P	P	P	R	R	S	S	R	R	R
40	512900	40yrs	M	Pus	3201	GPC in clusters	P	P	P	P	P	R	R	S	S	R	S	R
41	514071	7yrs	F	Pus	3249	GPC in clusters	P	P	P	P	P	R	R	R	S	R	R	R
42	**515013**	**29yrs**	**F**	**Pus**	**3272**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**R**	**R**	**S**	**S**	**S**	**S**	**R**
43	513288	65yrs	M	Pus	3282	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
44	515010	30yrs	F	Pus	3284	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S

Case No.	In / Out patient number	Age	Sex	Type of sample	Investigations							Antibiogram						
					Lab Number	Gram stain	Catalase	Slide Coagulase	Tube coagulase	Urease	Mannitol fermentation	Amp	Amc	Cip	Cd	Gen	E	Cefoxitin Disc Diffusion Test
45	515016	34yrs	F	Pus	3302	GPC in clusters	P	P	P	P	P	R	R	R	S	S	S	R
46	513828	40yrs	M	Pus	3304	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
47	515561	23yrs	F	Pus	3342	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
48	**515803**	**72yrs**	**M**	**Pus**	**3364**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**R**	**R**	**S**	**S**	**S**	**S**	**R**
49	508913	63yrs	M	Pus	3366	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
50	515721	45yrs	M	Pus	3374	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
51	513288	65yrs	M	Pus	3396	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
52	516264	35yrs	M	Pus	3399	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
53	516213	40yrs	M	Pus	3403	GPC in clusters	P	P	P	P	P	R	R	R	S	S	R	R
54	**514828**	**-**	**M**	**Pus**	**3417**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**R**	**R**	**S**	**S**	**S**	**S**	**R**
55	515302	45yrs	M	Pus	3423	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
56	516213	50yrs	M	Pus	3594	GPC in clusters	P	P	P	P	P	R	R	R	S	R	R	R
57	2423006	63yrs	F	Pus	3596	GPC in clusters	P	P	P	P	P	S	R	S	S	S	R	S
58	516665	50yrs	M	Pus	3609	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
59	-	50yrs	M	PUS	4214	GPC in clusters	P	P	P	P	P	R	S	R	S	S	S	S
60	**517883**	**23yrs**	**F**	**Pus**	**4501**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**R**	**R**	**S**	**S**	**S**	**S**	**R**
61	516424	27yrs	F	Pus	4503	GPC in clusters	P	P	P	P	P	S	R	R	S	S	S	S
62	516602	20yrs	F	Pus	4533	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
63	518030	35yrs	M	Pus	4537	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
64	519001	5yrs	F	Pus	4837	GPC in clusters	P	P	P	P	P	R	R	S	S	S	S	R
65	518617	32yrs	F	Pus	4839	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
66	**2457596**	**60yrs**	**F**	**Pus**	**5083**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**R**	**R**	**S**	**S**	**S**	**R**	**R**
67	519467	85yrs	M	Pus	5087	GPC in clusters	P	P	P	P	P	S	R	S	S	S	S	S
68	519278	1yr	F	Pus	5089	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
69	2458133	-	-	Pus	5091	GPC in clusters	P	P	P	P	P	R	S	R	S	S	R	S
70	518903	24yrs	M	Pus	5178	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
71	519948	55yrs	M	Pus	5204	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
72	**519034**	**-**	**-**	**Pus**	**5311**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**R**	**R**	**S**	**S**	**S**	**S**	**R**
73	517945	-	M	Pus	5482	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
74	519105	8yrs	F	Catheter tip	5589	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
75	520661	60yrs	F	Pus	5593	GPC in clusters	P	P	P	P	P	R	R	S	S	S	S	S
76	519105	-	-	Central line tip	5603	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
77	635962	50yrs	M	Pus	5605	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
78	**520289**	**25yrs**	**F**	**Pus**	**5618**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**R**	**R**	**S**	**S**	**R**	**S**	**R**
79	521095	18yrs	M	Pus	5646	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
80	520731	28yrs	F	Pus	5692	GPC in clusters	P	P	P	P	P	R	R	S	S	S	S	R
81	521583	21yrs	F	Pus	5697	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
82	519078	45yrs	F	Pus	5699	GPC in clusters	P	P	P	P	P	R	R	R	S	S	R	R
83	519680	32yrs	M	Pus	5819	GPC in clusters	P	P	P	P	P	R	R	R	R	R	R	R
84	**522425**	**50yrs**	**M**	**Pus**	**5821**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**R**	**R**	**S**	**S**	**S**	**S**	**R**
85	517150	-	-	Pus	5829	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
86	522994	20yrs	F	Pus	6150	GPC in clusters	P	P	P	P	P	S	S	S	S	R	R	S
87	522261	-	-	Pus	6157	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S

Case No.	In / Out patient number	Age	Sex	Type of sample	Investigations							Antibiogram						
					Lab Number	Gram stain	Catalase	Slide Coagulase	Tube coagulase	Urease	Mannitol fermentation	Amp	Amc	Cip	Cd	Gen	E	Cefoxitin Disc Diffusion Test
88	522659	35yrs	M	Pus	6209	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
89	523180	35yrs	M	Pus	6250	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
90	**2463858**	**29yrs**	**M**	**Pus**	**6370**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**R**	**R**	**R**	**S**	**R**	**S**	**R**
91	523758	35yrs	F	Pus	6372	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
92	523586	60yrs	M	Pus	6376	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
93	523566	24yrs	M	Pus	6520	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
94	523640	70yrs	M	Pus	6522	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
95	523659	56yrs	M	Pus	6524	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
96	**521794**	**55yrs**	**M**	**Pus**	**6706**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**R**	**R**	**S**	**S**	**R**	**S**	**R**
97	524813	1mth	M	Blood	6726	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
98	524821	D10	M	Blood	6727	GPC in clusters	P	P	P	P	P	R	R	R	S	R	R	R
99	524796	21yrs	F	Pus	6736	GPC in clusters	P	P	P	P	P	S	S	R	S	S	S	S
100	523610	62yrs	M	Urine	6743	GPC in clusters	P	P	P	P	P	S	S	S	S	S	R	S
101	-	-	F	Pus	6745	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
102	**524865**	**27yrs**	**M**	**Pus**	**6751**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**R**	**R**	**S**	**S**	**S**	**S**	**R**
103	525111	25yrs	F	Pus	6845	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
104	523015	45yrs	M	Pus	6847	GPC in clusters	P	P	P	P	P	S	R	R	S	S	S	S
105	524968	32yrs	M	Pus	6849	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
106	523184	-	-	Pus	6853	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
107	525337	14yrs	M	Urine	6872	GPC in clusters	S	S	S	S	S	S	S	S	S	S	S	S
108	**524965**	**24yrs**	**F**	**Catheter tip**	**6890**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**R**	**R**	**R**	**S**	**S**	**R**	**R**
109	525336	D11	F	Blood	6893	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
110	525336	D11	F	Pus	6893	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
111	522825	22yrs	M	Pus	6972	GPC in clusters	P	P	P	P	P	R	R	R	S	R	R	R
112	525360	48yrs	F	Pus	6976	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
113	525705	-	-	Pus	7054	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
114	**525485**	**15yrs**	**M**	**Pus**	**7052**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**R**	**R**	**S**	**R**	**S**	**S**	**R**
115	-	35yrs	F	Pus	7062	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
116	5258431	21yrs	F	Pus	7080	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
117	525946	56yrs	M	Pus	7322	GPC in clusters	P	P	P	P	P	R	R	R	S	R	R	R
118	526006	D10	M	Blood	7328	GPC in clusters	P	P	P	P	P	R	R	S	S	R	R	R
119	525798	37yrs	M	Pus	7482	GPC in clusters	P	P	P	P	P	S	S	R	S	S	R	S
120	**526060**	**55yrs**	**M**	**Pus**	**7484**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**R**	**R**	**R**	**S**	**S**	**S**	**R**
121	525949	58yrs	M	Urine	7593	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
122	526264	55yrs	M	Pus	7597	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
123	526235	58yrs	M	Pus	7724	GPC in clusters	P	P	P	P	P	R	R	R	S	R	R	R
124	526366	70yrs	F	Pus	7726	GPC in clusters	P	P	P	P	P	R	R	R	S	R	R	R
125	526097	70yrs	F	Pus	7864	GPC in clusters	P	P	P	P	P	R	R	S	S	S	S	R
126	**526812**	**40yrs**	**F**	**Urine**	**7878**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**R**	**R**	**S**	**S**	**S**	**S**	**R**
127	527051	9yrs	F	Pus	7892	GPC in clusters	P	P	P	P	P	S	R	R	S	S	S	S
128	526811	28yrs	M	Pus	7894	GPC in clusters	P	P	P	P	P	R	R	R	R	R	R	R
129	597160	12yrs	M	Pus	7927	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
130	2508489	12yrs	M	Pus	8051	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
131	527136	34yrs	F	Pus	8053	GPC in clusters	P	P	P	P	P	S	S	R	S	S	S	S

Case No.	In / Out patient number	Age	Sex	Type of sample	Investigations							Antibiogram						Cefoxitin Disc Diffusion Test
					Lab Number	Gram stain	Catalase	Slide Coagulase	Tube coagulase	Urease	Mannitol fermentation	Amp	Amc	Cip	Cd	Gen	E	
132	**527571**	**D18**	**-**	**-**	**8060**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**R**	**R**	**S**	**S**	**R**	**S**	**R**
133	527836	19yrs	F	Pus	8148	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
134	527281	60yrs	F	Pus	8154	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
135	527559	49yrs	M	Pus	8158	GPC in clusters	P	P	P	P	P	S	R	S	S	S	S	S
136	527893	20yrs	F	Pus	8328	GPC in clusters	P	P	P	P	P	S	R	R	R	S	S	S
137	526460	39yrs	M	Pus	8420	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
138	**524863**	**26yrs**	**M**	**Pus**	**8594**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**S**	**R**	**S**	**S**	**R**	**S**	**S**
139	528860	42yrs	F	Pus	8704	GPC in clusters	P	P	P	P	P	S	S	S	S	S	R	S
140	528691	34yrs	M	Pus	8830	GPC in clusters	P	P	P	P	P	S	S	S	S	S	R	S
141	524768	50yrs	M	Pus	8836	GPC in clusters	P	P	P	P	P	S	R	S	S	S	S	S
142	2893204	9yrs	M	Pus	8842	GPC in clusters	P	P	P	P	P	S	R	R	R	R	R	S
143	519680	40yrs	M	Pus	8873	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
144	**529567**	**D70**	**F**	**Blood**	**8975**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**R**	**R**	**R**	**R**	**S**	**R**	**R**
145	529561	D90	F	Blood	8976	GPC in clusters	P	P	P	P	P	R	R	R	R	S	R	R
146	529626	42yrs	M	Pus	9079	GPC in clusters	P	P	P	P	P	S	R	R	S	S	S	S
147	529261	18yrs	M	Pus	9081	GPC in clusters	P	P	P	P	P	S	R	R	S	S	S	R
148	-	50yrs	M	Pus	9085	GPC in clusters	P	P	P	P	P	S	R	R	S	S	S	R
149	529224	8yrs	M	Pus	9091	GPC in clusters	P	P	P	P	P	R	R	R	R	R	S	R
150	**528658**	**22yrs**	**F**	**Urine**	**9096**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**R**	**R**	**S**	**S**	**S**	**S**	**R**
151	529665	-	F	Pus	9102	GPC in clusters	P	P	P	P	P	S	R	R	S	S	R	R
152	529546	21yrs	F	Pus	9213	GPC in clusters	P	P	P	P	P	S	R	S	S	S	S	S
153	530538	52yrs	M	Pus	9273	GPC in clusters	P	P	P	P	P	R	R	R	R	R	R	R
154	-	65yrs	M	Pus	9293	GPC in clusters	P	P	P	P	P	R	R	R	R	S	S	R
155	530910	50yrs	M	Pus	9360	GPC in clusters	P	P	P	P	P	R	R	R	R	S	R	R
156	**531102**	**27yrs**	**M**	**Pus**	**9362**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**R**	**R**	**R**	**R**	**S**	**R**	**R**
157	531437	6yrs	F	Pus	9366	GPC in clusters	P	P	P	P	P	R	R	R	R	S	S	R
158	524768	40yrs	M	Pus	9371	GPC in clusters	P	P	P	P	P	R	R	R	R	S	S	R
159	529049	12yrs	F	Pus	9588	GPC in clusters	P	P	P	P	P	R	R	R	R	S	S	R
160	530842	24yrs	F	Pus	9590	GPC in clusters	P	P	P	P	P	R	R	S	R	S	S	R
161	2031636	22yrs	M	Pus	9726	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	R
162	**528844**	**51yrs**	**M**	**Pus**	**9813**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**S**	**R**	**R**	**R**	**S**	**R**	**R**
163	531743	4yrs	F	Pus	9815	GPC in clusters	P	P	P	P	P	R	R	R	R	S	S	R
164	531898	55yrs	M	Pus	9817	GPC in clusters	P	P	P	P	P	R	R	R	R	S	S	R
165	2550762	45yrs	F	Pus	9824	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
166	531986	20yrs	M	Pus	9941	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
167	531437	-	-	Pus	9943	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
168	**532303**	**60yrs**	**F**	**Pus**	**9998**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**R**	**R**	**S**	**R**	**S**	**S**	**R**
169	531898	65yrs	M	Pus	10096	GPC in clusters	P	P	P	P	P	R	R	R	R	R	S	R
170	527364	-	M	Pus	10135	GPC in clusters	P	P	P	P	P	R	R	R	R	R	R	R
171	528844	51yrs	M	Pus	10221	GPC in clusters	P	P	P	P	P	R	R	R	R	S	R	R
172	2559025	14yrs	F	Pus	10357	GPC in clusters	P	P	P	P	P	R	R	R	R	S	R	R
173	531343	-	F	Pus	10359	GPC in clusters	P	P	P	P	P	R	R	R	R	R	R	R
174	**519680**	**40yrs**	**M**	**Pus**	**10361**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**R**	**R**	**S**	**S**	**S**	**S**	**R**
175	2532972	32wks	M	Pus	10374	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S

Case No.	In / Out patient number	Age	Sex	Type of sample	Investigations							Antibiogram						Cefoxitin Disc Diffusion Test
					Lab Number	Gram stain	Catalase	Slide Coagulase	Tube coagulase	Urease	Mannitol fermentation	Amp	Amc	Cip	Cd	Gen	E	
176	533194	-	F	Pus	10489	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
177	533164	70yrs	M	Pus	10497	GPC in clusters	P	P	P	P	P	S	R	R	S	S	S	R
178	533557	55yrs	F	Pus	10518	GPC in clusters	P	P	P	P	P	S	R	R	S	S	S	R
179	533584	65yrs	M	Pus	10524	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
180	**534032**	**17yrs**	**M**	**Blood**	**10601**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**R**	**R**	**S**	**R**	**S**	**S**	**R**
181	532639	72yrs	M	Pus	10621	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
182	534032	17yrs	M	Pus	10625	GPC in clusters	P	P	P	P	P	R	R	R	S	S	R	R
183	534032	17yrs	M	Pleural fluid	10627	GPC in clusters	P	P	P	P	P	R	R	R	S	S	R	R
184	534082	D20	F	Pus	10639	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
185	534272	-	F	Pus	10641	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	R
186	**534032**	**12yrs**	**M**	**Pus**	**10643**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**R**	**R**	**S**	**S**	**S**	**S**	**R**
187	533249	55yrs	M	Urine	10729	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
188	534173	-	F	Pus	10733	GPC in clusters	P	P	P	P	P	S	R	R	S	S	S	S
189		20yrs	M	Pus	10861	GPC in clusters	P	P	P	P	P	S	R	S	S	S	S	R
190	-	40yrs	M	Pus	10863	GPC in clusters	P	P	P	P	P	S	R	R	S	S	S	R
191	534173	-	F	Pus	10737	GPC in clusters	P	P	P	P	P	S	R	S	S	S	R	S
192	**534918**	**41yrs**	**M**	**Pus**	**10959**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
193	535134	D1	M	Blood	10962	GPC in clusters	P	P	P	P	P	S	R	R	S	S	S	R
194	534419	D30	M	Blood	10963	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
195	534947	35yrs	F	Blood	10964	GPC in clusters	P	P	P	P	P	S	R	R	S	S	S	R
196	535936	1yr	F	Pus	11464	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
197	532808	16yrs	M	Pus	11594	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
198	**536985**	**32yrs**	**M**	**Pus**	**11906**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
199	536194	26yrs	M	Pus	11912	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
200	534987	39yrs	F	Pus	11984	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
201	536467	50yrs	M	Pus	12040	GPC in clusters	P	P	P	P	P	R	R	R	S	S	S	R
202	537247	36yrs	M	Pus	12042	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
203	537768	36yrs	M	Pus	12070	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	R
204	**536080**	**52yrs**	**F**	**Pus**	**12135**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**R**	**R**	**S**	**S**	**S**	**S**	**R**
205	537391	-	M	Urine	12141	GPC in clusters	P	P	P	P	P	R	R	R	S	S	S	R
206	538751	D80	F	Pus	12366	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
207	538679	30yrs	F	Pus	12368	GPC in clusters	P	P	P	P	P	R	R	R	S	S	S	R
208	538751	D80	F	Blood	12371	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
209	-	50yrs	F	Pus	12466	GPC in clusters	P	P	P	P	P	R	R	R	S	S	S	R
210	**538297**	**85yrs**	**M**	**Pus**	**12470**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**R**	**R**	**S**	**S**	**S**	**S**	**S**
211	538789	30yrs	F	Pus	12472	GPC in clusters	P	P	P	P	P	R	R	R	S	R	R	R
212	2031636	22yrs	M	Pus	12474	GPC in clusters	P	P	P	P	P	R	R	R	S	R	R	R
213	538954	14yrs	F	Pus	12488	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
214	-	4yrs	F	Pus	12495	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
215	539153	-	F	Pus	12674	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
216	**-**	**50yrs**	**F**	**Pus**	**12680**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
217	539494	45yrs	M	Pus	12726	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
218	-	61yrs	F	Pus	12728	GPC in clusters	P	P	P	P	P	R	R	S	S	S	S	R
219	534903	53yrs	M	Pus	12744	GPC in clusters	P	P	P	P	P	R	R	S	S	S	S	R

Case No.	In / Out patient number	Age	Sex	Type of sample	Investigations							Antibiogram						Cefoxitin Disc Diffusion Test
					Lab Number	Gram stain	Catalase	Slide Coagulase	Tube coagulase	Urease	Mannitol fermentation	Amp	Amc	Cip	Cd	Gen	E	
220	539782	55yrs	F	Pus	12985	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
221	539998	48yrs	M	Pus	12996	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
222	**540243**	**-**	**F**	**Pus**	**13009**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**R**	**R**	**S**	**S**	**S**	**S**	**R**
223	538789	30yrs	F	Pus	13024	GPC in clusters	P	P	P	P	P	R	R	S	S	S	S	R
224	540310	60yrs	F	Pus	13026	GPC in clusters	P	P	P	P	P	R	R	S	S	S	S	S
225	540318	80yrs	F	Pus	13028	GPC in clusters	P	P	P	P	P	R	R	S	S	S	S	R
226	540197	50yrs	F	Urine	13062	GPC in clusters	P	P	P	P	P	R	R	S	S	S	S	R
227	2609917	43yrs	F	Pus	13085	GPC in clusters	P	P	P	P	P	R	R	S	S	S	S	S
228	**540342**	**30yrs**	**M**	**Pus**	**13101**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**R**	**R**	**S**	**S**	**S**	**S**	**R**
229	538902	22yrs	F	Pus	13123	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	R
230	540793	27yrs	M	Skin biopsy	13170	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
231	534297	55yrs	F	Pus	13189	GPC in clusters	P	P	P	P	P	S	R	S	S	S	S	R
232	540985	66yrs	M	Pus	13209	GPC in clusters	P	P	P	P	P	S	R	R	S	R	S	S
233	2617529	60yrs	F	Pus	13224	GPC in clusters	P	P	P	P	P	S	R	R	S	R	S	S
234	**538509**	**65yrs**	**M**	**Pus**	**13230**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**R**	**S**	**S**	**S**	**S**	**S**	**R**
235	541629	27yrs	F	Pus	13248	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
236	-	27yrs	F	Pus	13261	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
237	541506	23yrs	F	Urine	13272	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
238	546645	12yrs	F	Pus	13922	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
239	-	45yrs	M	Pus	13926	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
240	**546834**	**60yrs**	**M**	**Pus**	**13932**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**R**	**R**	**S**	**R**	**S**	**S**	**R**
241	2652863	60yrs	M	Pus	13934	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
242	546462	30yrs	M	Pus	13948	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
243	547100	42yrs	M	Pus	13958	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
244	546465	40yrs	M	Blood	13973	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
245	544852	34yrs	M	Pus	13980	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
246	**547286**	**60yrs**	**M**	**Pus**	**13991**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**S**	**R**	**S**	**S**	**S**	**S**	**R**
247	544309	35yrs	M	Pus	14002	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
248	539782	55yrs	F	Pus	14004	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
249	546919	D90	M	Blood	14159	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
250	547673	49yrs	M	Sputum	14190	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
251	547856	54yrs	F	Sputum	14192	GPC in clusters	P	P	P	P	P	S	S	S	S	R	S	S
252	**543912**	**60yrs**	**F**	**Pus**	**14552**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
253	2667364	60yrs	F	Pus	14584	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
254	546456	30yrs	M	Pus	14586	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
255	548449	5yrs	M	Urine	14634	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
256	548724	26yrs	F	Urine	14638	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
257	548687	-	F	Pus	14656	GPC in clusters	P	P	P	P	P	S	S	S	S	R	S	S
258	**548880**	**-**	**F**	**Urine**	**14661**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**R**	**R**	**S**	**S**	**S**	**S**	**R**
259	548880	-	F	Pus	14663	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
260	547829	50yrs	M	Pus	14667	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
261	544309	35yrs	M	Pus	14768	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	R
262	548786	12yrs	F	Pus	14758	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	R
263	2642709	22yrs	F	Pus	14659	GPC in clusters	P	P	P	P	P	S	S	S	R	S	S	S

Case No.	In / Out patient number	Age	Sex	Type of sample	Investigations						Antibiogram						Cefoxitin Disc Diffusion Test
					Lab Number	Gram stain	Catalase	Slide Coagulase	Tube coagulase	Urease	Mannitol fermentation	Amp	Amc	Cip	Cd	Gen	E	
264	**548783**	**10yrs**	**M**	**Pus**	**14764**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**R**	**S**	**S**	**S**	**S**	**S**	**S**
265	548724	24yrs	F	Urine	14776	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	R
266	548394	52yrs	M	Pus	14780	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	R
267	548512	65yrs	M	Pus	14829	GPC in clusters	P	P	P	P	P	S	S	S	S	S	R	R
268	549109	-	F	Pus	14831	GPC in clusters	P	P	P	P	P	S	S	S	S	S	R	S
269	549114	38yrs	M	Pus	14837	GPC in clusters	P	P	P	P	P	S	S	R	S	S	S	S
270	**545472**	**32yrs**	**F**	**Pus**	**14848**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**S**	**R**	**S**	**S**	**S**	**S**	**S**
271	545629	48yrs	M	Pus	14950	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
272	549126	54yrs	M	Synovial fluid	14953	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
273	547673	56yrs	M	Sputum	15021	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	R
274	549148	10yrs	M	Sputum	15031	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
275	549161	27yrs	M	Pus	15103	GPC in clusters	P	P	P	P	P	R	R	S	S	S	S	R
276	**548287**	**20yrs**	**F**	**Pus**	**15105**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**S**	**R**	**S**	**S**	**R**	**S**	**S**
277	546462	30yrs	M	Pus	15250	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
278	549950	29yrs	F	Pus	15256	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
279	548116	26yrs	F	Pus	15609	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
280	-	60yrs	F	Pus	15629	GPC in clusters	P	P	P	P	P	S	S	S	S	R	S	S
281	551517	24yrs	F	Pus	15913	GPC in clusters	P	P	P	P	P	S	S	P	S	S	S	S
282	**551582**	**40yrs**	**M**	**Pus**	**15931**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**R**	**R**	**S**	**S**	**S**	**S**	**R**
283	546639	41yrs	M	Pus	15933	GPC in clusters	P	P	P	P	P	R	R	R	S	S	S	R
284	-	40yrs	F	Pus	16018	GPC in clusters	P	P	P	P	P	R	R	R	S	S	S	R
285	551825	34yrs	F	Pus	16030	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
286	551809	60yrs	F	nasal crust	16035	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
287	546462	34yrs	M	Pus	16214	GPC in clusters	P	P	P	P	P	R	R	S	S	S	S	R
288	**552544**	**24yrs**	**M**	**Pus**	**16310**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
289	547530	35yrs	M	Pus	16322	GPC in clusters	P	P	P	P	P	R	R	S	S	S	S	R
290	552799	19yrs	F	Pus	16420	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	R
291	552511	-	M	Pus	16527	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	R
292	552477	35yrs	F	Pus	16742	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	R
293	2701181	-	F	Urine	16759	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	R
294	**-**	**65yrs**	**F**	**Sputum**	**16858**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**S**	**R**	**S**	**S**	**R**	**S**	**S**
295	553400	49yrs	M	Pus	16968	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
296	551441	-	M	Pus	16966	GPC in clusters	P	P	P	P	P	R	R	S	S	S	S	R
297	549186	2mths	M	long line tip	16970	GPC in clusters	P	P	P	P	P	S	R	S	S	S	S	S
298	553389	-	M	Pus	16972	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
299	-	49yrs	M	Pus	17126	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
300	**554432**	**66yrs**	**M**	**Pus**	**17156**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**R**	**R**	**R**	**S**	**R**	**R**	**R**
301	553956	35yrs	F	Blood	17203	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
302	554677	11yrs	M	Pus	17210	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
303	554010	60yrs	M	Pus	17543	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
304	555151	52yrs	M	Pus	17566	GPC in clusters	P	P	P	P	P	R	R	R	S	R	R	R
305	-	36yrs	F	Pus	17717	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
306	**553202**	**45yrs**	**F**	**Pus**	**17751**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**S**	**R**	**S**	**S**	**S**	**S**	**S**
307	554215	60yrs	M	Synovial fluid	17757	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S

Case No.	In / Out patient number	Age	Sex	Type of sample	Investigations							Antibiogram						
					Lab Number	Gram stain	Catalase	Slide Coagulase	Tube coagulase	Urease	Mannitol fermentation	Amp	Amc	Cip	Cd	Gen	E	Cefoxitin Disc Diffusion Test
308	-	64yrs	M	Pus	17824	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
309	555042	8mths	M	Pus	17872	GPC in clusters	P	P	P	P	P	R	R	R	S	S	S	R
310	556565	9mths	M	Pus	17876	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
311	2293289	39yrs	M	Sputum	17878	GPC in clusters	P	P	P	P	P	S	R	R	S	S	S	S
312	**556820**	**35yrs**	**F**	**Pus**	**17947**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**R**	**R**	**S**	**S**	**S**	**S**	**R**
313	556965	50yrs	F	Pus	17949	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
314	556928	6yrs	F	Pus	17968	GPC in clusters	P	P	P	P	P	R	R	S	S	S	S	R
315	557130	8yrs	F	Pus	18085	GPC in clusters	P	P	P	P	P	S	R	S	S	S	S	S
316	557771	33yrs	M	Pus	18211	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
317	557900	75yrs	F	Pus	18243	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
318	**557811**	**65yrs**	**F**	**Pus**	**18247**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**S**	**R**	**S**	**S**	**S**	**S**	**S**
319	555658	55yrs	M	Pus	-	GPC in clusters	P	P	P	P	P	S	R	S	S	S	S	S
320	557691	40yrs	M	Pus	18308	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
321	2662376	24yrs	M	Pus	18321	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
322	556235	65yrs	F	Pus	18355	GPC in clusters	P	P	P	P	P	R	R	R	S	R	R	R
323	558731	-	F	Urine	18360	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
324	**557567**	**-**	**M**	**Pus**	**18393**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**S**	**S**	**S**	**S**	**R**	**S**	**S**
325	-	43yrs	F	Pus	18441	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
326	559656	25yrs	F	Pus	18551	GPC in clusters	P	P	P	P	P	R	R	S	S	S	S	R
327	560061	8yrs	M	Pus	18594	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
328	560000	36yrs	M	Pus	18596	GPC in clusters	P	P	P	P	P	R	R	R	S	S	S	R
329	560189	86yrs	M	Pus	18659	GPC in clusters	P	P	P	P	P	R	R	R	S	R	R	R
330	**560501**	**55yrs**	**F**	**Pus**	**18786**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**S**	**S**	**R**	**S**	**S**	**S**	**R**
331	-	30yrs	M	Pus	19695	GPC in clusters	P	P	P	P	P	R	R	R	S	S	S	R
332	562929	40yrs	M	Pus	19772	GPC in clusters	P	P	P	P	P	R	R	R	R	S	S	S
333	562019	19yrs	M	Sputum	19778	GPC in clusters	P	P	P	P	P	R	R	R	S	S	S	S
334	-	20yrs	M	Pus	19919	GPC in clusters	P	P	P	P	P	R	R	S	R	S	R	R
335	561496	25yrs	F	Pus	20163	GPC in clusters	P	P	P	P	P	R	R	R	S	S	R	R
336	**562674**	**58yrs**	**M**	**Pus**	**20202**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**S**	**R**	**S**	**R**	**S**	**S**	**R**
337	561938	28yrs	M	Pus	20200	GPC in clusters	P	P	P	P	P	R	R	R	S	R	S	R
338	564516	26yrs	M	Pus	20344	GPC in clusters	P	P	P	P	P	R	R	S	S	S	S	R
339	563829	60yrs	M	Pus	20340	GPC in clusters	P	P	P	P	P	R	R	S	S	S	S	R
340	-	45yrs	M	Pus	20556	GPC in clusters	P	P	P	P	P	R	R	S	S	S	R	R
341	562531	40yrs	F	Pus	20645	GPC in clusters	P	P	P	P	P	R	R	R	R	S	S	R
342	**566328**	**38yrs**	**F**	**Pus**	**20816**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
343	2031636	22yrs	M	Pus	20823	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
344	563645	-	M	Pus	20825	GPC in clusters	P	P	P	P	P	R	R	R	S	S	S	R
345	567061	-	M	Pus	20891	GPC in clusters	P	P	P	P	P	R	R	R	S	R	R	R
346	558586	50yrs	F	Pus	20932	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
347	557567	35yrs	M	Pus	20934	GPC in clusters	P	P	P	P	P	R	R	R	S	S	R	R
348	**567189**	**48yrs**	**M**	**Pus**	**20938**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**S**	**S**	**S**	**R**	**S**	**S**	**R**
349	566477	52yrs	M	Pus	21072	GPC in clusters	P	P	P	P	P	R	R	S	S	S	R	R
350	-	24yrs	F	Pus	21074	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
351	-	22yrs	F	Pus	21107	GPC in clusters	P	P	P	P	P	R	R	R	S	R	R	R

Case No.	In / Out patient number	Age	Sex	Type of sample	Investigations							Antibiogram						Cefoxitin Disc Diffusion Test
					Lab Number	Gram stain	Catalase	Slide Coagulase	Tube coagulase	Urease	Mannitol fermentation	Amp	Amc	Cip	Cd	Gen	E	
352	567623	59yrs	M	Sputum	21111	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
353	566417	45yrs	M	Pus	21119	GPC in clusters	P	P	P	P	P	R	R	R	S	S	S	R
354	**567799**	**70yrs**	**F**	**Pus**	**21123**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**R**	**R**	**S**	**S**	**S**	**S**	**R**
355	567754	70yrs	F	Pus	21125	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
356	563976	-	-	Pus	21135	GPC in clusters	P	P	P	P	P	R	R	R	S	S	S	R
357	-	30yrs	F	Pus	21200	GPC in clusters	P	P	P	P	P	R	R	R	S	R	R	R
358	568616	-	F	Urine	21264	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
359	568616	-	F	Pus	21266	GPC in clusters	P	P	P	P	P	R	R	S	S	S	S	R
360	**568464**	**16yrs**	**F**	**Pus**	**21268**	**GPC in clusters**	**P**	**P**	**P**	**P**	**P**	**R**	**R**	**S**	**S**	**S**	**S**	**R**
361	565858	40yrs	F	Pus	21347	GPC in clusters	P	P	P	P	P	R	R	R	S	S	S	R
362	568791	4yrs	F	Blood	21401	GPC in clusters	P	P	P	P	P	R	R	R	S	S	S	R
363	-	52yrs	F	Pus	21278	GPC in clusters	P	P	P	P	P	R	R	S	S	S	S	R
364	567807	21yrs	M	Pus	21518	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
365	569166	-	M	Pus	21520	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
366	569277	-	F	Pus	21526	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
367	569137	yrs6mth	F	Pus	21550	GPC in clusters	P	P	P	P	P	R	R	S	S	S	S	R
368	569391	30yrs	F	Pus	21551	GPC in clusters	P	P	P	P	P	S	R	S	S	S	S	R
369	569446	20yrs	F	Urine	21553	GPC in clusters	P	P	P	P	P	S	S	S	S	S	S	S
370	569636	60yrs	F	Pus	21573	GPC in clusters	P	P	P	P	P	R	R	R	S	R	R	R
371	569636	45yrs	F	Pus	21735	GPC in clusters	P	P	P	P	P	R	R	R	S	R	S	R
372	569732	20yrs	F	Pus	21755	GPC in clusters	P	P	P	P	P	R	R	R	S	S	S	R

MASTERCHART continued…….

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	Cefoxitin	**PCR**
6	R	R
12	S	S
18	R	R
24	R	R
30	S	S
36	S	S
42	R	R
48	R	R
54	R	R
60	R	R
66	R	R
72	R	R
78	R	R
84	R	R
90	R	R
96	R	R
102	R	R
108	R	R
114	R	R
120	R	R
126	R	R
132	R	R
138	S	R
144	R	R
150	R	R
156	R	R
162	R	R
168	R	R
174	R	R
180	R	R
186	R	R
192	S	R
198	S	S
204	R	R
210	S	S
216	S	S
222	R	R
228	R	R
234	R	R
240	R	R
246	R	R
252	S	S
258	R	R
264	S	S
270	S	R
276	S	S
282	R	R
288	S	S
294	S	S
300	R	R
306	S	S
312	R	R
318	S	R
324	S	R
330	R	R
336	R	R
342	S	R
348	R	R
354	R	R
360	R	R