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Research Article

Isolation, antibiotic sensitivity and molecular characterization of Multi Drug Resistance *Staphylococcus aureus* using Polymerase Chain Reaction

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Abstract

Multiplex polymerase chain reaction (PCR) strategy is described for rapid identification of clinically relevant methicillin resistant *Staphylococcus aureus* (MRSA) that targets mecA and coagulase genes. In our study totally 15 samples was collected out of 15 samples 11 samples showing positive results for culture and biochemical testes. Culture and biochemical positive isolates (11isolates) was subjected to the antimicrobial sensitivity testing. In antimicrobial testing out 11 isolates 9 isolates are resistance to the methicillin and two isolates are sensitive for methicillin. Methicillin resistant strains (MRSA) strains were detected using multiplex PCR with coag and mecA gene specific oligonucleotides. For MRSA strains, two discrete DNA fragments, a 533 bp mecA and 810 bp coag specific products were obtained. The mecA fragment was amplified in all the methicillin resistant Staphylococcus isolates. The coag gene fragment was observed in all *Staphylococcus aureus* strains, whereas such amplification did not occur in any of Methicillin sensitive strains.

Keywords: *Staphylococcus aureus*; PCR; culture and biochemical test; antimicrobial sensitivity.

Introduction

Staphylococcus is a very well known genus of bacteria. Colonies are “gold”, or yellow on sheep blood agar solid media, the golden appearance is the etymology root of the bacteria’s name aureus means “golden” in latin. *S.aureus* literally the ‘golden cluster seed’or’the seed gold’ and also known as the golden staphi. A common pathogen, boils, acne, wound infections ,food poisoning are among a host of conditions caused by the organism. The organism is both pathogenic and invasive.

It produces leukotoxin which kills the white blood cells and a wide variety of other toxins. *S.aureus* is quite pyogenic and in decades past was named *Staphylococcus pyogenes*. Increasingly ,and especially in hospital, strains of both *S.aureus* and *S.epidermitis*. Have become resistant to the antibiotic, methicillin. Such strains have been labeled MRSA(Methicillin Resistant *Staphylococcus aureus*).Other clinically significant species include, (MRSA) also produce

penicillinase. Methicillin resistant confers resistants to all the b-lactam antibiotics. Unfortunately, most MRSA nad now also resistant to aminoglycosides, microlides, tetraciclines, fluoroquinolones, and rifampin in many countries. Therefore, MRSA strains are multiple resistant strains with therapeutic options that are largely limited to vancomycin. The Detection of vancomycin resistant in clinical isolates of coagulase – negative Staphylococci raises the worrisome possibility that resistant to vancomycin may yet emerge in S.aureus, emphasizing the need for the therapeutic alternatives to this antibiotic.

Nosocomial Methicillin Resistant *Staphylococcus aureus* isolates are mostly multi – drug resistant *Staphylococcus aureus* is a community associated pathogen causing a wide range of disease, including endocarditis, osteomyelitis, toxic-stock syndrome, pneumonia, food poisoning and carbuncles (Savitha Nadiy et al ., 2006).

Drug resistant is the reduction in effect (Mesh, 2010). When the drug is not intended to kill (or) inhibit a pathogen. The term is used in the context of resistance acquired by pathogens (Daniel. Et al., 2007).

When an organism is resistant to more than one drug it is said to be as multi drug resistant the condition enabling the disease causing organism resist distant drug (or) chemicals wide variety of structure and function targeted at eradicating the organism (Mash 2010).

Bacterial resistant to antibiotic various micro organisms have survived for thousands of years by the one able to adapt to antimicrobial agents (Bennot 2008). Microorganism employs several mechanisms in attaining multi drug resistant ,ex:- no longer relying on a glycoprotein cell wall , enzymetic deactivation of antibiotics etc., (Nikadio 2009). Many different bacterium exhibit multi – drug resistantce such as staphylococcus aureus infection is penicillin-resistance is extremely common and first line therapy is most commonly a penicillinase – resistant b.lactum antibiotic oxacillin (or) combination therapy with gentamycin may be used to treat serious infections like endocarditis (Korzeniuslci et al., 1982) (Bayer et al.,1998)

A multiplex PCR assay for detection of genes for staphylococcul enterotoxins a to z (ent a,ent b and ent c) toxic shock synderome, exfoliative toxins a and b and intrinisic methicillin resistant (mec a) and fema developed (Manisha Menotra 2000). Multiple antibiotic resistant *Staphylococcus aureus* strains constitute a major health care problem therefore the availability of sensitive and specific methods for the accurate detection of antibiotic resistance in these bacteria has become an important tool in clinical diagnosis (Perezroth et al.,2001).

The use of PCR for the sensitive and specific detection of microorganism and antibiotic Resistance genes in increasing in clinical microbiology laboraties .Drug resistance staphylococcus isolates amplifies this escalating problem (Smtitikana biswar et al. 2010). To investigate the prevalence of Multi Drug Resistance Staphylococcus aureus isolated from patients having throat infection and detection of mac A and coag gene using multiplex PCR.

Materials and Methods

Collection of throat specimen

The mouth is held wide open and the tongue depressed. Swabs are firmly rubbed over the tonsils and pharyngeal mucosa, an attempt should made to collect any purulent material that is present

Throat swab culture

Throat swab is inoculated on to Blood agar, MacConkey agar, and Mannitol salt agar and DNase agar and incubated at 37^{o}C for 48hours. After incubation the colony morphology was observed.

Presumptive *Staphylococcus aureus* colonies were then subjected to gram staining and a series biochemical tests such as motility , oxidation and fermentation , catalase , indole production ,methyl red test ,voges proskaur test , and cultures which matched typical reaction of standard

Staphylococcus aureus were confirmed as *Staphylococcus aureus* .

Isolation and identification of staphylococcus aureus

Grams staining

Bacterial smears of 16-18 hrs old were made on clean grease free slides , heat fixed and stained as follows . Slide was flooded with crystal violet for a minute , drained and rinsed with water; followed by grams iodine for one minute , drained and rinsed with water . Decolourised with acetone alcohol for 30 seconds and later counter stained with safranin for one minute and observed under an oil immersion microscope. violet or purple cell were identified as gram positive and pink ones as gram negative.

Indole production test

Indole test demonstrate the ability of certain bacteria to decompose amino acid tryptophan to indole which accumulates in the medium indole is than tested by a color reaction with kovac's reagent. The tubes of tryptone broth were inoculated with the organism, and incubated for 24-48hrs at 37◦c.After incubation 0.2ml of kovac's reagent was added and observed gently. It was about to stand for 10 min and the result is observed. A dark red or pink color on the top layer of the solution indicates the positive reaction and the original color of the reagent indicates the negative reaction.

Methyl red test

Glucose is the major substrate oxidized by all organisms for energy production. The end process of this process will depend upon the specific enzymatic pathway present in the bacteria. The organism were inoculated in 5ml of MRVP broth and incubated for 48hrs at 37^{o} C After incubation 5 drops of methyl red indicator was added gently along the sides of the tubes. Bright red color formation indicates positive result were has yellow color indicates negative result.

Voges -proskauer test

Voges-proskauer test depends upon production of acetyl methyl carbinol from pyruvic acid. This test is usually done in conjugation with methyl red test. The organism was inoculated with 5ml propotion of MRVP medium and incubated the tubes for 48hrs at 37^{o} C. To 1ml of culture 0.6ml of alpha napthol solution and 0.2ml of potassium hydroxide solution was added to each tube. Development of pink to crimson color indicates a positive test.

Citrate utilization test

Citrate agar was prepared, dispensed in tubes and then sterilized. Later slants were prepared, by allowing it to solidify in a slanting position. The organism was inoculated and incubated overnight37^{o} C, color changes were observed. The color of the medium changes from green to blue shows positive result and the absence of the color shows negative result.

Catalase test

A small amount of culture was placed on a clean slide a drop of 3% of hydrogen peroxide was placed over the culture and observed for effervescences. The production of effervescences showed the ability of the organism that produces enzyme catalase.

Coagulase test

A loopful of culture aseptically transferred to human plasma. It was than incubated to 2-3hrs at 37^{o}C The incubated test tube was examined periodically for coagulation (or) Fibrin formulation.

Urease test

In urease test the organism producing urease, which hydrolysis the urea into ammonia and CO_2 it is done in Christensen's urease medium. The organism was inoculated in the slant and incubated at 37^{o} C Urease producing organism produce pink color in positive tube and no change in negative tubes.

Sugar fermentation test

90ml of sterile peptone water was prepared and 1gm of sugar was weighed and dissolved in 10ml of distilled water. This 10ml of sugar solution was added to 90ml of prepared peptone water. 0.1ml of bromocresol purple indicator dye was added. This sugar solution was dispensed in tubes and Durham's tubes were placed and then sterilized and incubate the tubes at 37^{o} C for 24hrs. Both acid and gas production were noticed. Formation of yellow color, acid production, gas formation in Durham's tube indicated the positive results and no change in the tube indicated negative results.

Antibacterial Susceptibility Testing

Procedure for Performing the Disc Diffusion Test

The growth method is performed as follows

At least three to five well-isolated colonies of the same morphological type are selected from an agar plate culture. The top of each colony is touched with a loop, and the growth is transferred into a tube containing 4 to 5 ml of a suitable broth medium, such as tryptic soy broth. The broth culture is incubated at 35°C until it achieves or exceeds the turbidity of the 0.5 McFarland standard (usually 2 to 6 hours).

The turbidity of the actively growing broth culture is adjusted with sterile saline or broth to obtain a turbidity optically comparable to that of the 0.5 McFarland standard. This results in a suspension containing approximately 1 to 2 x 10^{8} CFU/ml for E.coli ATCC 25922. To perform this step properly, either a photometric device can be used or, if done visually, adequate light is needed to visually compare the inoculum tube and the 0.5 McFarland standard against a card with a white background and contrasting black lines.

Direct Colony Suspension Method

As a convenient alternative to the growth method, the inoculum can be prepared by making a direct broth or saline suspension of isolated colonies selected from a 18- to 24-hour agar plate (a nonselective medium, such as blood agar, should be used). The suspension is adjusted to match the 0.5 McFarland turbidity standard, using saline and a vortex mixer. This approach is the recommended method for testing the fastidious organisms, Haemophilus spp., N. gonorrhoeae, and Streptococci, and for testing Staphylococci for potential methicillin or oxacillin resistance.

Inoculation of Test Plates

Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab is dipped into the adjusted suspension. The swab should be rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This will remove excess inoculum from the swab. The dried surface of a Müeller-Hinton agar plate is inoculated by streaking the swab over the entire sterile agar surface. This procedure is repeated by streaking two more times, rotating the plate approximately 60^{o} C, each time to ensure an even distribution of inoculum. As a final step, the rim of the agar is swabbed. The lid may be left ajar for 3 to 5 minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks.

NOTE: Extremes in inoculum density must be avoided. Never use undiluted overnight broth cultures or other unstandardized inocula for streaking plates.

Application of Discs to Inoculated Agar Plates

The predetermined battery of antimicrobial discs is dispensed onto the surface of the inoculated agar plate. Each disc must be pressed down to ensure complete contact with the agar surface. Whether the discs are placed individually or with a dispensing apparatus, they must be distributed evenly so that they are no closer than 24 mm from center to center. Ordinarily, no more than 12 discs should be placed on one 150 mm plate or more than 5 discs on a 100 mm plate. Because some of the drug diffuses almost instantaneously, a disc should not be relocated once it has come into contact with the agar surface. Instead,

place a new disc in another location on the agar. The plates are inverted and placed in an incubator set to 35°C within 15 minutes after the discs are applied. With the exception of Haemophilus spp., Streptococci and N. gonorrhoeae, the plates should not be incubated in an increased CO_2 atmosphere, because the interpretive standards were developed by using ambient air incubation, and CO_2 will significantly alter the size of the inhibitory zones of some agents.

Reading Plates and Interpreting Results

After 16 to 18 hours of incubation, each plate is examined. If the plate was satisfactorily streaked, and the inoculum was correct, the resulting zones of inhibition will be uniformly circular and there will be a confluent lawn of growth. If individual colonies are apparent, the inoculum was too light and the test must be repeated. The diameters of the zones of complete inhibition (as judged by the unaided eye) are measured, including the diameter of the disc. Zones are measured to the nearest whole millimeter, using sliding calipers or a ruler, which is held on the back of the inverted petriplate. The petriplate is held a few inches above a black, nonreflecting background and illuminated with reflected light. If blood was added to the agar base (as with streptococci), the zones are measured from the upper surface of the agar illuminated with reflected light, with the cover removed. If the test organism is a Staphylococcus or *Enterococcus* spp., 24 hours of incubation are required for Vancomycin and Oxacillin, but other agents can be read at 16 to 18 hours. Transmitted light (plate held up to light) is used to examine the oxacillin and Vancomycin zones for light growth of Methicillin- or Vancomycin- resistant colonies, respectively, within apparent zones of inhibition. Any discernable growth within zone of inhibition is indicative of Methicillin or Vancomycin resistance. The zone margin should be taken as the area showing no obvious, visible growth that can be detected with the unaided eye. Faint growth of tiny colonies, which can be detected only with a magnifying lens at the edge of the zone of inhibited growth, is ignored. However, discrete colonies growing within a clear zone of inhibition should be subcultured, re-identified, and retested. Strains of Proteus spp. may swarm into areas of inhibited growth around certain antimicrobial agents. With Proteus spp., the thin veil of swarming growth in an otherwise obvious zone of inhibition should be ignored. When using blood-supplemented medium for testing Streptococci, the zone of growth inhibition should be measured, not the zone of inhibition of hemolysis. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth), and measure the more obvious margin to determine the zone diameter.

The sizes of the zones of inhibition are interpreted by referring to Tables 2A through 2I (Zone Diameter Interpretative Standards and equivalent Minimum Inhibitory Concentration Breakpoints) of the NCCLS M100-S12: Performance Standards for Antimicrobial Susceptibility Testing:

Isolation of geonomic DNA from *Staphylococcus aureus*

The stored isolated cultures were inoculated in the fresh Nutrient Broth (NB) which provides the rich turbid growth. The inoculated broth was incubated at 37ºC for 24 – 48 hours. Nutrient broth (NB) is used for the extraction.

The sub cultures *Staphylococcus aureus* species in Nutrient Broth (NB) was taken in the centrifuge tube and it was centrifuged at 10000 rpm for 10 minutes. The supernatant was discarded and the pellet obtained after centrifugation is used for DNA isolation. The pellet was then suspended in 300 µl of solution A at room temperature. The solution was vortexes completely. It was then incubated at 60ºC for 20 minutes and then solution B was add, then solution is completely (vortexing). It was centrifuge at 10,000 rpm for 10 minutes. About 500 µl of the aqueous supernatant solution was collected and add to that equal volume of isopropanol was added into the fresh vials then they are mixed by inverting then the vials where kept for centrifugation at 10,000 rpm for 10 minutes. About 200 µl of ethyl alcohol was added and mixed than by inverting the tube till the white strands of DNA precipitation are seen. It was then centrifuged at

10,000 rpm for 10 minutes and the supernatant was discarded. Then the alcohol was decanted without dislodging the pellet, it was completely air dried to remove the ethyl alcohol smell from the vials. To the final pellet about 20 µl of TE buffer was added and mixed completely by tapping the tube, till the solution settle at the bottom. The isolated DNA was separated and visualized with the help of agraose gel electrophoresis and viewed in the UV transilluminator.

PCR Protocal

Two sets of primers were used for the mPCR. The first pair of primers was derived from the region of the mecA gene, the forward primer corresponded to nucleotides 1282 to 1303(5. AAAATCGATGGTAAAGGTTGGC) and the reverse primer was complementary tonucleotides 1793to1814(5.AGTTCTGCAGTACCGGATTTGC). The second pair of primers was derived from the region of the coag gene, the forward primer was 5. CGA GAC CAAGAT TCAACA AG and the reverse primer was 5.AAA GAA AACCACTCA CAT CAG T.

Bacterial genomic DNA (aliquot of 1µL containing 50 ng of genomic DNA) was added to PCR mixture consisting tenfold concentrated reaction buffer (500mM KCl, 100mM Tris-HCl, pH 8·3), with final concentrations of 0.5 mM each dNTP, 2.5 mM MgCl2, 0.15 µM of each coag primer and 0.1 µM of each mecA primer. This mixture was supplemented with 2U of Taq DNA polymerase. The final reaction volume for PCR was 20 µL. DNA amplif ication was carried out in an automated thermo cycler (MJ Research PTC-200). After an initial denaturation step for 5 minutes at 95°C, 40 cycles of amplification were performed as follows: denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds and DNA extension at 72°C for 90 seconds, followed by an additional cycle of 5 minutes at 72°C to complete partial polymerizations. Amplified products were analyzed using horizontal 1·5% agarose gel electrophoresis

Gel electrophoresis

1% agarose was prepared and dissolved in 1 x TBE buffer. After cooling 2 µl Ethidium bromide was added in dissolved agarose. Prepared agarose was poured on to gel boat, wells can made by using thecomb. 20 µl of amplified PCR product was loaded along with 3µl geloading buffer and Electrophoresis was performed

Results and Discussion

A total of 15 throat specimens were analyzed for detection of *Staphylococcus aureus* via culture on Blood agar, Mannitol salt agar, DNase agar and Biochemical tests. Out of 15 samples 11 samples shows positive results for Staphylococcus aureus .Colony morphology of isolates on selective media as follows Beta haemolytic colonies blood agar golden yellow colonies on mannitol salt agar.(Table1,2).

Biochemical tests for identification of *Staphylococcus aureus*

The isolates from the clinical samples showed positive results for Methyl red test, Voges proskaur and Urea's tests .It showed negative results to Indole test and Citrate test .It suggests that the isolates are *Staphylococcus aureus*. The results in Triple sugar iron test is the colour change to yellow and yellow in both slant butt region .Sugar fermentation test showed the result from Acid production without gas production .From the positive result of coagulase and catalase test ,it is clearly found that the 13 isolates are Staphylococcus aureus. (Table. 3).

Antibiotic sensitivity tests for *Staphylococcus aureus*

The 11 isolates obtained from clinical sample were made to undergo antibiotic susceptility test using commercially available antibiotics with the concentration. The antibiotic discs used and the concentration (µg) present in the discs (Table .4) .

The results of antibiotic sensitivity test for *Staphylococcus aureus* isolated from clinical samples (Table .5). The percentage of antibiotics , 81 and 100% resistant to Methicillin , Cefpodoxime, Penicilin-G, 72and 81% , sensitive to Gentamycin , Kanamycin and Vancomycin (Table. 6). The obtained percentage of all 12 antibiotics plotted standard graph (fig .1). 11 positive isolates are subjected to antimicrobial sensitivity testing .Out of 11 positives 9 isolates are resistant to the methicillin only two isolate is sensitive to the methicillin

PCR detection of coag and mecA gene

MRSA strains were unequivocally detected within three hours using multiplex PCR with coag and mecA gene specific oligonucleotides. For MRSA strains, two discrete DNA fragments, a 533 bp mecA and 810 bp coag specific products were obtained. The mecA fragment was amplifiedin all the methicillin resistant *Staphylococcus* isolates. Amplification product was also detected for methicillin susceptible strains. The coag gene fragment was observed in all staphylococcus aureus strains, whereas such ampliÞ cation did notoccur in any of the CoNS. No non-specific background amplification products were observed.

Discussion

Methicillin resistant Staphylococci are significant pathogens causing both nosocomial and community acquired infections. High prevalence of methicillin Resistant Staphylococcus aureus (MRSA) in hospitals has been reported from many states of India. (Rajaduraipandi.et al., 2006) Methicillin resistance among *S. aureus* isolates has reached phenomenal proportions in Indian hospitals, with some cities reporting 70% of the strains to be resistant to methicillin. In the last few years sensitive molecular typing techniques are leading the way to track the source and transmission route of bacterial pathogens. They have also helped in establishing epidemiological investigations and comparing strains across continents. Detection of mecA gene by PCR has been shown to be highly discriminatory in analyzing hospital outbreaks and tracking genetic changes which occur in a relatively short time. The aim of this study was to validate the multiplex polymerase chain reaction (mPCR) technique in Indian isolates by which India specific data will be of immense benefit for the optimal application in our patients.(Rallapalli et al., 2008).

Beta-lactam antibiotics are the preferred drugs for serious *S.aureus* infections. Since the introduction of methicillin into clinical use, the occurrence of MRSA strains has increased steadily and nosocomial infections have become a serious problem worldwide. Indiscriminate use of multiple antibiotics, prolonged hospital stay, intravenous drug abuse and carriage of MRSA in nose are all important risk factors for MRSA acquisition.(Chin etal.2005). In addition, MRSA infected patients require expensive and intensive isolation measures and strict hygiene. To date, the only standardized means of identifying methicillin resistance in the clinical microbiology laboratory are susceptibility tests such as disk diffusion, agar or broth dilution and agar screen methods. The performance of these tests has many drawbacks because factors such as inoculums size, incubation time and temperature, pH of the medium, salt concentration of the medium and exposure to beta lactam antibiotics influences the phenotypic expression Of resistance. Rapid and accurate identification of S.aureus and its methicillin susceptibility pattern has important implications for therapy and management of both colonized and infected patients.

In this study, we have developed and evaluated an mPCR method which allows the detection of MRSA by using primers specific for methicillin resistance and coagulase genes. The purpose of our study was to set up a rapid and reliable identification procedure for MRSA through the amplification of specific gene determinants by PCR in order to efficiently support therapy and eradication of the Pathogen. The results of the multiplex PCR amplification strategy are shows in the 9strains determined as MRSA by phenotypic methods, the results were 100%Consistent with PCR results.

Table.1 Sample taken and presence of Staphylococcus aureus

sample	Sample	Result
S_1	Patient 1	+
S_2	Patient 2	+
S_3	Patient 3	+
S_4	Patient 4	+
S_5	Patient 5	-
S_6	Patient 6	+
S_7	Patient 7	+
S_8	Patient 8	-
S_9	Patient 9	+
S_{10}	Patient 10	-
S_{11}	Patient 11	+
S_{12}	Patient 12	+
S_{13}	Patient 13	-
S_{14}	Patient 14	+
S_{15}	Patient 15	+

Table.2 Preliminary Identification of *Staphylococcus aureus*

S.NO	Test	***Staphylococcus aureus***
1	Mannitol salt agar	Yellow color colonies
2	Gram staininig	Gram positive ,cocci (spherical shaped)
3	Blood agar	β - heamolysis

Table. 3 Biochemical test for identification of *Staphylococcus aureus*

Sample No S.no	Inodole test	MR Test	VP test	TSI		Coagulae test	Citrate test	Urease test	Catalase test	Sugar fermentation
				Acid Production	H_2S/ gas production					
S1	-	+	+	y/y	-	+	-	+	+	A^+G^-
S2	-	+	+	Y/Y	-	+	-	+	+	A^+G^-
S3	-	+	+	Y/Y	-	+	-	+	+	A^+G^-
S4	-	+	+	Y/Y	-	+	-	+	+	A^+G^-
S6	-	+	+	Y/Y	-	+	-	+	+	A^+G^-
S7	-	+	+	Y/Y	-	+	-	+	+	A^+G^-
S9	-	+	+	Y/Y	-	+	-	+	+	A^+G^-
S11	-	+	+	Y/Y	-	+	-	+	+	A^+G^-
S12	-	+	+	Y/Y	-	+	-	+	+	A^+G^-
S14	-	+	+	Y/Y	-	+	-	+	+	A^+G^-
S15	–	+	–	Y/Y	-	+	–	+	+	A^+G^-

Table.4 Concentration of Antibiotics

S.No	Antibiotic	Symbol	Disc concentration(µg/disc)
1..	Amoxyclav	AC	30
2.	Ampicillin	A	10
3.	Bacitracin	B	10
4.	Cefpodoxime	CEP	10
5.	Kanamycin	K	30
6.	Gentamycin	GEN	10
7.	Methicillin	M	30
8.	Novobiocin	NV	30
9.	Pefloxacin	PF	5
10.	Penicillin-G	P-G	10
11.	Tobramycin	TB	10
12.	vancomycin	VA	30

Table. 5 Results of Antibiotic sensitivity tests for staphylococcus aureus isolates

sample	AC	A	B	CEP	K	GEN	M	NV	PF	P-G	TB	VA
S_1	R	R	I	R	S	S	R	R	I	R	I	S
S_2	R	R	R	R	S	I	R	R	I	R	R	S
S_3	R	I	R	R	S	S	R	R	R	R	R	S
S_4	R	I	R	R	R	S	R	R	R	R	S	S
S_6	R	R	R	R	I	S	I	R	R	R	R	S
S_7	R	R	R	R	S	S	R	R	I	R	R	I
S_9	R	I	R	R	S	S	R	R	S	R	R	S
S_{11}	I	I	R	R	S	I	R	R	S	R	I	S
S_{12}	R	R	I	R	I	I	R	R	I	R	R	I
S_{14}	R	R	R	R	S	S	I	R	R	R	I	S
S_{15}	I	R	I	R	S	S	R	R	R	R	R	S

R- Resistance; I-Intermediate; S- Sensitive

Table.6 percentage of resistance of antibiotics

Antibiotics	R	I	S
AC	81.81	18.18	-
A	63.63	36.36	-
B	72.72	27.27	-
CEP	100	-	-
K	9.09	18.18	72.72
GEN	-	27.27	72.72
M	81.81	18.18	-
NV	100	-	-
PF	45.45	36.36	18.18
P-G	100	-	-
TB	63.63	27.27	9.09
VA	-	18.18	81.81

Figure.1 Percentage of resistance of antibiotics

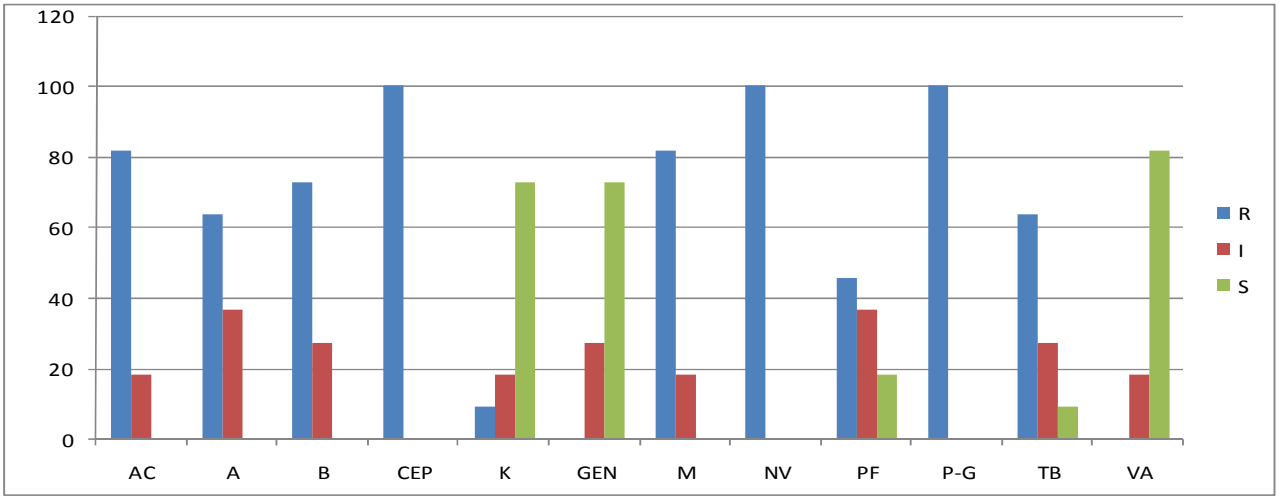
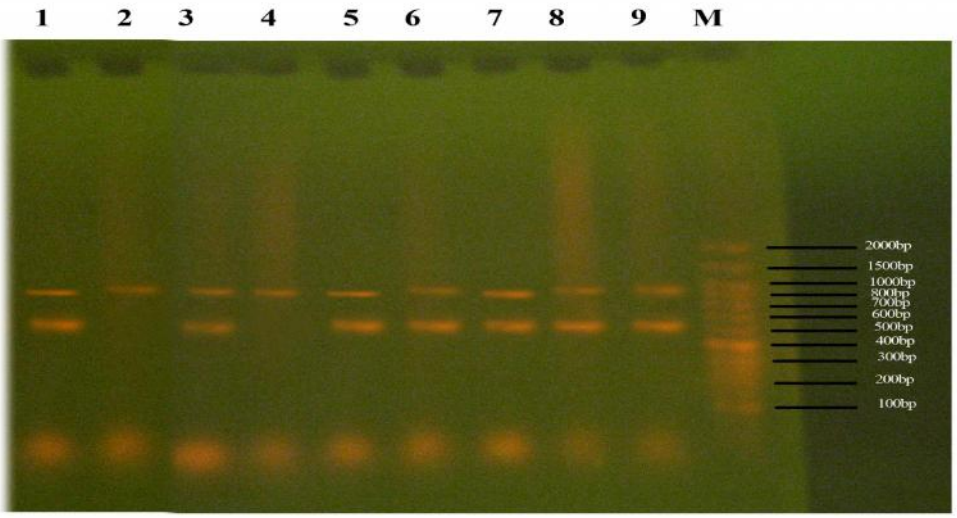


Fig: PCR DETECTION OF Mec A & Coag A GENE In *Staphylococcus aureus*



Lane-1 *Staphylococcus aureus* Sample- 1; Lane-2 *Staphylococcus aureus* Sample- 2; Lane-3 *Staphylococcus aureus* Sample- 3; Lane-4 *Staphylococcus aureus* Sample- 4; Lane-5 *Staphylococcus aureus* Sample- 7; Lane-6 *Staphylococcus aureus* Sample- 9; Lane-7 *Staphylococcus aureus* Sample- 11; Lane-8 *Staphylococcus aureus* Sample- 12; Lane-9 *Staphylococcus aureus* Sample- 14.

References

Abeer, Russdy, Mohamed, Salama , Amal and Othman. 2007. Detection of Methicillin /Oxacillin Resistant *S.aureus* isolated from some clinical hospitals in cairo using Mec A/nuc genes . Agriculture and Biology .9,P .1560-8530

Awadalla, Khalil , Bassim, Ahmed and Wahba,2010 Molecular typing of Methicillin Resistant *S.aureus* isolates at Ain Shams University hospital, Epypt,African J Micro Res , vol.4,P.1639-1646.

Bej, et al., 1990. Multiplex PCR amplification and immobilized capture probesfor detection of bacterial pathogens and indicators in water. Mol. Cell.Probes**4:**353–365.

Chung, M., Dickenson, G., de Lencastre, H. and Tomasz, A., International clones of methicillin resistant *Staphylococcus aureus* in two hospitals in Miami, Florida. J. Clin. Microbiol., 2004, **42**,542–547.

Enright , Robinson , Randle , Feil , Grumman Spratt. The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). Proc Natl Acad Sci USA

Fang H, Hedin G. Rapid screening and identification of Methicillin-Resistant *Staphylococcus aureus* from clinicalamples by selective-broth and real-time PCR assay. J ClinMicrobiol 2003; 41 : 2894-9.

Hashimoto, Inouye, and . Hayashi. 1994. A survey of *Staphylococcus aureus* by typing and drug-resistance in various areas of Japan during 1992 and 1993. Jpn. J. Antibiot. **47:**618–626.

Ito, Katayama, Asada, Mori, Tsutsumimoto, Tiensasitorn, and Hiramatsu, 2001 Structural comparison of three types of Staphylococcal Cassette Chromosome mec integrated in the chromosome in methicillin resistant *Staphylococcus aureus*. Antimicrob. Agents Chemotherapy., , **45**, 1323–1336.

Mehndiratta, Bhalla , Ahmed , Ydsharma, 2009, Molecular typing of Methicillin- Resistant

S.aureus strains by PCR- RELP of spa A gene , Indian J Med Micro Biology, vol. 27,P.116-122

Mehrotra,Wang,and Johnson,2000 Multiplex PCR for detection of genes for *Staphylococcus aureus* enterotoxins, exfoliative toxins, toxic shock syndrome toxin and methicillin resistanceJ.Clin.Microbiol,, 38**:** 1032–1035.

Micek S, et al., 2005. Pleuropulmonary complications of Panton-Valentine leukocidin-positive community-acquired methicillin-resistant *Staphylococcus aureus*: importance of treatment with antimicrobials inhibitingexotoxin production. Chest 2005; 128 : 2732-8.

Perez-Roth, Claverie-martin and Villar, 2001, Multiplex PCR for simultaneous identification of *S.auerus* and detection of Methicillin and mupirocin Resistance .clinical Micro Biology .39 P 4037-4041

Rallapalli , Verghese and Verma .2008 Validation of multiplex PCR strategy for simultaneous detection and identification of methicillin resistant Staphylococcus aureus. Indian J MedMicrobiol ; 26 : 361-4.

Rosenbach, 1884. described the two pigmented colony types of Staphylococci and proposed the appropriate nomenclature: *Staphylococcus aureus* (yellow) and staphylococcus albus (white) JMed Micro Biology .15 P.302-354

Savitha Nadig , Prasanthi Namburi and Dasarathy Ragunath. 2006, Genotyping of Methicillin – Resistant *S. aureus* isolates from Indian hospitals, current science 91, P.1364-1369

Schmitz**,**et al**.,** 1997. Specific information concerning taxonomy, pathogenicity and methicillin resistance of staphylococci obtained by multiplex PCR. J. Med. Microbiol.13: 234-241.

Shiv sekhar Chatterjee, Pallabray, Arun agarwal, Anindita Das and Meera Sharma. 2009. A community based study on nasal carriage of S.aureus, Indian J Med Res,.130,P.742-748

Shrestha, 2009. Phenotypic characterization of nosocomial isolates of Staphylococcus aureus with reference to MRSA. J Infect Dev Ctries. 99 : 7687-92.

Smortikana Biswas, Kumari Dipti Rani, Prithwiraj Mukherjee and Chandradipa ghosh. 2010. Pathogenic *S.aureus* isolates from post operative wounds of hospitalized patients, J. Med Science.3: 219-227

Sutherland, R., and G. N. Rolinson. 1964. Characteristics of methicillin Resistant Staphylococci. J. Bacteriol. 87**:**887–899 11.