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AMPLIFICATION AND SEQUENCING OF mecA GENE FROM METHICILLIN RESISTANCE *STAPHYLOCOCCUS AUREUS*

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ABSTRACT:

The antibiotic resistance is common among pathogenic bacteria associated with both community acquired and nosocomial infections. In view of the present serious problem of resistance to antibiotics from the organism *Staphylococcus aureus*, the present investigation is undertaken to investigate the control of antibiotic resistant in *S. aureus*, specifically methicillin resistance *Staphylococcus aureus* (MRSA) and amplification of mecA gene from *S. aureus*, which are isolates of infected patients and carried molecular characterization of Staphylococcus aureus strains like plasmid typing, primer designing for the amplification of mecA gene, PCR amplification of mecA gene and sequencing of the amplified product. The amplified product of *S. aureus* mecA gene shows partial homology with *Staphylococcus aureus* strain M600 penicillin binding protein 2a (mecA) gene, partial cds, which is available in public databases. Amplification of specific gene and sequencing of mecA gene gives insight into pharmaceutical aspects to design new effective drugs for treatment of methicillin resistance *S. aureus.*

Key words: *Staphylococcus aureus*, MRSA, mecA gene, PCR amplification, Sequencing.

[I] INTRODUCTION:

Staphylococcus aureus is Gram positive, facultative, anaerobic, non motile and non spore forming bacterium, it causes a wide spectra of diseases that include bacteremia, endocarditis, osteomyelitis, nosocomial infection. This pathogenic bacterium could also causes from mild conditions such as skin and soft tissue infections, to life threatening debilitations like toxic shock syndrome (TSS) [1]. This pathogen has persisted and is now resurging as an important hospital and community acquired

pathogen [2]. The development of resistance to a wide range of antibiotics in *S. aureus* is diversified, such as resistance to methicillin that takes the account of *S. aureus* to most β-lactams, macrolides and aminoglycosides [3]. MRSA (methicillin resistant *staphylococcus aureus*), most profound in community craves for the bacteriological surveillance that includes the study encompassing origin and spread of MRSA Molecular study of antibiotic resistance gene from *Staphylococcus aureus* its amplification and sequencing of mecA gene which responsible for most of the β-lactams [4] antibiotics resistance including methecillin will give insight in to design new synthetic drugs to control in community acquired infections of *S. aureus.*

[II] MATERIALS AND METHODS:

2.1. Isolation of *Staphylococcus aureus*:

The clinical samples were collected selectively form the patients who are infected from *S. aureus* in around Bagalkot region of Karnataka state India [5]. The clinical specimens like pus, blood, urine, samples from biomedical waste etc., were selected for the source of organisms and carried in 18.2% peptone water to the laboratory [6]. Mannitol salt agar was used as selective medium for primary isolation of the Staphylococci [7]. Then set of biochemical tests like, oxidative fermentation, manitol

fermentation, phosphatases and catalase test were performed. The coagulase test was performed to confirm *S. aureus* strains for twenty isolates.

2.2. Antibiotic Assay:

Initially antibiotic sensitivity studies were done by disc diffusion method in Mueller Hinton agar for eight [SA2, SA3, SA5, SA9, SA10, SA12, SA17 and SA19] coagulase positive samples by using different antibiotics like, Methicillin (5mcg), Rifampicin (5mcg) and Vancomycin (30mcg) [8]. MIC (Minimum inhibitory concentration) was also done for selecting SA5, SA9, SA12, SA17 and SA19 because these samples were shown maximum résistance to the above said antibiotics in disc diffusion. For MIC we have chosen methicillin antibiotic with concentration of 4mg/l. The five strains of *S. aureus* were inoculated into Brain Heart Infusion (BHI) broth in test tubes and grown to stationary phase for 24 hours at 37°C up to 105-110 CFU/ml. 75 µl of overnight growth culture diluted to 95-100 CFU/ml was inoculated into fresh BHI (75µl) containing varying concentrations, serially diluted (500-2 µg/ml) antibiotics listed in the results are shown in table1 [9].

2.3. Plasmid isolation and Amplification of mecA gene:

For isolation of plasmid and amplification of mecA gene from MRSA we have selected SA17 sample, since it had shown maximum resistance to methicillin. Then before targeting the gene of interest, we have isolated plasmid DNA, because antibiotic genes are present on extra chromosomal DNA. To isolate plasmid DNA 1.5 ml fresh bacterial culture of methicillin resistant SA17 was centrifuged to harvest cells, then the cell pellet was suspended in 0.3 ml of solution I (50 mg glucose, 25 mM tris pH 8.0, 10 mM EDTA), and gently vortexed and kept for cooling at 4°C for 10mins, 0.6 ml of solution II (0.2 N NaOH, 1% SDS) was added, mixed well and incubated for 10mins at room temperature, then 0.450 ml of solution III (5 M sodium acetate) was added and mixed gently and kept in cool for 10 min and was centrifuged at 12000 rpm for 10mins at 4°C. To the supernatant 1ml of isopropanol was added, mixed well, plasmid was allowed to precipitate by keeping it to incubation for 10mins and then the plasmid was separated by centrifuging at 10,000rpm for 10mins. This can be stored by dissolving the dried pellet in TE buffer. 15µl of isolated plasmid were mixed with 2µl assay buffer, 1µl of restriction enzyme HindIII and 2µl of distilled water is aded in eppendrof tube and mixed gently. The mixture was first incubated for 1 hour at 37°C and then for 10mins at 60°C and subjected to agarose gel electrophoresis for separation of digested plasmid DNA fragments (figure1) [10]. To amplify mecA gene we have selected forward and backward primers respectively (5'-GTGGAATTGGGCAATACACC and 5'-AGTTCTGCAGTACCGGAT) and after the primers were designed and ordered, these were made to amplify through PCR (polymerase chain reaction) technique [11]. These primers are present in lyophilized condition which was made viable by thawing. The PCR reaction starts with denaturation step, is the first regular cycling event and consists of heating the reaction to 94-98°C for 20-30 seconds. The reaction temperature is lowered to 50-65°C for 20-40 seconds allowing annealing of the primers to the single stranded DNA template. The temperature used in this step 72°C. At this step the DNA polymerase synthesizes a new DNA strand complimentary to the DNA template strand by adding dNTPs that are complimentary to the template in 5`to3` direction, condensing the 5` phosphate group of the dNTPs with the 3` hydroxyl group at the end of the nascent (extending) DNA strand [12].

[III] RESULTS:

3.1. Isolation of *Staphylococcus aureus*:

Initially there are twenty samples were isolated from patients, out of twenty, eight samples were shown positive for coagulase test, called as SA2,

SA3, SA5, SA9, SA10, SA12, SA17 and SA19. The demonstration of coagulase production is the best single test to identify a *Staphylococcus* belonging to the pathogenic species, *Staphylococcus aureus*. Further the above said samples are chosen for further studies.

3.2. Antibiotic Assay:

The antibiotic resistance zone of inhibition (in diameter) is shown for different antibiotics with respective concentration that we have used. For analysis of antibiotic assay the table1 shows that all the sample that we have used are showing minimum zone of inhibition and it indicates that samples are resistance to antibiotics because the ring diameters must be above 18mm to our antibiotic concentration(M-5mcg, R-5mcg and V-30mcg) according to standard resistance pattern and MIC also showing maximum resistance to antibiotics.

S. No	M (5 mcg)	R (5 mcg)	V (30 mcg)	MIC for (Methicillin) (µg/ml)
SA2	04±0.6mm	10±0.3mm	12±0.4mm	-
SA3	05±0.4mm	12±0.5mm	13±0.3mm	-
SA5	02±0.3mm	10±0.5mm	09±0.4mm	>20±2.00
SA9	02±0.5mm	10±0.6mm	10±0.5mm	>21±3.00
SA10	05±0.5mm	12±0.2mm	13±0.5mm	-
SA12	02±0.5mm	09±0.2mm	09±0.4mm	>22±2.00
SA17	**00mm**	**08±0.4mm**	**09±0.5mm**	**>26±3.00**
SA19	03±0.5mm	10±0.3mm	14±0.5mm	>23±2.00

Table 1: M (methicillin), R (rifampicin) and V (vancomycin) showing zone of inhibition of diameter in mm. Sample SA17 showing maximum resistance for (M, R, and V) in Disc diffusion method and MIC for methicillin method.

3.3. Plasmid isolation and Amplification of mecA gene:

Among eight positive isolates, the sample number SA17 showed maximum resistance to methicillin antibiotic both in disc diffusion and minimum inhibitory concentration assay methods. The figure 1 shows the isolated plasmid DNA from SA17 and subjected to restriction mapping using Hind-III restriction endonuclease enzyme along with standard markers. The figure 2 shows after amplification of plasmid DNA from primers and subjected to agarose electrophoresis, the amplified part is corresponds to 2.3kb of standard DNA marker. The amplified part is sequenced and then 1232 bases have been found similar when compared with *Staphylococcus aureus* strain M600 penicillin binding protein 2a (mecA) gene, partial cds, which is available in the databases and shown in figure 3.

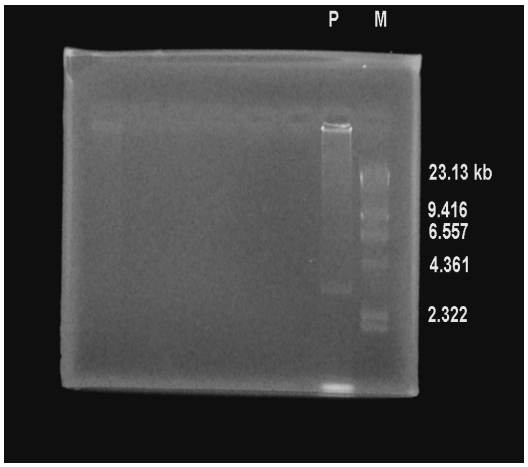

Figure1: The plasmid of S*taphylococcus aureus*, subjected to restriction mapping (RNase + HindIII) along with standard markers DNA.

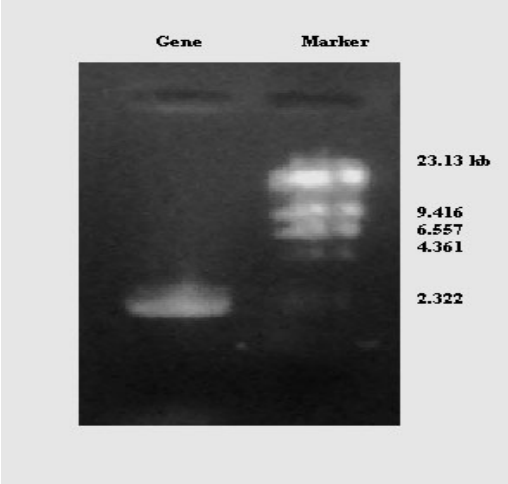

Figure2: The amplified part of the mecA gene which corresponds to the 2.3kb in standard marker.

tagttgtagttgtcgggtttggtatatatttttatgcttcaaaagataaagaaattaataatac
tattgatgcaattgaagataaaaatttcaaacaagtttataaagatagcagttatatttctaa
aagcgataatggtgaagtagaaatgactgaacgtccgataaaaatatataatagtttagg
cgttaaagatataaacattcaggatcgtaaaataaaaaaagtatctaaaaataaaaacg
agtagatgctcaatataaaattaaaacaaactacggtaacattgatcgcaacgttcaattt
aattttgttaaagaagatggtatgtggaagttagattgggatcatagcgtcattattccagg
aatgcagaaagaccaaagcatacatattgaaaaattaaaatcagaacgtggtaaaatttt
agaccgaaacaatgtggaattggccaatacaggaacagcatatgagataggcatcgtt
ccaaagaatgtatctaaaaaagattataaagcaatcgctaaagaactaagtatttctgaa
gactatatcaaacaacaaatggatcaaaagtgggtacaagatgataccttcgttccactt
aaaaccgttaaaaaaatggatgaatatttaagtgatttcgcaaaaaaatttcatcttacaac
taatgaaacagaaagtcgtaactatcctctagaaaaagcgacttcacatctattaggttat
gttggtcccattaactctgaagaattaaaacaaaaagaatataaaggctataaagatgat
gcagttattggtaaaaagggactcgaaaaactttacgataaaaagctccaacatgaaga
tggctatcgtgtcacaatcgttgacgataatagcaatacaatcgcacatacattaataga
gaaaaagaaaaaagatggcaaagatattcaactaactattgatgctaaagttcaaaaga
gtatttataacaacatgaaaaatgattatggctcaggtactgctatccaccctcaaacagg
tgaattattagcacttgtaagcacaccttcatatgacgtctatccatttatgtatggcatgag
taacgaagaatataataaattaaccgaagataaaaaagaacctctgctcaacaagttcc
agattacaacttcaccaggttcaactcaaaaaatattaacagcaatgattgggttaaataa
caaaacattagacgataaa

Figure3: Sequenced part of the mecA gene showing partial homology to *Staphylococcus aureus* strain M600 penicillin binding protein 2a (mecA) gene, partial cds.

[IV] DISCUSSION:

With the advent of antibiotics, it was thought that warrant for the treatment of the *S. aureus* related infection got issued but due to the development of antibiotic resistant gene in the plasmid of *S. aureus* could defend itself in a much secured manner [13]. In our current study we have made an attempt to isolate *S. aures* from patients in around Bagalkot district, Karnataka, India. Out twenty isolates, eight samples are shown positive coagulase test, and are named as SA2, SA3, SA5, SA9, SA10, SA12, SA17 and SA19. For above samples we studied antibiotic resistance by using both disc plate and minimum inhibitory concentration method by selecting specific β-lactamase antibiotics [14]. Our aim was to trace out specifically methicillin resistance *S. aureus* and we found maximum resistance at sample SA17 for both disc plate and MIC methods. SA17 is chosen for our further molecular studies and we have isolated plasmid DNA and subjected to restriction digestion using Hind-III endonuclease. Then we have designed primers for amplification of mecA gene which is present in the plasmid DNA of *S. aureus* from PCR. The amplified part has sequenced, 1232 bases have been found similar when compared with mecA gene of *Staphylococcus aureus* strain M600. The mecA gene plays an important role in β-lactamase antigen resistance including methicillin [15].

[V] CONCLUSION:

The current study of isolation, antibiotic study and sequencing of the mecA gene from MRSA strain will give insight in to the molecular diagnosis of *S. aureus* and to find better synthetic drugs (analogs) or organic molecules by computational biologist in pharmaceutical companies to control expression of antibiotic genes in different pathogenic organism in generally and in particularly MRSA strains.

REFERENCES:

1. Michal Bukowski, Benedykt Wladyka and Grzegorz Dubin, Exfoliative Toxins of *Staphylococcus aureus* journal of Toxins 2010, 2(5), 1148-1165.
2. HongHJ, LeeCH, Park CO, Jung IW Lee SH. Korean J.A clinical study on *Staphylococcus aureus* Bacterimia. medicine, 1997 Sep;53(3):359-370.
3. KennedyAD,OttoM, BraughtonKR Epidemic community-associated methicillin-resistant *Staphylococcus Aureus:* recent clonal expansion and diversification.,2008 Jan29;105(4):1327- 32.
4. Antibiogram and beta lactamase production of S.aureus isolates from different human clinical specimens in tertiary health institutions in ile-ife,Nigeria,A.A. akindele, I.K adewuyi, O.a. adefioye, ammerican –Eurasian journal of scientific research5(4):230-233,2010
5. Goldmann DA et al. (1996) Strategies to prevent and control the emergence and spread of antimicrobial-resistant microorganisms in hospitals: A challenge to hospital leadership. JAMA 275: 234-240.
6. J.O Isibor,P.U. Amadi Comparison of modified peptone water transport medium with two commercially available transport media for the recovery of aerobes from swab specimens. African journal of biotechnology Vol.9 (23) PP. 3467-3467, 7 June 2010

7. R. W. Smyth* and Gunnar Kahlmeter Mannitol Salt Agar-Cefoxitin Combination as a Screening Medium for Methicillin-Resistant *Staphylococcus aureus* J Clin Microbiol. 2005 August; 43(8): 3797–3799.
8. Courvalin P (January 2006). "Vancomycin resistance in gram-positive cocci". Clin. Infect. Dis. 42 Suppl 1: S25– 34
9. Asad U KhanAyesha Sultan, Anju Tyagi, Shazia Zahoor, Mohd Akram, Sukhminderjit Kaur, Mohd Shahid, Chetana V Vaishnavi Amplification of mecA gene in multi-drug resistant Staphylococcus aureus strains from hospital personnel J Infect Developing Countries 2007; 1(3):289-295.
10. Licitra, C.M., R.G Brooks, P.M terry, K.J. shaw, and R.S. Hare (1989). Use of plasmid analysis and determination of aminiglycoside modifying enzyme to characterize isolate from an outbreak of methicillin- resistance Staphylococcus aureus. J. Clin. Microbilogy. 27:2535- 3538.
11. Mesherf aawad Al-ruaoly and omer mohamad khalil. Detection of (mecA) gene in MRSA at prince A/- jout, Soudiarabia. Journal of medical genetics and genomics Vol. 3 (3) PP 41-45, March 2011.
12. Brakstad OG, Aasbakk K and Maeland JA (1992) Detection of Staphylococcus aureus by polymerase chain reaction amplification of the nuc gene. J Clin Microbiology 30: 1654-1660.
13. Khan AU, Musharaf A (2004) Plasmid-mediated multiple antibiotic resistance in Proteus mirabilis isolated from patients with urinary tract infection. Med Sci Monitor 10: 598-602.
14. Lowy FD (2003) Antimicrobial resistance: the example of Staphylococcus aureus. J. Clin Invest 111: 1265-1273.
15. Kolbert CP et al. (1998) Branched-DNA Assay for Detection of the mecA Gene in Oxacillin-Resistant and Oxacillin-Sensitive Staphylococci. J Clin Microbiol 36: 2640-2644.