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Cloning Antibiotic Resistance Plasmid of *Staph .aureus* Isolated From Clinical Sample

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Abstract: Bacterial transformation is an important process in recombinant DNA technology and determines the bacterial pathogenicity. Infections caused by antibiotic-resistant bacteria resulted in higher mortality and recognized as a major public health threat. *Staphylococcus.aureus* is a gram-positive bacterium with numerous virulence factors that cause impetigo diseases. The study aims to investigate that cloning plasmid isolated from bacteria that causes' S. auras pathogenicity. The pathogenic *S.aureus* isolated from clinical samples and identified on the basis of morphological and biochemical characteristics. Furthermore, isolation of plasmid and cloning according to manufactures procedure kit, and transform to *E.Coli.* Also, confirm the antibiotic sensitivity by disc diffusion method. The result shows that the highest antibiotic sensitivity is amoxicillin, while the lowest antibiotic sensitivity is tetracycline for both bacteria. The plasmid transformer to *E.coli* Bacteria appears same zone sensitivity of *S.auras,* and that indicates the plasmid pathogenicity transverse from *S.aures* into *E.coli.*

Keywords: Antibiotic resistance, *S. aureus*, Plasmid cloning, Clinical samples

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1 Introduction

Staphylococci are gram-positive bacteria, it has the following result catalase positive, oxidase negative, facultative anaerobic and non-motile cocci, has large round, golden yellow colonies, often when grown in blood agar plate has the positive hemolysis. *Staphylococcus.aureus* is a various pathogen process for the community as well as clinical related infections. S. aureus can be isolated from patient specimens (Petráš, 2004). Infections disease caused by invasion bacteria result in a peracute clinical sign, and antibiotic resistance are a major public health disturbance (Dheyab, 2013). Bacteria become more resistant to much-using antibiotics and many drugs because the deviance mechanisms required for antimicrobial susceptibility may be not working hard or because of mutation in the bacterial DNA or through the acquired of transformation genetic material(Quinn, Carter, Markey, & Carter, 1999), (Chambers & DeLeo, 2009). Virulence and antibiotic resistance are the two most important features of S. aureus which are associated with mobile genetic elements (MGE), including several prophages and the Staphylococcal Cassette Chromosome mec (SCCmec) (Feng et al., 2007).

Approximately in all bacterial species have plasmids genetic material. These additional genetic elements typically account for only small pieces of all bacterial genome corresponding and roughly extend between 1 and 200 kb (Rohde & Henze, 2011). The aim of the study is determining the antibiotic resistance plasmid of S. aureus isolates and transformation into *E.coli* anther Bactria by molecular typing techniques. It will be very important to introduce all genes necessary for antibiotic production into *E.coli* by gene cloning (Okanishi et al., 1983).

2. Material & Method:

2.1 Isolation & identification of microorganism:

The pathogenic S. aureus were obtained from clinical spacemen people suffering from scalded skin staphylococcus syndrome. And identified on the basis of microscopically and biochemical characteristics .

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2.2 Determination of Antibiotic Sensitivity:

The overnight grown bacterial culture was transferred to Mueller Hinton (MH) agar plates and spread. The antibiotic disc was placed, and the plates were incubated at 37 °C for 24 hrs. The Antibiotic Resistance was determined by measuring the zone of inhibition.

2.3 Isolation of plasmid :

After determining the zone of antibiotic sensitivity we took which sample was more resistance for isolation the random plasmid. On the basis of technique procedure .

2.4 Preparation of bacterial competent:

The bacterial competence cell is artificially occurring by adding calcium chloride Inoculate DH5α strain into 5ml of LB broth and incubates at 37ºC for 24hours. Added The CaCl2 ions exchange the membrane situation and calcium phosphate-DNA complex is formed that attached to the cell surface. The DNA is taken up during heat shock, and immediate chilling on ice ensures closure of the pores. Aseptically transfer of the competent cells into 5 Eppendorf vials. All transferring should be done on the ice.

2.5 Bacterial Transformation:

This process occurs naturally, or bacteria can be made competent to accept foreign pieces of DNA in the laboratory. Bacterial transformation is a process of cloning foreign DNA inserted into a plasmid .

1. The 100µl of the competent cells prepared to add 20 µl of plasmid DNA Provided. Gently tap and keep the vial in ice for 20minutes .

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2. The competent cells are subjected to a heat-shock treatment at 42ºC (on a water bath) for 2minutes.

3. Remove the vials after heat shock and chill the vial on ice for 20 minutes.

4. Add 10 ml of LB broth aseptically to the vial and incubate the culture for 24 hours at 37ºC to allow the bacteria to express and recover the antibiotic resistance.2.1 Title and author names

2.2 Results and Discussion

In this clinical study samples isolated were taken from different Patients in Bangalore city - India and these specimens were identified as S. aureus depended on microscopically and biochemical procedure. The identification was made based on their culture morphology, bacterial Gram staining, mannitol fermentation test, yellow pigmentation test and catalase test. Five isolated *S.aureus* were subjected to many laboratory analysis (Fig1, 2, 3 and4).

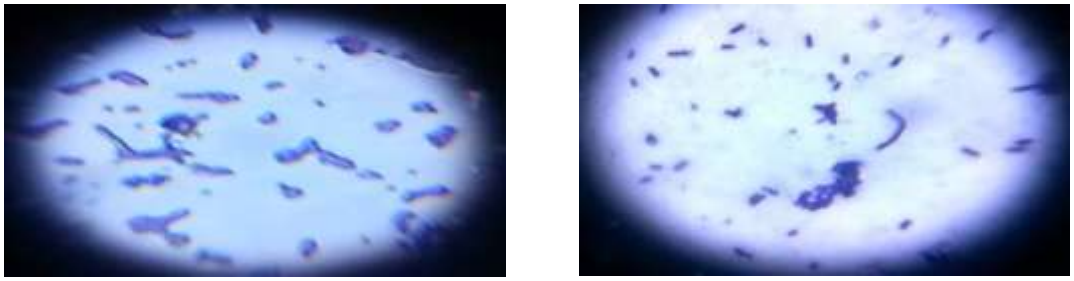

Figure 1: *S.aureus* isolated from clinical samples and staining by Gram Stain

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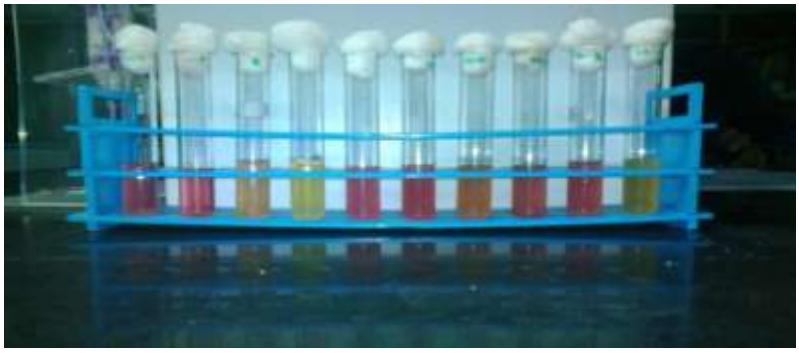

Figure 2. Mannitol Fermentation test for *S.aureus* biochemical identification

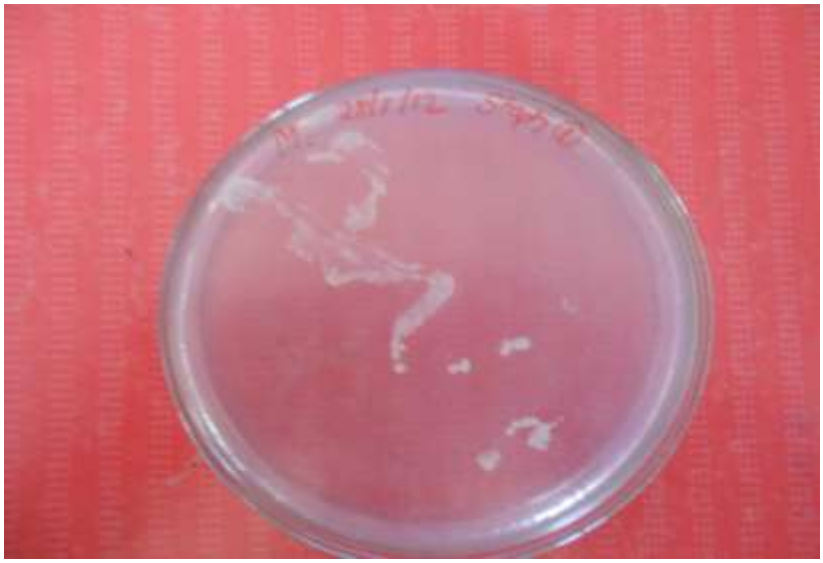

Figure 3: Yellow pigment test for *S.aureus* biochemical identification

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Figure 4: Catalase test for *S.aureus* biochemical identification

The antibiotic sensitivity test for these 5staphylococcus aureus samples. The bacteria that inhibition of zone 10mm or less are considered as bacterial resistance and denoted as (R) In antibiotic susceptibility test all bacteria were resistant to Fluconazol and sensitivity to Rifampicin. For other many antibiotics, these isolated samples showed different results for susceptibility and resistance

Figure 5: Antibiotic sensitivity test for sample four

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Table1

Antibiotics Resistant and Zone of Inhibition

Antibiotics	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
Amoxicillin	**28**	**42**	**17**	**R**	**R**
Gentamicin	**13**	**24**	**23**	**R**	**16**
Methionine	**28**	**31**	**28**	**23**	**R**
Cloxacillin	**36**	**25**	**28**	**R**	**R**
Fluconazol	**R**	**R**	**R**	**R**	**R**
Ampicillin	**32**	**31**	**18**	**R**	**R**
Rifampcin	**25**	**24**	**14**	**14**	**13**
Tetracycline	**11**	**12**	**R**	**R**	**R**
Azithromycin	**12**	**R**	**R**	**R**	**15**
Cefixime	**14**	**15**	**13**	**R**	**R**

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After the get, it results of antibiotic sensitivity we did the plasmid isolation from sample 4 by Alkali lysis method found to be very effective. Because the bacterial sample4 very antibiotic resistance depended on above table result. In this method, the bacterial cells are lysed using alkali and sodium dodecyl sulfate. Which denatures the plasmid as well as chromosomal DNA. Chromosomal DNA along with cell debris gets precipitated which is removed by centrifugation. And running by gel electrophoresis machines for confirmation of the plasmid purify. (Figure 6)

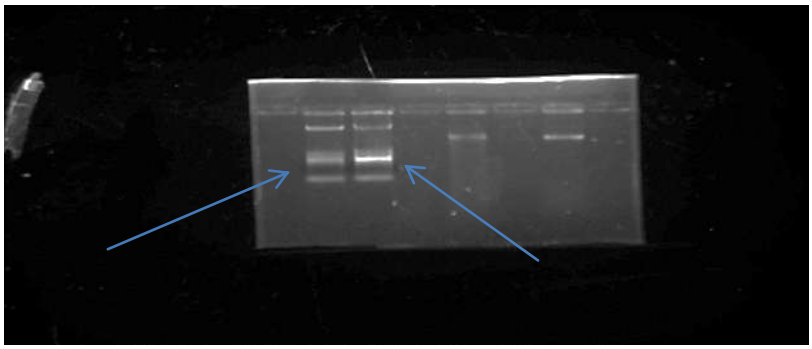

Figure 6: Plasmid Isolation 2 % agarose gel for sample4

The competent cell is artificially induced by treating with calcium chloride. Bacteria can be made competent to accept foreign pieces of DNA in the laboratory. After isolating the plasmid from the bacterial sample 4, we artificially transferred them to DH5α strain and then replanted them on the sensitivity plates to show the same results as in Figure 5 in terms of antibiotic resistance. That was implanted in the laboratory. Figure 7, 8

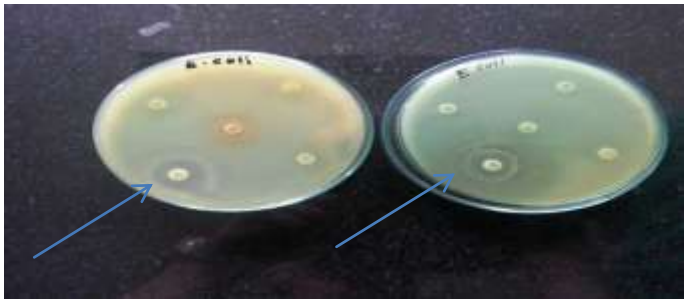

Figure 7: Antibiotic Sensitivity before Cloning for *E.coli* DH5α strain

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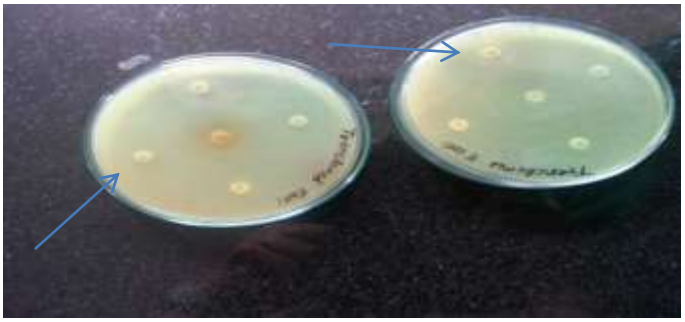

Figure 8: Antibiotic Sensitivity after Cloning for *E.coli* DH5α strain

4. Conclusion

This is evidence that the isolated plasmid is the main cause of these type pathogenic bacteria in the resistance of antibiotics

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