

## DEVELOPMENT OF BACTERIAL STRAINS BY INDUCED MUTATIONS

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**ABSTRACT:** Antibiotic resistance is a serious and growing phenomenon in contemporary medicine and has emerged as one of the pre-eminent public health concern. In this work we have isolated, identified the bacteria from Burn wound samples. Later the antibiotic genes were transferred to *E.Coli* to study the antibiotic mechanism. Grams staining, Endospore staining test, capsulated staining test, Motility test were carried out for the morphology of cell. Catalase, ONPG, Lysine decarboxylase, Ornithine, Urease, Phenyl alanine deamination, Nitrate reduction, H<sub>2</sub>S production, Citrate utilization, Vogesproskaeurs, Methyl red, Indole and Malonate were suited for biochemical studies. Plasmid DNA was isolated and Plasmid curing was performed in order to identify the position of Antibiotic genes. The microorganism identified was staphylococcus and the antibiotic resistant genes were present in the Plasmid. The genes were isolated and transferred to *E.coli* and *E.coli* which was sensitive to antibiotics showed antibiotic resistance.

**Key Words:** Antibiotic resistance, staphylococcus aureus, PCR and Genetic Transformation

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### INTRODUCTION

The development of resistance is inevitable following the introduction of a new antibiotic. Initial rates of resistance to new drugs are normally on the order of 1% [1]. However, modern uses of antibiotics have caused a huge increase in the number of resistant bacteria. In fact, within 8-12 years after wide-spread use, strains resistant to multiple drugs become widespread [2-5]. Multiple drug resistant strains of some bacteria have reached the proportion that virtually no antibiotics are available for treatment. Antibiotic resistance in bacteria may be an inherent trait of the organism that renders it naturally resistant, or it may be acquired by means of mutation in its own DNA or acquisition of resistance-conferring DNA from another source [6, 7]. Taxonomically, the genus *Staphylococcus* is in the Bacterial family *Staphylococcaceae*, which includes three lesser known genera, *Gamella*, *Macroccoccus* and *Salinicoccus* [8, 9]. The best known of its nearby phylogenetic relatives are the members of the genus *Bacillus* in the family *Bacillaceae*, which is on the same level as the family *Staphylococcaceae* [10]. *Staphylococcus aureus* forms a fairly large yellow colony on rich medium, *S. epidermidis* has a relatively small white colony. *S. aureus* is often hemolytic on blood agar; *S. epidermidis* is non hemolytic. *Staphylococci* are facultative anaerobes that grow by aerobic respiration or by fermentation that yields principally lactic acid. The bacteria are catalase-positive and oxidase-negative. *S. aureus* can grow at a temperature range of 15 to 45 degrees and at NaCl concentrations as high as 15 percent [11, 12]. *Staphylococcus aureus* causes a variety of suppurative (pus-forming) infections and toxinoses in humans. It causes superficial skin lesions such as boils, styes and furunculosis; more serious infections such as pneumonia, mastitis, phlebitis, meningitis, and urinary tract infections; and deep-seated infections, such as osteomyelitis and endocarditis [13, 14]. *S. aureus* is a major cause of hospital acquired (nosocomial) infection of surgical wounds and infections associated with indwelling medical devices [15]. *S. aureus* causes food poisoning by releasing enterotoxins into food, and toxic shock syndrome by release of superantigens into the bloodstream.

## MATERIALS AND METHODS

### Sample Collection

Burn wound sample collected from hospital by wearing sterile gloves. These samples were taken for serial dilutions. Grams staining, Endospore staining test, capsulated staining test, Motility test were carried out for the morphology of cell. Catalase, ONPG, Lysine decarboxylase, Ornithine, Urease, Phenyl alanine deamination, Nitrate reduction, H<sub>2</sub>S production, Citrate utilization, Vogesproskaeurs, Methyl red, Indole and Malonate were suited for biochemical studies.

### DISC DIFFUSION METHOD

The disk-diffusion method (Kirby-Bauer) is more suitable for routine testing in a clinical laboratory where a large number of isolates are tested for susceptibility to numerous antibiotics. An agar plate is uniformly inoculated with the test organism and a paper disk impregnated with a fixed concentration of an antibiotic is placed on the agar surface. Growth of the organism and diffusion of the antibiotic commence simultaneously resulting in a circular zone of inhibition in which the amount of antibiotic exceeds inhibitory concentrations. The diameter of the inhibition zone is a function of the amount of drug in the disk and susceptibility of the microorganism

### Isolation of Plasmid DNA by Alkaline Lysis

Transferred a single bacterial colony in to LB medium Containing the 50 mg. per ml. penicillin. Incubated the culture overnight at 37°C with vigorous shaking. Poured the 1.5 ml of the culture into a clean microfuge tube & Centrifuged at 10,000 rpm for 10 minutes. When centrifugation is completed, decant the medium leaving the bacterial pellet was dried as possible. Resuspended the bacterial pellet in 100 µl of ice-cold Alkaline Lysis solution I by vigorous vortex. Kept on ice. Added 200 µl of freshly prepared Alkaline Lysis Solution II to each bacterial suspension. Close the tube tightly and mixed the contents by inverting the tube rapidly five times. Stored the micro centrifuge tube on ice for 5 minutes. Added 150 µl of ice-cold Alkaline Lysis Solution III. Closed the tube and dispersed Solution III through the viscous bacterial lysate by inverting the tube several times. Stored the tube on ice for 3-5 minutes. Centrifuged the bacterial lysate at 10000 rpm for 5 minutes at 4°C in microfuge. To the supernatant add 5 µl of 10 mg/ml DNase free Rnase; incubate at 37°C. for 1 hour. Added an equal volume of phenol: chloroform: isoamylalcohol (25:24:1). Mixed the organic and aqueous phases by vortexing and then centrifuged the emulsion at 10,000 rpm for 5 minutes. Transferred the aqueous upper layer to a fresh tube. Added equal volume of chloroform: isoamylalcohol (24:1) to the aqueous phase and mix it by inverting. Centrifuged the emulsion at 10,000 rpm for 5 minutes. Transferred the aqueous upper layer to a fresh tube. Added 1/10th volume of 3 M Sodium acetate and 2.5 volumes of ethanol to the aqueous layer, Mixed vigorously. Keep the tube in -70°C for 1 hour. Collected precipitate DNA by centrifugation at 12,000 rpm for 12 minutes. Decant the ethanol. Wash the pellet with 1 ml. of 70% ethanol by centrifuged at 12,000 rpm for 5 minutes. Decant the 70% ethanol and dried the pellet at 65°C till the alcohol evaporates completed. Air-dried the pellets for 5-10 minutes and redissolved the DNA in a 1x T.E. buffer. Check purity of Plasmid DNA by 1% Agarose gel Electrophoresis.

### Plasmid purity Assessment Protocol

Size and purity of Plasmid DNA isolated were determined by agarose gel electrophoresis. Prepared 0.7% agarose gel (0.92 g agarose dissolved in 130 ml. of 1x TAE buffer by boiling.

Pour on gel costing tray with appropriate comb and allowed to set for 45 minutes at room temperature. After solidified the gel, the comb and gel-costing tray were removed and the gel was transferred to in an electrophoresis tank containing 1x TAE running buffer. Mixed DNA sample with 6x loading dye (10:1 V/V) and loaded wells carefully along with 100 b.p. molecular marker with micro pipette. Carry out electrophoresis for until the tracking dye migrated to the end of the gel at a constant voltage of 50 volts using power pack. Ethidium bromide stained (90.5 µg/ml) DNA bands were visualized under UV transilluminator.

### Curing of Plasmid DNA:

To detect whether PNP degraded genes are Plasmid borne or main chromosomal borne. Curing of the plasmid DNA is done to remove the plasmid from the cells. To remove the plasmid, curing agents like Sodium lauryl sulphate, Sodium dodecyl sulphate, Acridine orange, Mitomycin-C, Streptomycin Ampicillin can be used at various concentrations. For this study Sodium lauryl sulphate, was used for curing plasmid from the bacterium. Fresh cultures of overnight incubated bacterium was taken and the organism was inoculated in the LB-Broth along with the Sodium lauryl sulphate at increasing concentrations of 100 µg/ml. 250 µg/ml., 500 µg/ml., 750 µg/ml., 1000 µg/ml., 3000 µg/ml., 5000 µg/ml and 7000 µg/ml. All the flasks were incubated orbital shaker at 200 rpm at 30°C for 48 hrs. A concentration that is below MIC (Minimum Inhibitory Concentration) is selected as curing concentration. After incubation, the organism was incubated at selected curing concentration of Sodium lauryl Sulphate for 24 hours.

### Polymerase chain reaction (PCR)

Primer sequences used for amplifying the Resistance genes in plasmid DNA

**F – 5' – AGC TTG GCA ATC AGT TTA – 3'**

**R – 5' – TCC CAC CAT AAA AGA TGA – 3'**

### PCR Mixture

The PCR mixtures were prepared with 2 µl of 20 pmol of both forward and reverse primers, 1µl of 10 mM dNTP, 5 µl of 1U Taq DNA polymerase, 5 µl of 10X PCR buffer, 4.0 µl of 25 mM MgCl<sub>2</sub>, 1µl DNA Sample. Water was added to adjust the final reaction volume to 50µl. PCR Products were analyzed with 2% agarose gel electrophoresis.

### *E.coli* Transformation.

Transformation of *E.coli* with antibiotic resistant plasmid isolated from *Staphylococcus aureus*. Through the application of biotechnology, it has become apparent that interspecies gene transfer between unrelated organisms may be occurring naturally. Researchers have thoroughly demonstrated that prokaryotic and eukaryotic cells will uptake and incorporate foreign DNA from any other organism. If the genetic material is inserted adjacent to the necessary upstream regulatory sequences, then expression of foreign genes will also occur. There are several procedures reported for transformation among which heat shock procedure is a simple and widely used one. Other procedures utilize electrical pulses, sonication, or agitation with glass beads or fibers. All of these techniques are simply used to improve the transformation efficiency by increasing the frequency of contact between the foreign DNA and the host cell. In the present study, heat shock procedure was used to transform plasmid isolated from *staphylococcus aureus* into *E.coli* cells. The transformation procedure was carried out by standard procedure.

### Competent *E.coli* cells are taken

Competent cells are bacteria, which can accept extra-chromosomal DNA or plasmids. Cells can be made competent in several ways. One such way is to "shock" it, which involves cooling the bacteria in a Calcium Chloride bath at 0 degrees Celsius, then quickly heating it to around 47 degrees Celsius for approximately 90 seconds (too long will denature the cell membrane, killing the bacteria). The calcium chloride ions neutralize the repulsion between the negatively charged phospholipid heads of the cell membrane and the negatively charged phosphate groups on the DNA. The quick heat shock creates a thermal gradient, which, in turn, creates a draft leading into the cell, allowing extra-chromosomal DNA (such as plasmids) to enter the cell, allowing the bacterium to be genetically modified. In addition, cells are most competent during their mid-log phase. In an inoculum of *E. coli* cells, competent cells are found at an absorbency of 0.25 using a standard spectrophotometer at 660 nm. The time required to reach mid-log, thus competent cells, depends on the size of the initial inoculum. *E.coli* cells are added to 25 ml of LB broth and incubated at 37 °C on a shaker incubator (200rpm) overnight. LB broth (100 ml) is added to 2.5 ml of overnight culture and initial O.D was recorded at 600 nm with continuous shaking. The bacterial cells are allowed to grow for 5 hrs until to an O.D of 0.5. Cells are spun at 2500 rpm for 5 min at 4°C and the cell pellet thus obtained is gently resuspended in 20 ml of 50 mM calcium chloride. This flask is incubated once for 30°C. After incubation the cells are centrifuged at 2500 rpm at 4°C after suspending in 1.5 ml of 50 mM calcium chloride.

### Transformation of plasmid DNA to competent *E. coli* cells

Plasmid (0.1 µg) is added to 20 µl of competent cells in a sterile eppendorf tubes. The tubes are incubated on ice for 30 min. These cells are given heat shock by transferring tubes to a 42°C water bath and treated for 40 seconds. LB medium (1 ml) is added to the tubes and incubated at 37°C in an incubator shaker at 200 rpm. Cells (50 µl, 100 µl and 200 µl) from the above medium are spreaded on LB agar medium using a bent end glass rod. Plates are incubated at 37°C for overnight. Sensitivity of transformed cells to Penicillin is checked by inoculating the cells in Penicillin containing medium (with yeast extract 50 mgL<sup>-1</sup>, to support growth) containing Penicillin (20mg) as sole source of carbon and nitrogen. *E.coli* cells from stock solution (controls) are plated on above medium and checked for its sensitivity to Penicillin.

### Cells grown on Penicillin containing medium so that transformed cells will only grow

Now the cells were taken, grown on the nutrient medium, enriched with Penicillin. This enrichment was made in order to obtain the transformed cell. Here, the transformed *E.coli* cells will only contain the Antibiotic resistance gene. So it will successfully survive on this medium, all the other non-transformed cells cannot grow on this medium. In this way we can separate transformed cells from other cells. Now these cells will be tested for the stability of the transformation. And so they will be grown on the Penicillin medium for 2-3 generations.

## RESULTS AND DISCUSSION

### Streak plate Technique

Color less colonies were observed over the NAM medium. Colonies of isolated organism on Nutrient agar medium. On Gram staining blue colored cocci were observed. Hence it is a Gram positive Bacterium.

### Negative Staining

On negative staining spherical cells occurring in clusters appear transparent (colorless) against a blue-black ground. From above observation isolated organism is capsulated.

### Fermentation of Carbohydrates

After 48 hrs of incubation it was observed that sugars that are glucose, sucrose and lactose were utilized by *isolated organism* acid was produced in glucose, lactose and sucrose (Figure 4).As isolated organism utilized all the three sugars and produced to the acid so it is positive. Whereas *Staphylococcus aureus* did not utilized the any sugars so it is negative.

### Catalase Activity

After 48 hours of incubation when four drops of hydrogen peroxide was added to the slants slow appearance of gas bubbles was observed (Figure 5).After the addition of hydrogen peroxide gas bubbles were observed which the indication of positive test is. Hence *isolated organism* is positive for catalase test.

### Hydrogen Sulphide Production test

No black coloration along the line of stab inoculation was observed (Figure 6).Black coloration along the line of stab inoculation was not observed. Hence the Organism may be H<sub>2</sub>S negative.

### Indole Production test

Development of cherry (deep) red color in the top layer of the tube is not observed. Hence, isolated organism is indole – negative bacterium (Figure 7).As development of cherry red color is not observed in the top layer of the tube so *Isolated organism* is negative test.

### Methyl-red and Voges-Proskauer tests

The tubes in which methyl red was added no red color was observed in the V-P test tubes when V-P reagents I & II were added no red color was observed (Figure 8 & 9 ).As in the methyl red test red color is observed hence, it is positive test. In the VP test, red color is not observed hence, it is negative test.

### Citrate Utilization test

After 48 hours of incubation it was observed that there is no change in the medium colour.From the above observation it is said that *isolated organism* is negative to this test.

### Urease Test

After 48 hours of incubation it was observed that there is no change in the medium.From the above observation it is said that *isolated organism* shows positive test.

### Disc diffusion Method

**Result:** This type of antibiotic sensitivity is observed.

**Table 1: antibiotic sensitivity test**

Number of samples	Action of micro organism	CLR	AN	CR	CFP	CIP	ACX	CF	P
	Sensitive	Sen	Sen	Sen	Sen	Sen	Sen	Res	Res

CLR= clarithromycin, AN = Amikacin, CR = Cefuroxime, CFP = Cefoperazone, CIP = Ciprofloxacin, ACX = Ampiclox, P. = penicillin, CF=cefotaxime.

Isolated unknown organism is identified as *Staphylococcus aureus* based on morphology and biochemical tests compare to BERGEY'S manual.

**Plasmid DNA Isolation:**

The Plasmid DNA was isolated and can be confirmed by the 1% running agarose gel electrophoresis and visualized the bands under UV trans illuminator. (Fig. 1)



**Fig 1: Plasmid DNA**

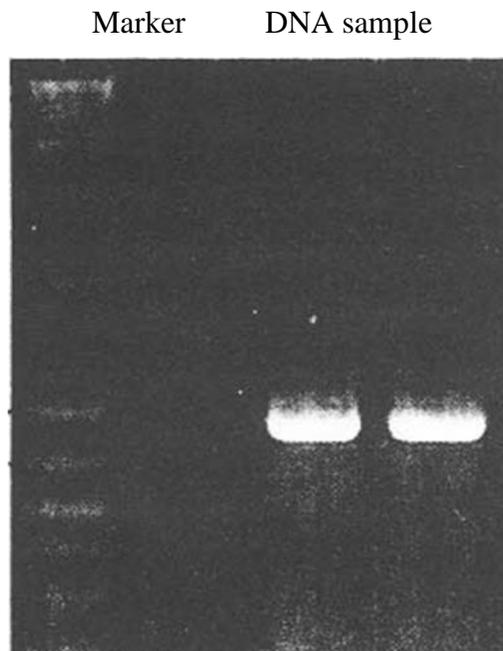
**Location of Penicillin resistance Genes:**

**Plasmid Curing from Isolated Penicillin resistance genes in Bacteria**

Curing concentration was determined by observing turbidity in test tubes after treating the isolates at different concentrations. A concentration less than lethal concentration was selected as curing concentration. An SDS concentration above 750µ g/ml was lethal to the isolates. Hence, 750µg/ml was used for plasmid curing studies. Absences of plasmid from cured isolates are confirmed by 1% Agarose gel electrophoresis.

**PCR**

The strong bands which were obtained near the well is the indication of good quality of pure DNA without any RNA and protein contamination. (Fig. 2).



**Fig 2: PCR product**

## Cloning of Antibiotic genes

### Transformed competent *E.coli* cells:

Plasmid of *Staphylococcus aureus* was taken and transformation of the competent *E.coli* cells was made. And then these cells were made to grow on to the nutritive medium containing penicillin.

Normal cells i.e., non-transformed cells will die, and as normal *E.coli* cells are not resistant to penicillin, as it has no resistance gene in it. Where as the transformed cells have Resistance gene in it, so they survive on Resistance medium.

The transformed cells were cultured for 2-3 generations in order to check the stability of the transformation. And the cells were found to be stable. This is the photograph of the cell culture in 3<sup>rd</sup> generation (Fig 3).



**Fig 3: Cloning of Antibiotic genes into *E.coli***

## DISCUSSION

Antibiotics are among the most powerful and important medicines known. When used properly they can save lives, but often they are used unnecessarily for illnesses that do not respond to antibiotics and for infections that our body can handle without medication. Our immune systems have evolved (developed) to enable us to recover from most infections.

One of the foremost concerns in modern medicine is antibiotic resistance. Simply put, if an antibiotic is used long enough, bacteria will emerge that cannot be killed by that antibiotic. This is known as antibiotic resistance. Infections exist today that are caused by bacteria resistant to some antibiotics. The existence of antibiotic-resistant bacteria creates the danger of life-threatening infections that don't respond to antibiotics. There are several reasons for the development of antibiotic-resistant bacteria. One of the most important is antibiotic overuse. This includes the common practice of prescribing antibiotics for the common cold or flu. Even though antibiotics do not affect viruses, many people expect to get a prescription for antibiotics when they visit their doctor. Although the common cold is uncomfortable, antibiotics do not cure it, nor change its course. Each person can help reduce the development of resistant bacteria by not asking for antibiotics for a common cold or flu.

Antibiotics are an important class of medications that save many lives. However, antibiotics are often over prescribed and recommended when simple natural remedies would do. Antibiotics also have many side effects. A more balanced approach is to begin living so as to enhance and keep your immune system strong. If an infection occurs, apply harmless and simple natural measures immediately. Most often, the natural methods work very well. Reserve the antibiotics for when they are appropriate, which is usually only as a last resort. One way to circumvent the origin of antibiotic resistance is to treat an infection with multiple drugs. Such drug combinations like trimethoprim-sulfamethoxazole can inhibit the development of antibiotic resistance. Perhaps of greater importance is the realization that widespread antibiotic resistance is a product of how we use antibiotics, and it can be decreased by changes in antibiotic prescription practices. Antibiotics are often prescribed for mostly viral infections such as colds and acute bronchitis. Another problem is the sale of antibiotics as over the counter drugs in certain countries and the widespread use of antibiotics in agriculture. Changes in antibiotic prescribing practices can have a tremendous impact. In Finland, for instance, a nationwide effort was undertaken to fight the rapid increase in the prevalence of group A streptococci resistant to macrolides. The total consumption of these antibiotics was decreased from 2 daily doses per 1000 inhabitants to 1.4 in 1992, subsequently the prevalence of group A streptococci resistance to macrolides declined from 19% in 1993 to 0.6% in 1996.

Antibiotics are an important class of medications that save many lives. However, antibiotics are often over prescribed and recommended when simple natural remedies would do. Antibiotics also have many side effects. A more balanced approach is to begin living so as to enhance and keep your immune system strong. If an infection occurs, apply harmless and simple natural measures immediately. Most often, the natural methods work very well. Reserve the antibiotics for when they are appropriate, which is usually only as a last resort.

## CONCLUSION

Both the number and the specific combinations of virulence- and antibiotic resistance-associated genes in *S. aureus* isolates from the burn wound sample were found to various hospitals. This suggests that more accurate assessments of health risks to patient may require not only total colony-forming units of *S. aureus*, but also an assessment of the virulence gene content of those colonies. Further work to determine accurate molecular indicators of antibiotic resistance will be useful for rapid non-cultivation based diagnostic assays.

In summary, the results demonstrate that conjugation does occur between different genera of bacteria. This implies that conjugation depends very little on the specific attributes of the host. In other words, a plasmid can transfer from *Escherichia* to *Serratia* and work because the necessities of the plasmid are met by both genera. One could hypothesize that a plasmid can be utilized by different types of host because all host use similar polymerases with which they transcribe proteins from the plasmid.

However, the results also show that conjugation does not occur or occurs with low frequency between a Gram (-) bacteria and a Gram (+). The first part of this experiment demonstrated that conjugation can occur between different genera, thus implying that the recipient has very little influence in conjugation. However, conjugation most likely does not occur between a Gram (-) and a Gram (+) due to the excessive thickness of the peptidoglycan layer found in Gram (+) cell walls. This would inhibit the sex pilus from entering the cytoplasm of the recipient Gram (+) cell and thus prevent the plasmid from entering. Thus the experiment demonstrated conjugation between different bacterial genera, and supplied evidence that conjugation does not occur through the gram (+) cell wall.

There are several improvements upon this experiment which would aid in stabilizing these results. The first comes from the problem that the experiment lacks in repetition. Because each step in the experiment takes roughly 2 days, each experiment was only attempted once, as time allowed. If time allowed for more experimental runs of the Gram (-) to Gram (+) transfers, perhaps a clearer picture of the actual results could be presented.

This experiment did prove the ease at which Gram (-) bacteria can transfer resistance among one another. This can be extremely problematic in cases where frequent use of antibiotics causes some people to have host flora resistant to antibiotics. These bacteria could then transfer this resistance to other invasive genera. On the other hand, these results could be useful to companies working with bacteria, as they could transfer protein plasmids between different genera to suit their needs.

## REFERENCES

- [1] Zigelboim S, Tomasz A. Penicillin-binding proteins of multiply antibiotic-resistant South African strains of *Streptococcus pneumoniae*. *Antimicrob Agents Chemother*. 1980. Penicillin-binding proteins of multiply antibiotic-resistant South African strains of *Streptococcus pneumoniae*. *Antimicrob Agents Chemother* Mar;17(3):364–371
- [2] Saiki RK, Gelfand DH, Stoffel S, Scharf SJ, Higuchi R, Horn GT, Mullis KB, Erlich HA. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science*. Jan 29; 239(4839):487-91
- [3] Dougherty TJ, Koller AE, Tomasz A. 1980. Penicillin-binding proteins of penicillin-susceptible and intrinsically resistant *Neisseria gonorrhoeae*. *Antimicrob Agents Chemother*. Nov;18 (5):730-7
- [4] Parr TR Jr, Bryan LE. 1984. Mechanism of resistance of an ampicillin-resistant, beta-lactamase-negative clinical isolate of *Haemophilus influenzae* type b to beta-lactam antibiotics. *Antimicrob Agents Chemother*. Jun;25 (6):747-53
- [5] Latorre C, Juncosa T, Sanfeliu I. 1985. Antibiotic resistance and serotypes of 100 *Streptococcus pneumoniae* strains isolated in a children's hospital in Barcelona, Spain. *Antimicrob Agents Chemother*. Aug;28 (2):357-9
- [6] Song MD, Wachi M, Doi M, Ishino F, Matsubashi M. 1987. Evolution of an inducible penicillin-target protein in methicillin-resistant *Staphylococcus aureus* by gene fusion. *FEBS Lett*. Aug 31;221(1):167-71
- [7] Brakstad OG, Aasbakk K, Maeland JA. 1992. Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the *nuc* gene. *J Clin Microbiol*. Jul; 30(7): 1654–1660.

- [8] Henze, UU, Berger-Bachi, B. 1995. Staphylococcus aureus penicillin-binding protein 4 and intrinsic beta-lactam resistance. *Antimicrob Agents Chemother*; 39:2415.
- [9] Tomasz, A, Drugeon, HB, de Lencastre, HM, 1989. New mechanism for methicillin resistance in Staphylococcus aureus: clinical isolates that lack the PBP 2a gene and contain normal penicillin-binding proteins with modified penicillin-binding capacity. *Antimicrob Agents Chemother* 33:1869.
- [10] Chambers, HF, Sachdeva, M, Kennedy, S. 1990. Binding affinity for penicillin-binding protein 2a correlates with in vivo activity of  $\beta$ -lactam antibiotics against methicillin-resistant Staphylococcus aureus. *J Infect Dis* 162:705.
- [11] Bush LM, Calmon J, Cherney CL, 1989. High-level penicillin resistance among isolates of enterococci: implications for treatment of enterococcal infections. *Ann Intern Med.* 110:515–20.
- [12] Hartman BM, Tomaz A. (1984. Low-affinity penicillin binding protein associated with beta lactam resistance in *Staphylococcus aureus*. *J Bacteriol.* 158:513
- [13] Voyich, J. M., Braughton, K. R., Sturdevant, D. E., Whitney, A. R., Said-Salim, B., Porcella, S. F., Long, R. D., Dorward, D. W., Gardner, D. J., Kreiswirth, B. N., Musser, J.M., DeLeo, F. R. 2005. Insights into Mechanisms Used by Staphylococcus aureus to Avoid Destruction by Human Neutrophils. *J. Immunol.* 175: 3907-3919
- [14] Amon RB, Dimond RL. 1975. Toxic epidermal necrolysis. Rapid differentiation between staphylococcal- and drug-induced disease. *Arch Dermatol. Nov*; 111 (11):1433–1437
- [15] de Azavedo J, Arbuthnott JP. 1981. Prevalence of epidermolytic toxin in clinical isolates of Staphylococcus aureus. *J Med Microbiol.* Aug ;14(3):341–344.

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