

Comparison of Acidometric Method And Clover Leaf Method For The Detection of Beta Lactamase In Staphylococcus aureus

Mr. Ravi Vashistha¹, Dr. Atul Kumar², Dr.(Col) Paramjit Singh³, Mrs.Ankita⁴

¹Tutor In Microbiology Department At Muzaffarnagar Medical College, Muzaffarnagar, UP, India

² Assistant Professor In Microbiology Department At Muzaffarnagar Medical College, Muzaffarnagar, UP, India

³Professor & HOD In Microbiology Department At Muzaffarnagar Medical College, Muzaffarnagar, UP, India

Corresponding Author: Dr. Atul Kumar, C-44, Faculty Campus, Muzaffarnagar Medical College, Muzaffarnagar, UP

Corresponding Author: Dr. Atul Kumar²

Abstract: *Staphylococcus aureus* is also known as "golden staph" and *Oro staphira*, it is facultative anaerobic Gram-positive cocci. It is frequently found as part of the normal skin flora on the skin and nasal passages. *S. aureus* is the most common species of *Staphylococcus* to cause Staph infections. Total 105 *Staphylococcus aureus* isolates were identified from various samples. Organisms were identified by various tests like Catalase, Slide and Tube Coagulase, Mannitol fermentation tests, Phosphatase test, and DNase test. MRSA and MSSA test were also done. In this study total 105 *Staphylococcus aureus* isolates were processed. The incidence of Staphylococcal infections was more in males (62%) as compared to females (38%). Out of 105 samples, 93 (88.5%) were MSSA whereas 12 (11.5%) were MRSA. The need to properly detect beta lactamase production in clinical isolates becomes apparent after the first reports of the emergence of beta lactamase production in pathogenic bacteria.

Keywords: *Isolate Staphylococcus aureus, AST, Detection of beta lactamase, Sensitivity of clover leaf and Acidometric methods*

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I. Introduction

Staphylococcus aureus very commonly causes infections in humans, virtually every person will have one or more *Staphylococcus aureus* infections in his or her lifetime¹. *S. aureus* ranks second as the cause of nosocomial blood stream infections, that leads to increased morbidity, mortality, hospital stay, and costs². Nasal carriers of *Staph. aureus* are also at increased risk of developing *S. aureus* infection³. Beta-lactams such as penicillin are the most widely used antibiotics, and beta-lactamases are the greatest source of resistance to penicillin's. Infections that cause mortality and morbidity were treated easily with penicillin, but after the extensive usage of beta-lactam antibiotics, bacteria have developed resistance via different mechanisms. Beta lactamase enzyme production is the first developed and the greatest source of resistance to beta-lactams. Staphylococcal beta-lactamase is not chromosomal and is carried on plasmids and can be non-inducible or inducible with antibiotic contact.⁴ To overcome this resistance, β -lactam antibiotics are often given with β -lactamase inhibitors such as clavulanic acid. β -Lactam antibiotics are indicated for the prophylaxis and treatment of bacterial infections caused by susceptible organisms. At first, β -lactam antibiotics were mainly active only against Gram-positive bacteria, yet the recent development of broad-spectrum β -lactam antibiotics active against various Gram-negative organisms has increased their usefulness⁵. Beta-Lactamases are the commonest single cause of bacterial resistance to beta-lactam antibiotics. Numerous chromosomal and plasmid-mediated types are known and may be classified by their sequences or phenotypic properties. Hence, in this study we shall try to detect the production of beta lactamase enzyme in *S. aureus* using two different methods⁶.

II. Material And Method

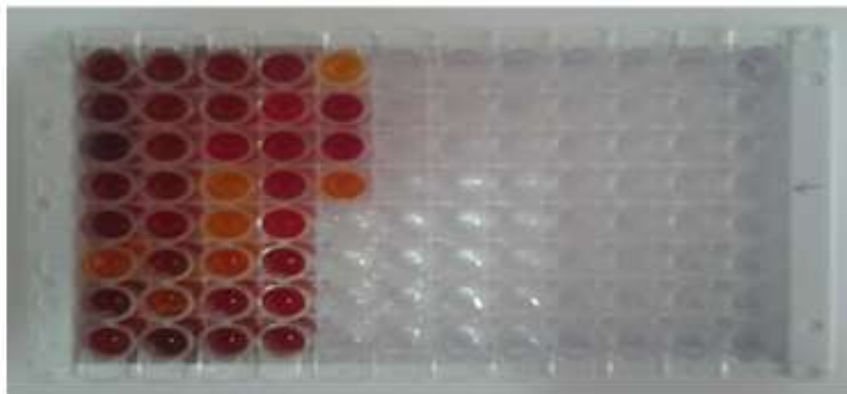
This retrospective study was conducted at Microbiology department, MGM Medical college & Hospital, Navi Mumbai, a tertiary care teaching institute in the state of Maharashtra for a period of one year (June 2012 – May2013). During this period, 105 samples were taken from patients admitted to the hospital. The patients included children, adults, and elderly people. A battery of culture media including blood, Mannitol salt agar (MSA) were used for the isolation of organisms. All media were incubated at 37°C for 24-48 h. The susceptibility of all the isolated *Staphylococcus aureus* to different antibiotics was determined by Kirby-Bauer's disk diffusion technique as per the Clinical and Laboratory Standards Institute (CLSI) guidelines. The

antibiotics used were Ampicillin/sulbactam (20mcg), Cefotaxime (30mcg), Cloxacillin(1mcg), Cefazolin (30mcg), Cefaclor (30mcg), Azithromycin, (30mcg), Teicoplanin (30mcg), Co-trimoxazole (25mcg), Tetracycline(30mcg), Ciprofloxacin(5mcg), Linezolid(30mcg), Gentamicin(10mcg), Vancomycin (30mcg) , Rifampicin(30mcg).

Beta lactamase detection :-

1. **In acidometric tube method:-** Phenol red solution was prepared. Penicillin was dissolved in this solution and pH was adjusted to 8.5 with NaOH. 0.1 ml of this solution was taken to micro titration plate and bacteria were transferred to this solution by loop. If the violet color disappeared, bacteria were beta lactamase positive⁷.

Acidometric Method



YELLOW COLOUR SHOWING POSITIVE TEST AND PURPLE COLOUR SHOWING NEGATIVE TEST.

2. **Cloverleaf-lactamase test –** In this method 5% blood agar plate is first lawn cultured with the *S.aerus* ATCC 25923 as the beta lactamase negative control, 1U Penicillin disk is placed in the center, then test strain is streaked as a cross. Plates are incubated at 37° C for 18-24 hours. If the test strain is beta lactamase positive then clover leaf shaped zone of inhibition will be observed⁸.



Comparison of β - lactamase production by Clover leaf and Acidometric method. From these 105 Staphylococcus aureus isolates, 93 were MSSA and 12 were MRSA and out of the total isolates 105, 62 was positive by Cloverleaf method and 56 was positive by Acidometric method. In MSSA, 54 isolates were beta lactamase positive by cloverleaf method and 49 by Acidometric method.

III.Result

In this study total 105 Staphylococcus aureus isolates were identified, 65 (62%) were isolated from males and 40 (38%) from females. Staphylococcus aureus were isolated from patients with age maximum i.e. 20 (19%) were isolated from age group 21-30 years and least 05 (4.7%) were isolated from the age group 61-70 years and 71-80 years respectively. It was seen that maximum numbers of Staphylococcus aureus strains were isolated from pyogenic infections. The most common sample from which the Staphylococcus aureus was isolated was pus 51(48.57%) followed by Blood 25(23.80%), Urine 15(14.28%), Pleural fluid 04(3.80%), Throat swab 06(5.71%), Ear swab 04(3.80%). Out of 105 Staphylococcal isolates 12(11%) were methicillin resistant and 93(89%) were methicillin sensitive and 62 (59.04%) was positive by Cloverleaf method and 56 (53.33%) was positive by Acidometric method. In MSSA, 54 (58.06%) isolates were beta lactamase positive by cloverleaf method and 49 (52.68%) by Acidometric method. In MRSA, 8 (66.66%) isolates were beta lactamase positive by cloverleaf method and 7 (58.33%) by Acidometric method. {TABLE1} This indicates that Cloverleaf method was more sensitive for detection of beta lactamase as compared to Acidometric method.

[Table 1]: Comparison of β - lactamase production by Cloverleaf and Acidometric method

Isolates	Total number of isolates	Number of isolates showing beta lactamase production.	
		Cloverleaf	Acidometric
MSSA	93	54 (58.06%)	49 (52.68%)
MRSA	12	8 (66.66%)	7 (58.33%)
Staphylococcus aureus	105	62 (59.04%)	56 (53.33%).

[Table2]: Comparing sensitivity of beta lactamase producers to a beta lactam antibiotic (Ampicillin) and beta lactam antibiotic with an beta lactamase inhibitor (Ampicillin + Sulbactam)

Beta lactamase producers	Sensitive to only Ampicillin	Sensitive to Ampicillin-Sulbactam
62 (59.04%)	07 (11.29%)	52 88.70%)

IV. Discussion & Conclusion

Ampicillin is a beta lactamase inhibitor and acts primarily by irreversible inactivation of beta lactamases. The combination of Amipicillin-Sulbactam inhibits beta lactamase activity and increases the spectrum of activity. In this study total 105 Staphylococcus aureus isolates were processed.

The incidence of Staphylococcal infections were more in males (62%) as compared to females (38%) which was also supported by the study done by Bidhya Shrestha et al⁹, which showed higher percentage of Staphylococcus aureus strains in male 24.9% (47/189) as compared to females 22.9% (37/161).

In this study, the most common age group from which the Staphylococcus aureus was isolated was 21-30 years, 20 (19%) and least from the age group 61-70 and 71-80 years respectively 05 (4.7%). It was seen that maximum numbers of Staphylococcus aureus strains were isolated from pyogenic infections.

The most common sample from which the Staphylococcus aureus was isolated was pus (55.43%) followed by blood (52.08%), urine (60%) and others which was also seen in the study of Jamshaid Ali Khan et al², who showed the highest percentage of Staphylococcus aureus isolates was from pus among all the clinical samples.

Out of 105 samples, 93 (88.5%) were MSSA whereas 12 (11.4%) were MRSA which correlates with the study done by Poonam Zsood et al¹⁰ who showed the incidence of MRSA as 8%.

This study was designed to highlight the current antimicrobial susceptibility pattern of staphylococcus with respect to β -lactamase production in order to guide clinician as to the choice of antimicrobial agents. By disk diffusion sensitivity testing, Vancomycin (100%) and Linezolid (100%) was the most effective antibiotic followed by Rifampicin (94.28%), Cefaclor (92.38%) and others were Gentamycin (80%), Cloxacillin (81.90%), Tetracycline (80%). Whereas in the study done by A.A. Akindeles et al¹¹ showed variation in antibiotic sensitivity pattern, susceptibility to antibiotics were: erythromycin (82.5%), Cloxacillin (66%), Others were, Gentamicin, tetracycline and streptomycin with 61%, 30% and 53.8% susceptible respectively. While cotrimoxazole shows the resistance of 28.57% and the study carried out by Okunye Olufemi L et al¹ also showed Cotrimoxazole, a double blocker antibiotics elicited 30.39% resistance. The combination of Ampicillin +

Sulbactam was more effective in beta lactamase producing strains of *Staphylococcus aureus* as compared to only Ampicillin. Study done by A.A. Akindele et al¹¹ showed variation in the sensitive percentage of Ampicillin (3.8%). So the Ampicillin sulbactam is the best choice of drug in beta lactamase producing Staphylococcal strains.

In this study we compared one biochemical i.e. acidometric method and one microbiological method i.e. Clover leaf technique for detection of β lactamase in Staphylococci. Clover leaf technique was found to be the more sensitive, easy, cost effective and reliable method for β lactamase detection which can be easily incorporated in the routine work in laboratories. In several publications, β lactamase production rates vary from 33.8% to 92.6% for Staphylococci. In a recent study Ekrem Killick et al⁴ have reported this incidence to be 83.9%. In this study, total beta lactamase enzyme producer's strains were 59.04% by Cloverleaf method and 53.33% were by Acidometric method which was also supported by study of SA Samant et al¹²,

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