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Detection of Beta Lactamase Production in *Staphylococcus aureus* Isolated from Pus Samples at Tertiary Care Hospital

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A B S T R A C T

The β-lactamase enzymes produced by *Staphylococcus aureus* confer resistance against β-lactam antibiotics like penicillin; and are encoded in the blaZ gene located on a transposable part of the large plasmid within the bacterial cells. Clover Leaf Method was performed on all penicillin resistant *Staphylococcus aureus* isolates to confirm the Beta lactamase production. This is an important confirmatory method to detect the production of beta-lactamase enzyme. Results : Out of 76 isolates 92%(70) were found to be the producers of Beta lactamase enzyme which are resistant to penicillin and 08% (6) didn't show cloverleaf pattern which are sensitive to penicillin. Out of 76 isolates maximum sensitivity was shown with Vancomycin and Linezolid i.c 100% followed by Minocycline (96%), Teicoplanin (88%), Tetracycline (83%), Doxicycline (80%), Chloramphenicol (77%), Gentamicin (76%), Clindamycin (59%), Erythromycin (21%) and less sensitive were Ciprofloxacin (20%), Levofloxacin (20%) & Penicilin-G (08%). Resistant isolates was higher among Penicilin-G (92%) followed by Ciprofloxacin (80%), Levofloxacin (80%), Erythromycin (79%), Gentamicin (24%), Clindamycin (41%), Chloramphenicol (23%) and less Resistant was shown to Teicoplanin (12%), Tetracycline (17%), Doxicycline (20%) and Minocycline (04%). Conclusion: *Staphylococcus aureus* continues to be a health problem for both community-acquired and hospital-associated infections. Finally concluded that continuous monitoring of antibiotic susceptibility pattern of S. aureus and use of a right and proper antimicrobial drug will be helpful for minimizing the resistance to beta lactams and other group of antibiotics.

Introduction

Beta Lactamases can be broadly divided into enzymes with a serine residue at the active site, similar to bacterial penicillin binding proteins, from which they probably evolved (Joris *et al.,* 1988) and metalloenzymes with zinc ion as a cofactor and with a separate heritage (Garau *et al.,* 2004). Both are ancient enzymes. Since β-lactam antibiotics came into clinical use, β-lactamases have coevolved with them (Medeiros, 1997). Hundreds of β-lactamases have been described and have been given a bewildering variety of names. Fortunately, the enzymes can be classified. On the basis of their

primary structure into four molecular classes (A through D) (Ambler, 1980) or on the basis of their substrate spectrum and responses to inhibitors into a larger number of functional groups (Bush *et al.*, 1995). Class A and class C β-lactamases are the most common and have a serine residue at the active site, as do class D β -lactamases. Class B comprises the metallob-lactamases.

Twenty years ago, plasmids mediating resistance to β -lactam antibiotics in *Escherichia coli* and other Enterobacteriaceae most often carried genes encoding class A enzymes such as TEM-1 or SHV-1 or class D enzymes such as OXA-1 (Livermore, 1998). Class B and C enzymes had a broader spectrum of activity but were almost always encoded by chromosomal genes and hence were confined to particular bacterial species. Penicillinase is a specific type of β-lactamase, showing specificity for penicillins, again by hydrolysing the β-lactam ring. Molecular weights of the various penicillinases tend to cluster near 50 kiloDaltons. Penicillinase was the first β-lactamase to be identified (Abraham and Chain, 1988).

We have selected gram positive cocci *Staphylococcus aureus* for our present study which is a member of Micrococcaceae family and is increasingly recognized as causing Nosocomial infections and continues to be a major cause of community-acquired infections (Washington *et al.*, 2007). *Staphylococcus aureus* possesses several properties which are believed to contribute to their ability to causes diseases such as Capsular polysaccharides, biofilm formation, Adhesins, Peptidoglycan teichoic, lipoteicoic acid, Protein A, Enzymes, Hemolysins and leukocidins Toxins and Superantigens but these virulence factors are not found in all strains of *Staphylococcus aureus* (Watkins *et al.*, 2012). The advent of penicillin in the 1940s immediately improved this prognosis, with over 94% of strains exhibiting susceptibility (Medeiros, 1997). This was short-lived. The use of penicillin quickly selected *Staphylococcus aureus* that were resistant as a result of β-lactamase expression (Barber and Rozwadowska-Dowzenko, 1948). Penicillin-resistant *S. aureus* emerged in hospitals around 1942, with ultimate proliferation in the community (Rammelkamp and Maxon, 1942; Kirby, 1944). By 1950, more than 50% of all staphylococcal isolates were resistant to penicillin.

The pattern of resistance – first hospitals and then the community – is the common pattern for each new antibacterial (Chambers, 2001; Lowy, 2003). Prior to the antibiotic era, the mortality of patients infected with pathogenic *Staphylococcus aureus* exceeded 80%, and over 70% developed metastatic infections (Skinner and Keefer, 1941).

The developed antibiotic resistance will be disseminated to the soil, water, animal and humans. This antibiotic resistance in microorganisms may interfere with the effective functioning of antibiotics, both in humans and animals by creating the microorganisms causing diseases to be unresponsive to antibiotics (Afewerki *et al.*, 2020; Kolawole and Shittu, 1997).

Staphylococcus aureus is a bacterium widely spread in the environment, such as air, dust and on the household items and has evolved resistance to all antibiotic classes used for its treatment through different mechanisms (Akindolire *et al.*, 2018; Cockfield *et al.*, 2007; Kiliç and Çirak, 2006).

The β-lactamase enzymes produced by *Staphylococcus aureus* confer resistance against β-lactam antibiotics like penicillin; and are encoded in the blaZ gene located on a transposable part of the large plasmid within the bacterial cells (Hugo and Russell, 1987; Bidya and Suman, 2014). Due to its location, the gene is easily movable to surrounding the cells through horizontal gene transfer.

The Staphylococci have two primary resistance mechanisms with respect to the β-lactam antibiotics. One is the expression of beta Lactamases enzymes which destroy β-lactamase by hydrolysis, and are expressed by activation of the blaZ gene. Higher level β-lactam resistance (MRSA) results from the acquisition of the mecA gene, which encodes the penicillin-binding protein 2a (PBP 2a). Strains of *Staphylococcus aureus* exhibiting either β-lactamase

or PBP 2a-directed resistance (or both) have established a considerable presence in the exotic world of human pathogens (Rosato *et al.,* 2003) β - lactam antibiotics, the major class of antibiotics used against *Staphylococcus aureus,* develop resistance and are most often due to a plasmid-encoded penicillinase/β-lactamase (Babic *et al.,* 2006; Quinn *et al.,* 2011). Staphylococcal beta lactamase is not chromosomal and is plasmid mediated and can be non-inducible or inducible with antibiotics (Maddux, 1991).

Materials and Methods

The study was entitled with the 76 isolates of *Staphylococcus aureus* which was carried out from various clinical samples collected from outpatient and inpatients attending in National Institute of Medical Sciences and Research hospital, Jaipur on our study period from march 2021 to march 2022. *Staphylococcus aureus* isolated from pus samples. All samples except pus sample and multiple samples from the same patient were excluded.

Culture characteristic were observed on Blood agar and MacConkey's agar, gram stating, biochemical tests like Catalase and coagulase test were performed. Antimicrobial susceptibility testing was performed on Muller-Hinton agar using Kirby-Bauer disc diffusion method.

The antibiotic discs were selected according to the protocol as recommended by Clinical laboratory Standards Institute (CLSI). Clover Leaf Method was performed on all penicillin resistant *Staphylococcus aureus* isolates to confirm the Beta lactamase production. This is an important confirmatory method to detect the production of beta-lactamase enzyme.

Results and Discussion

Our study was carried out in the time period of march 2021 to march 2022. 76 *Staphylococcus aureus* were isolated from pus samples, patients of all ages and both sexes attending various outpatients, inpatients at NIMS hospital.

Results analysed as per the clinical and laboratory findings from our hospital. Our statistical analysis carried out based on Demographic details of *Staphylococcus aureus* associated Samples analysed for *Staphylococcus aureus*.

As shown in table 1 and graph 1 out of total 76 isolates of *Staphylococcus aureus*, 8(10.52%) isolated from age 0-20 yrs, 21-40 yrs 24(31.57%), 41-60 yrs 26(34%) and above 60yrs 18(23%)

As shown in table 4 and graph 4, maximum sensitivity was shown i.c 100% sensitive to Vancomycin and Linezolid. Followed by Chloramphenicol (77%), Clindamycin (59%), Gentamicin (76%), Erythromycin (21%), Teicoplanin (88%), Tetracycline (83%), Minocycline (96%), Doxicycline (80%), and less sensitive were Ciprofloxacin (20%), Levofloxacin (20%) & Penicilin-G (08%) And the resistant pattern of *Staphylococcus aureus* isolate were Resistant isolates was higher among Penicilin-G (92%) followed by Ciprofloxacin (80%), Levofloxacin (80%), Erythromycin(79%), Gentamicin (24%), Clindamycin (41%), Chloramphenicol (23%) and less Resistant was shown to Teicoplanin (12%), Tetracycline (17%), Doxicycline (20%) and Minocycline (04%).

Table.1 Age wise distribution of *Staphylococcus aureus*

Age Group	Isolates(n=76)	Percentage (%)
0-20	8	**10.52%**
21-40	24	**31.57%**
41-60	26	**34.21%**
>60	18	**23.68%**

Table.2 Sex Wise distribution of *Staphylococcus aureus*.

Sex	No. of isolates (n=76)	Percentage (%)
Male	57	75%
Female	19	25%
Total	**76**	**100%**

Table.3 Distribution of Penicillin resistance in *Staphylococcus aureus*

Penicillin Resistance (R)	Penicillin Sensitive(S)
70(92%)	6(8%)

Table.4 Antibiotic sensitivity pattern of Staphylococcus isolates.

Antibiotic	Sensitive Isolates (S)	Resistance isolates (R)
Vancomycin	76	0
Linezolid	76	0
Penicilin g	6	70
Erythromycin	16	60
Clindamycin	45	31
Chloramphenicol	58	18
Gentamicin	58	18
Teicoplanin	67	09
Minocycline	73	03
Tetracycline	63	13
Doxycycline	61	15
Ciprofloxacin	15	61
Levofloxacin	15	61

Table.5 Detection of Beta lactamase production by clover leaf method

Clover Leaf Method	Positive	Negative
	70	**6**

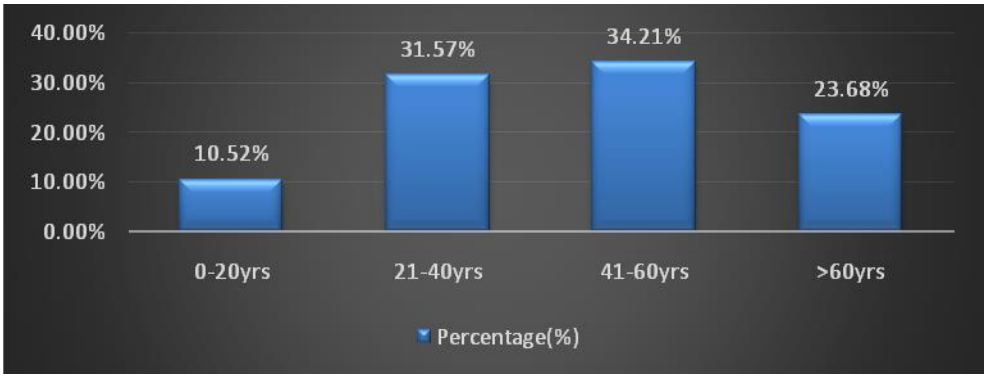
Table.6 Area wise distribution of *Staphylococcus aureus*

OPD/IPD	Total Samples	Penicillin	
		Sensitive	Resistance
Surgery	35	2	33
Orthopedics	18	1	17
Medicine	17	3	14
MICU	3	-	3
Pediatric	2	-	2
ENT	1	-	1
Total	76	6	70

Table.7 Showing prevalence of Penicillin Resistance *Staphylococcus aureus* as per previous studies

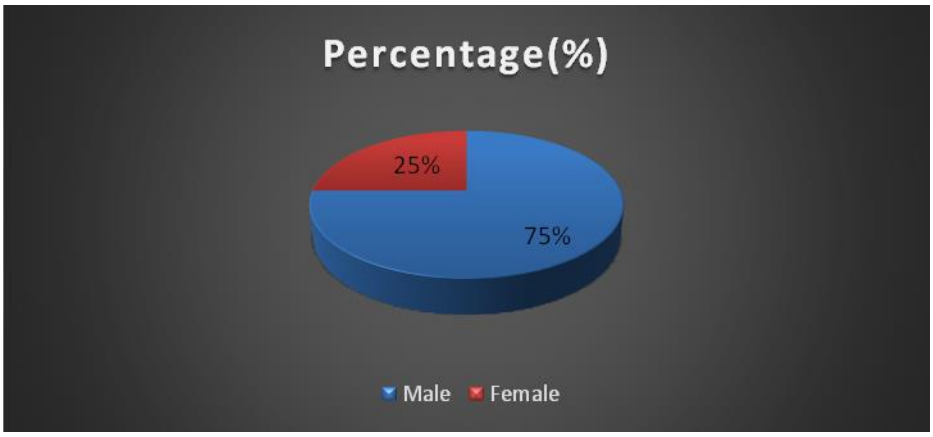
S.No	Author	Percentage
1.	Kawsar NM *et al.*, (2008)	85.7%
2.	A.A. Akindele *et al.*, (2010)	80%
3.	Ihsan Edan Abdulkareem AlSaimary *et al.*, (2012)	68.9%
4.	Lito E. Papanicolas *et al.*, (2014)	64%
5.	Mr. Ravi Vashistha *et al.*, (2018)	59.04%
6.	Arivalagan Pugazhendhi *et al.*, (2020)	100%
7	**Present study**	**92%**

Graph.1 Age wise distribution of *Staphylococcus aureus*.



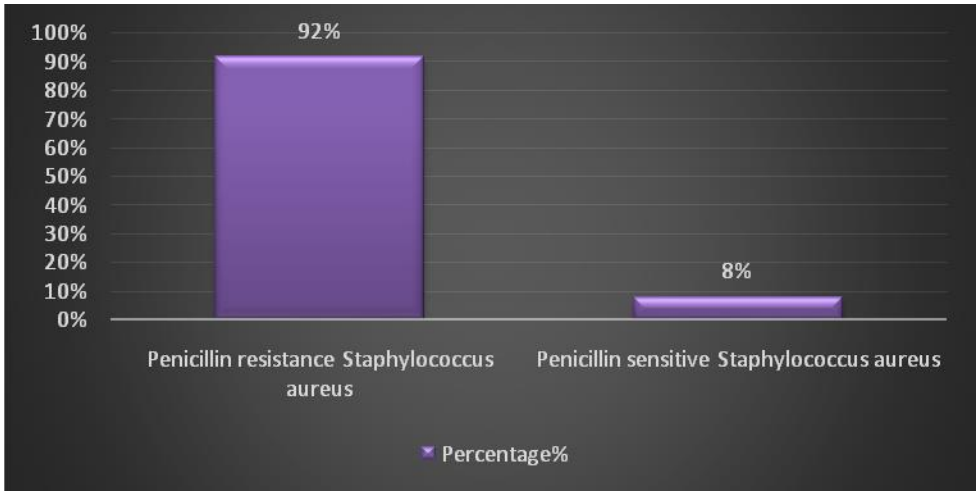
Out of total 76 isolates of *Staphylococcus aureus*, 57(75%) isolates from male while 19(25%) from female patients as shown in table 2 and graph 2.

Graph.2 Sex Wise distribution of *Staphylococcus aureus*.

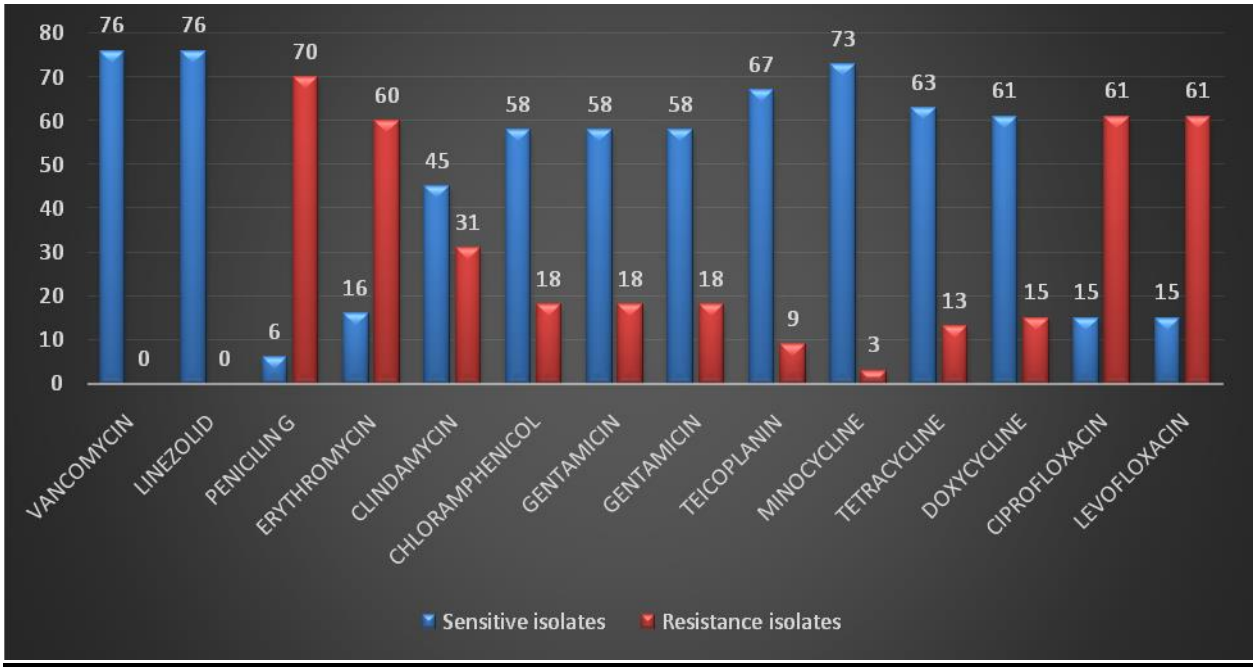


As shown in table 3 and graph 3 Penicillin resistance was detected in 92% (70) of isolates while 8% (6) were sensitive

Graph.3 Distribution of Penicillin resistance in *Staphylococcus aureus*



Graph.4 Antibiotic sensitivity pattern of Staphylococcus isolates



Out of 76 isolates 92% were found to be the producers of Beta lactamase enzyme which are resistant to penicillin and 08% didn't show cloverleaf pattern which are sensitive to penicillin as shown in table 5.

S. aureus among various Surgery units were the most prevalent 46% (35) followed by orthpopedics and medicine unit 24% (18) & 22% (17), MICU was 4% (3), Pediatric department 3% (2) and ENT 2% (1). 70 isolates of *S. aureus* in percentage 92 were isolated as β-lactamase producers, while 6 isolates (8%) were β-lactamase non-producers as shown in table 6.

Staphylococcus aureus continues to be a health problem for both community-acquired and hospital-associated infections. *Staphylococcus aureus* resistant to Penicillin was reported soon after its introduction in 1942 in hospitals, with ultimate proliferation in the community. By 1950, more than 50% of all staphylococcal isolates were resistant to penicillin. Penicillin resistant *Staphylococcus aureus*

is now endemic in India, so it is necessary to assess the prevalence of penicillin resistant *Staphylococcus aureus*.

Different mechanisms of drug resistance in bacterial pathogens are the major hurdle in their treatment. With emerging resistance, it became a serious concern to look into drug resistance mechanism, which can help us to prescribe a specific medication to effectively overcome the problem of resistance.

In this study we found that mostly *StaphylococcuS. aureus* isolates were resistant to penicillin and identified as beta-lactamase production which was confirmed by Clover Leaf Method. The percentage of beta-lactamase producing *Staphylococcus aureus* isolates is greater than other earlier reports (Kesah, 1994-1996; Akindele *et al.,* 2010). Beta-lactamases are a family of enzymes produced by many bacteria that inactivates beta-lactam antibiotics by opening the beta-lactam ring (Khan *et al.,* 2018). Beta-lactamases are enzymes that are responsible for many failures of antimicrobial therapy by the hydrolysis of beta lactam ring of these antibiotics (Bush *et al.,* 2015). The spread of beta-lactamase genes had been enhanced by their integration within mobile genetic elements such as plasmids and transposon which facilitate the rapid transfer of genetic materials between microbes (Wilke *et al.,* 2005)

Staphylococcus aureus is one of the most widely spread and virulent nosocomial pathogen and is usually resistant to multiple antibiotics making infections difficult to treat. Infections caused by *Staphylococcus aureus* pose serious threat in Health Care settings. In present study total of 92% (70) Penicillinase production was detected in *Staphylococcus aureus* from pus samples from various departments Surgery (94%), Orthopedics (82%), Medicine (94%), Micu (100%), Pediatric (100%) and ENT (100%) were found as producers of penicillinase by using penicillin (10u) disc diffusion technique which was confirmed by cloverleaf method.

The rate of infection of *Staphylococcus aureus* was higher among Male than Female. The patients were found to be frequently associated with *Staphylococcus aureus* with an infection rate of 75% and 25% respectively. The phenomenon of drug resistance was first observed when beta lactam antibiotics became ineffective after in discriminative uses and plasmid responsive beta lactamase (penicillinase) synthesis.

Production of Beta Lactamase was detected by using penicillin disc 10 µg which was confirmed by Clover Leaf method. Total of 76 *Staphylococcus aureus* isolates were processed and subjected for Beta lactamase detection. The majority of *Staphylococcus aureus* strains were the producers of beta lactamase i.e. 92% and Non producer strains of beta lactamase were 08%.

Finally conclude that continuous monitoring of antibiotic susceptibility pattern of *S. aureus* and use of a right and proper antimicrobial drug was helpful for minimizing the resistance to beta lactamase and other group of antibiotics. Judicial use of antibiotics may significantly decrease the further development of resistance.

The present study observations suggest that antibiotic susceptibility testing of *Staphylococcus aureus* must be done along with penicillin disc which will definitely help in distinguish between penicillin susceptible and penicillin resistant strains there by minimize the risk of treatment failure of patients who are on beta lactamase antibiotics therapy. The incidence of penicillin resistance varies from region to region and the population studied, hence determination of accurate statistics is of immense importance for therapy. It is important for laboratories to be aware of the local prevalence of beta lactamase producer isolates. On the basis of their data they can perform the penicillin disc test routinely. The D-test is an easy which differentiates sensitive and resistant for penicillin and reliable for detection of beta lactamase producing and non producing strains in a clinical laboratory setting without specialized testing facilities.

The prescription of penicillin depends upon antimicrobial susceptibility results, whether the patient is admitted in the hospital or is an outpatient and clinician's own experience. The pattern of penicillin resistance in Staphylococci varies in different regions. Depending upon this the prescription rate will not be uniform in different regions.

Expression of resistance to penicillin could limit the effectiveness of beta lactam antibiotics. In such cases, other antibiotics are considered for therapy.

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