

## Detection Of Beta Lactamase And Antibiotic Susceptibility Pattern Of Staphylococci Isolated From Various Clinical Samples In Tertiary Care Hospital, Jamnagar, Gujarat.



### Medical Science

**KEYWORDS :** Staphylococci, Beta lactamase, antimicrobial resistance.

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### ABSTRACT

Since the discovery of penicillin, beta-lactam antibiotics are among the most widely prescribed antibiotics. Resistance to beta-lactams is a common problem now a days and most common mechanism being the production of betalactamase enzyme. It was first detected in staphylococci in 1944. It has narrow spectrum of activity aimed mainly at penicillin, so also known as penicillinase. In this study various clinical samples are plated on nutrient and blood agar plates. Staphylococci were identified on basis of gram positive cocci that gives positive catalase test and further identification in to *S.aureus* and *CONS* is done with coagulase test. Production of betalactamase enzyme in these isolates was detected by cefinase disc (chromogenic cephalosporin method). Out of total 150 staphylococcal isolates, 114 (76%) showed the production of betalactamase enzyme. These isolates were subjected to antimicrobial susceptibility testing with various drugs including cefoxitin. Antibiogram results showed that betalactamase positive isolates were more resistant to various groups of antimicrobials than betalactamase negative isolates.

### 1) INTRODUCTION

Staphylococci are ubiquitous and amongst them *S.aureus* is the most important human pathogen. It is a leading cause of nosocomial infection and death in hospitals. Since the discovery of penicillin, betalactam antibiotics are important component of empirical therapy and are among the most widely prescribed antibiotics<sup>5</sup>. Penicillin was the drug of choice for staphylococcal infections, but soon resistant strains began to emerge. These resistant strains were capable of producing penicillinase (betalactamase) enzyme, which opens the beta-lactam ring and inactivate the drug. It is the most common mechanism of resistance to betalactam drugs<sup>6</sup>. Betalactamase of gram positive bacteria are extracellular, while that of gram negative bacteria are intracellular in periplasmic space. Genes that encodes betalactamase can be located on bacterial chromosomes, plasmid or transposable elements<sup>6</sup>. Betalactamase gene in staphylococci is typically located on plasmid. Staphylococcal betalactamase are molecular class A (according to Ambler classification), placed in Bush's group 2a (according to Bush-jacoby-medeiros functional classification). Betalactamase produced by staphylococci have been stable over several decades and have activity only against penicillin while betalactamase produced by gram negative bacilli have broaden their spectrum of activity against many of newly developed betalactam antibiotics and known as Extended Spectrum betalactamase (ESBLs)<sup>6</sup>. Resistant strains of staphylococci are increasingly being associated with hospital infections. Failure to identify these strains leads to serious therapeutic failure.

### MATERIAL AND METHOD

This study was conducted in the department of Microbiology, Gurugobindsingh hospital, Jamnagar, during June 2009 to December 2009. Various clinical samples received in the department were tested to isolate staphylococci.

### Specimen collection, culturing and identification :

Various clinical samples like urine, pus, blood, sputum, throat swab, vaginal swab, endotrectheal secretions were taken with aseptic precautions. Samples were inoculated on nutrient agar and blood agar. Nutrient agar is incubated aerobically, while Blood agar is incubated under 5-10% CO<sub>2</sub> in candle jar at 37°C for 24 hrs. Next day, nutrient agar shows large (2-4mm), circular, convex, smooth and easily emulsifiable colonies. Most strains of *S.aureus* produce golden yellow pigments while some coagulase negative strains produce

white or lemon yellow pigments. On blood agar most strain are hemolytic though some may be nonhemolytic. Gram stain is performed from colonies. Staphylococci appear as gram positive cocci in clusters. Further biochemical tests shows that staphylococci are nonmotile and gives positive catalase reaction. Coagulase positive isolates are taken as *S.aureus* and negative isolates as *CONS* (coagulase negative staphylococci).

### Antibiotic susceptibility testing

Antimicrobial susceptibility of these isolates was determined by KIRBY-BAUER disc diffusion method. Following antimicrobial agents were used (table-1). Result of antibiogram study was noted according to standard zone of inhibition.

**Table-1**

Antimicrobial	Disc content
Amikacin	30 µg
Gentamycin	10 µg
Roxithromycin	15 µg
Erythromycin	15 µg
Azithromycin	15 µg
Clarithromycin	15 µg
Cefotaxime	30 µg
Cefuroxime	30 µg
Cefoperazone	75 µg
Cefadroxil	30 µg
Cefaclor	30 µg
Cotrimoxazole	30 µg
Penicillin	10 units
Ampicillin	10 µg
Augmentin	20 µg
Ciprofloxacin	5 µg
Cefoxitin	1 µg

### Detection of betalactamase enzyme

Manifestation of this enzyme as a resistant mechanism is sometimes difficult to demonstrate in vitro. Enzyme must be induced by exposure to oxacillin before testing<sup>2</sup>. Cefinase disc (paper disc

impregnated with chromogenic cephalosporine, nitrocefine) was used to detect betalactamase enzyme, as it is very rapid and most convenient method<sup>7</sup>. Moistened the disc. Bacterial growth was taken from the periphery of the zone of inhibition around the oxacillin disc by sterile loop or applicator stick and smeared on to a cefinase disc surface. Development of red color indicates positive reaction (as shown in figure below) i.e. production of betalactamase by test strain.



(negative test) (positive test)

**RESULT AND ANALYSIS**

Out of total 150 isolates of staphylococci, 114 (76%) showed the production of betalactamase enzyme and out of these 114, 64 (56.14%) were S.aureus and 50 (43.86%) were CONS. A positive betalactamase test means that the organism is resistant to penicillin, ampicillin, amoxicillin, carbenicillin, mezlocillin and piperacillin, but a negative reaction does not always mean that the organism is susceptible to above mentioned agents because bacteria have multiple other mechanisms of resistance<sup>2</sup>. Maximum betalactamase positive isolates were from blood (45.61%) and pus (42.11%). Maximum no. of positive isolates were from paediatrics (38.60%) and surgery (36.84%) ward. These isolates were subjected to susceptibility testing with cefoxitin (better indicator of methicillin resistance than oxacillin)<sup>1</sup>. Out of 114 betalactamase positive strains, 83 (72.81%) were methicillin resistant while out of 36 betalactamase negative isolates only 12 (33.33%) were methicillin resistant. Comparison of antimicrobial susceptibility in betalactamase positive and negative isolates is shown in table-2.

**Table-2**

Antibiotics	% of sensitive strains in $\beta$ -lactamase positive isolates	% of sensitive strains in $\beta$ -lactamase negative isolates
Amikacin	37.14%	73.33%
Ciprofloxacin	14.29%	46.66%
Roxithromycin	22.86%	60.00%
Gentamycin	17.14%	53.33%
Clarithromycin	28.95%	72.22%
Cefotaxime	22.81%	66.67%
Cefuroxime	31.58%	69.44%
Cefoperazone	53.50%	80.55%
Cefadroxil	19.30%	66.67%
Cefaclor	9.09%	61.90%
Cotrimoxazole	20.45%	61.90%

Erythromycin	18.18%	66.67%
Penicillin	00%	52.38%
Ampicillin	4.54%	61.90%
Azythromycin	36.84%	72.22%
Augmentin	18.00%	66.67%
Cefoxitin*	27.19%	66.67%

\*tested for detection of methicillin resistance.

As shown in table-2 susceptibility to various antimicrobials is less in betalactamase positive isolates whereas it increases by 40-50% in betalactamase negative isolates. Methicillin resistance is much higher in betalactamase positive isolates than in negative isolates. Out of total 150 isolates of staphylococci, 27 (18%) were resistant to all above mentioned drug. Out of these 27 MDR isolates, 26 (96.3%) were from betalactamase positive isolates while only 1 (3.7%) is from betalactamase negative isolates. So, multidrug resistance is more common in betalactamase positive isolates.

**DISCUSSION**

The penicillinase was first reported in staphylococci in 1944, and by 1950, 80% of S.aureus showed resistance to benzyl penicillin<sup>8</sup>. The present study shows that 76% staphylococci produces betalactamase enzyme, which was comparable with the study of George et al (85%)<sup>4</sup>, Shible et al (75%)<sup>9</sup>, Ekrem methem (83.9%)<sup>3</sup>. In the present study betalactamase production is more common in s.aureus as compared to CONS. Maximum no. of positive isolates were from pus and blood samples and from pediatric and surgery ward. Methicillin resistance is also more common in betalactamase positive isolates (72.81%) than in negative (33.33%). Aminoglycosides, cephalosporines and macrolid group of antibiotics are more commonly used for treating staphylococcal infections. In our study betalactamase positive isolates are more susceptible to cefoperazone (53.50%), amikacin (37.14%), and azithromycin (36.84%) as compared to other drugs of similar group. The susceptibility pattern to these drugs is similar but percentage of sensitive strains are drastically increase in betalactamase negative isolates i.e. strains susceptible to cefoperazone is 80.55%, to amikacin is 73.33% and to azithromycin is 72.22%. Multidrug resistance is also more common in betalactamase positive isolates (96.3%) as compared to betalactamase negative (3.7%) isolates.

**CONCLUSION**

Each laboratory must adopt tests to detect resistant strains. A positive betalactamase test means that the organism is resistant to penicillin, ampicillin, amoxicillin, carbenicillin, mezlocillin and piperacillin, but a negative reaction does not always mean that the organism is susceptible to above mentioned agents because bacteria have multiple other mechanisms of resistance<sup>2</sup>. Betalactamase (penicillinase) produced by staphylococci have been stable over several decades and have narrow spectrum of activity aimed mainly at penicillin, so betalactamase detection tests determines whether narrow spectrum betalactam agents (e.g. penicillin, ampicillin) can be used to treat staphylococcal infections or not. Clavulanic acid inactivates staphylococcal betalactamase and a combination of amoxicillin plus clavulanic acid (co-amoxycylav) has a place in therapy. Penicillinase Resistant Penicillins (PRPs) like oxacillin, methicillin are also used to treat infections caused by betalactamase producing staphylococci. Resistance to other group of antibiotics is also much more common in betalactamase positive isolates, so formulations of appropriate hospital antibiotic policy and taking adequate precautionary measures to prevent spread of resistant strains in hospital environment is must for every hospital.

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