Study of β lactamase activity of *staphylococcus aureus* isolated from healthy nasal carriers and Hospital isolates

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ABSTRACT

Staphylococcus aureus (n=84) isolated from the nostrils of a healthy population from Kathmandu and from the infectious cases (n=100) from Tribhuvan University Teaching Hospital, Kathmandu, Nepal were tested from May 1996 to March 1997 in Central Department of Microbiology, Tribhuvan University, Kathmandu, Nepal by microbiological and chemical methods to find out their β Lactamase activity. Among the healthy population, in domiciliary conditions 21.4% of the isolates were found β Lactamase producers. The occurrence of β Lactamase producing *S. aureus* was greater among female (27.0%) than among male (17.0%), however it was not significant (X^2 = 1.2309, P>0.05). The occurrence of the same was observed high among 40 and above age groups (66.7%) and 0-9 age group (60.0%), however no association with any particular age group was observed (X^2 = 16.8674, P>0.05). The β lactamase activity of *S. aureus* hospital inpatients isolates was 75.0% showing high occurrence of β Lactamase activity in hospital isolates compared to *S. aureus* isolates from healthy carriers (X^2 = 52.4113, P<0.001). No association of β lactamase positive hospital isolates with gender (X^2 = 0.2158, P>0.05) and age group (X^2 = 1.5522, P>0.05) was observed. This study shows that the prevalence of β Lactamase positive *S. aureus* was greater in hospital cases than in nasal carriers in domiciliary condition indicating the requisition of further study in this field.

Keywords: *Staphylococcus aureus*, healthy, β Lactamase, penicillinase, hospital isolates.

INTRODUCTION

β Lactamases (penicillinase and cephalosporinase) are inducible enzymes produced by gram-negative and gram-positive microorganisms, usually plasmid-coded, which breaks open the β lactam ring of β lactam antibiotics rendering them inactive. Plasmid-borne β Lactamases are the most common cause of antibiotics resistance.[1] These enzymes have been reported by Yasushisa in 1994 in hospital strains of *S. aureus* (62.3%), *Escherichia coli* (84.7%), *Klebsiella pneumoniae* (65.4%), *Proteus, Pseudomonas* and a minority of *Haemophilus influenzae* (22.4%) and *Neisseria gonorrhoeae*.[2]

Before the introduction of benzylpenicillin Staphylococcus strains were seldom resistant to this antibiotics. From 1945 onwards penicillin-destroying enzymes were encountered with increasing frequency: first, in strains isolated from hospitals; and then in those isolated from the general population.[3] Skov *et al* in 1995 studied β Lactamase production and its genetic location in *S. aureus* in 184 penicillin-resistant phage group II from bacterimia cases.[4] Until 1977, all strains had a chromosomally located β Lactamase gene but in 1990, 84% of the group II strains contained plasmid, which produced more β Lactamase than strains without plasmid. The clinically important penicillin-resistance in *S. aureus* is almost always caused by plasmid, which has been reported to range from $12x10^6$ to $21x10^6$ in size.[5] Other resistance was linked along with penicillin-resistance on the same plasmid. The plasmid transfer normally occurs by transduction. However, plasmid transfer by transformation has also been reported.

In this study, the detection of β Lactamase producing *S. aureus* was aimed at testing the penicillinase activity on *S. aureus* isolated from hospital samples and those isolated from the nostrils of healthy domiciliary population from Kathmandu for a comparative study.

MATERIALS AND METHODS

A total of 100 isolates of *S. aureus* (48 from male and 52 from female) was collected during May 1966 to march 1997 from Tribhuvan University Teaching Hospital (TUTH), Kathmandu, Nepal from different sources like pus (84.0%), urine (12.0%), high vaginal swab (2.0%), prosthetic fluid (1.0%) and sputum (1.0%). For calculation the later three were categorized as "others". The inpatients at TUTH from which *S. aureus* was isolated were not treated with any antibiotics prior to the sample collection for isolation, identification and antibiotics sensitivity test. At the same time, nasal swabs were collected randomly from 189 male and 161 female from healthy domociliary Kathmandu dwellers having no infections. Consent for the test was taken and confidentiality of the result was assured to the subjects. The specimens were processed by following the standard methodology in Central Department of Microbiology, Tribhuvan University, Kathmandu, Nepal, to isolate and identify the nasal commensal *S. aureus* from the nostrils. Identification of all hospital isolates was confirmed by biochemical tests. All media used were manufactured by Hi-Media, India and all chemicals were manufactured by Glaxo, India and augmentin antibiotics disc was manufactured by Smithcline Beecham, Germany. After identification of the isolate as *S. aureus* by performing different biochemical tests like catalase, oxidation/fermentation, DNase, coagulase, mannitol fermentation, and growth in presence of 7.5% sodium chloride, they were tested for their β lactamase

activity especially penicillinase. β Lactamase test was done microbiologically by clover leaf method; and biochemically by acidimetry (filter paper strip and microtitre plate method) and iodometry (microtitre plate), the methods mentioned by Plested, Simpson and James in 1983.[6]

The β Lactamase-positive *S. aureus* isolates were further confirmed by performing antibiotic sensitivity test by Stoke's method. The central part of Mueller Hinton Agar was swabbed with the control strain *S. aureus* ATCC 25923 and upper and lower part with two different test microorganisms. Augmentin disc (20μg amoxycillin and 10 μg clavulanate) and penicillin G (10 units) discs were placed at 2-3 mm un-inoculated gap between the control and the test microorganisms in upper and lower part. The plate was left at room temperature for pre-diffusion for 30 minutes and incubated at 37^0 C for 24 hours. The radius of the zone of inhibition produced by the control and the test microorganisms were measured and the test microorganisms were categorized as sensitive, intermediate or resistant. Any microorganisms producing zone radius not more than 5 mm smaller than control was regarded as sensitive, the zone radius greater than or equal to 3 mm but not large enough to fall into sensitive group was regarded as intermediate sensitive and those producing zone radius 2 mm or less were regarded as resistant. Penicillin resistant and augmentin sensitive strains were regarded as β Lactamase positive.

The Chi Square Test was used on results obtained.

RESULTS

Out of 24.0% (84) isolates of nasal *S. aureus* from 350 samples from healthy male 24.9% (47/189) and 22.9% (37/161) healthy female 21.4% (18) were found β lactamase positive by clover leaf, acidimetric (filter paper and microtitre plate), and iodometric method. Antibiotics sensitivity test by Stoke's method using augmentin and penicillin disc also correlated with these tests. Among 47 and 37 isolates from healthy male and female 17.0% (8) and 27.0% (10) respectively were found β Lactamase producers. Though the occurrence of β Lactamase producing *S. aureus* was high in female subjects than in male, no association between gender and occurrence of β Lactamase producing nasal *S. aureus* was observed. (X^2 =1.2309, P>0.05). No significant association was observed between occurrence of β Lactamase producing *S. aureus* and age group (X^2 = 16.6874, P>0.05), although a greater occurrence was observed in 40 and above and 0-9 age groups (Table-1).

Among 100 hospital isolates, 75.0% (75) was found β Lactamase positive, which was very high compared to the isolates from healthy population in domiciliary condition 21.4% (18/84)(Table 2). Production of β Lactamase enzyme was found significantly associated with the hospital isolates (X^2 = 52.4113, P<0.001). Among the 52 isolates from female [pus 75.0% (39), urine 10 and 3 others] 73.0% (38) were β Lactamase positive and 87.2% (34/39) of β Lactamase positive was from pus alone. Similarly, from 48 *S. aureus* isolated from male [pus 93.7% (45) urine 2 and 1 other] 77.0% (37/48) was β Lactamase positive and 80.0% (36/45) of β Lactamase positive was from pus. The high occurrence of β Lactamase positive *S. aureus* among male in totality and among in female in pus was not significant (X^2 = 0.2158, P>0.05) and (X^2 = 0.7753, P>0.05) respectively. No association of age group with the β Lactamase positivity was observed (X^2 = 1.5522, P>0.05).

DISCUSSION

There is no published study of this nature for identification of β Lactamase producing *S. aureus* in domiciliary and hospital isolates in Nepal. Since, *S. aureus* in this study was positive for β Lactamase by microbiological (clover leaf), acidimetric and iodometric methods, it can be concluded that the enzymes produced were β Lactamase not acylase, which gives positive result in microbiological and acidimetric methods and negative result in iodometric method.[6] Twenty one percent of the isolates was β Lactamase producers in domiciliary condition. In contrary, 75.0% of the hospital isolates was β Lactamase producer, indicating the intensity of the problem in hospital cases, requiring alertness in treatment with multiple therapeutic interventions. The frequent combination for the treatment is the β Lactamase labile penicillin with specific β Lactamase inhibitor.[7] β Lactam antibiotics and β Lactamase inhibitor combination has been proven as one of the most successful antibiotic regimens for the treatment of common infections caused by β Lactamase producing gram negative bacteria. In this study, higher occurrence of β Lactamase positive strain in hospitals compared to domiciliary conditions was probably due to the exposure of microorganisms to the antibiotics in hospital settings. Easmon had reported that after 1945, the strains of *S. aureus* carried in hospitals were often more resistant to antibiotics than those carried by the members of the non-hospital isolates.[8] He has also stated that the frequency of penicillinase production by *Staphylococcus* strain in general population rose very much more slowly and did not reach 50.0% until 1960, which later approached to more than 80 %. β Lactamases are important weapons of β Lactam resistance *S. aureus*, where they are frequently found together with acquired low affinity penicillin binding protein PBP2a.[7]

Similar occurrences of β Lactamase activity in hospital isolates have been reported by different researchers. In a study carried out by Turner and Nord in 2004, *S. aureus* was isolated from 42.0% of the cases of recurrent tonsilitis and 98.0% of them were β Lactamase producers, where Clindamycin was used to eradicate them.[9] In a similar study carried out in Nigeria by Kolawole and Shittu in 1995, 100.0% of the hospital isolated *S. aureus* was reported as β Lactamase producer.[10]

Clavulanic acid is class A serine β Lactamase inhibitor, the suicide inactivator has mechanism of inhibition of β Lactamase similar to that of semi-synthetic inhibitors (sulbactam and tazobactam). All these are used successfully in combination with penicillin. Example: Clavulanic acid–amoxycillin, sulbactam–ampicillin, tazobactam–piperacillin. These combinations inhibit growth of bacteria producing β Lactamase such as the common TEM, SHV and OXA enzymes. These are often plasmid coded β Lactamase in gram-negative bacteria.[7] When such combinations were first introduced, multiple β Lactamase production was rare even in organisms with plasmid encoded β Lactamase genes.

Typically, extended spectrum β Lactamase (ESBL) are mutant plasmid mediated β Lactamase derived from older broad spectrum β Lactamase which has extended substrate profile.[11] ESBLs have extended substrate profile permitting hydrolysis of penicillin, cephalosporin and aztreonam. *E .coli* and *Klebsiella* are the common producers of ESBL. ESBL enzymes mediate resistance to extended spectrum (third generation) cephalosporins (eg: ceftizidine, cefotaxime and ceftriaxone) and monobactams but do not affect cephamycin (cefoxitin and cefototan) or (meropenam and imipenam) the carbepenams.[12] ESBL and plasmid mediated AmpC β Lactamases are associated with multi-drug resistance. Plasmid mediated AmpC β Lactamase has arisen through the transfer of chromosomal genes for the inducible AmpC β Lactamase onto plasmids. This also occurs in many gram negative bacteria.[11]

Multi-drug resistant plasmids are now being freely disseminated among the enterobacteriaceae resulting in accumulation of R factors. Today the plasmid have become much larger with acquisition of multiple drug determinant resulting in multi-drug resistance in gram negative bacteria with as many as five different β Lactamases inducing both serine and metalbo β Lactamases with multiple functional group.

β Lactamase only in gram-positive microorganism *S. aureus* was studied In present study. As the spread of ESBLs producing microorganisms has been in increase, the same may be the scenario in Nepal too, manifesting the need of further research in this field.

In present study, β Lactamases production by *S. aureus* was studied by microbiological method and biochemical methods (acidimetric and iodometric). The β Lactamases activity of *S. aureus* isolated from healthy individuals was quite low compared to those isolated from hospital cases. There was so association of carriage of β Lactamases producing *S aureus* with gender and age in domiciliary condition. Similarly, in hospital isolates no association of gender and age was observed with occurrence of β Lactamases positive *S. aureus.*

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Table-1: β Lactamase positive nasal *S. aureus* in different age groups and gender.

	Male (number)		Female (Number)		Total (Number)		
Age group	*S. aureus*	β lactam pos	*S. aureus*	β lactam pos	*S. aureus*	β lactam pos	%
0-9 yrs	3	1	2	2	5	3	60.0 %
10-19	29	3	22	1	51	4	7.8 %
20-29	9	2	11	5	20	7	35.0 %
30-39	4	1	1	1	5	2	40.0 %
40 & up	2	1	1	1	3	2	66.7 %
	47	8	37	10	84	18	21.4%

Table-2: β Lactamase positive hospital isolate *S. aureus* in different age groups and gender.

	Male (number)		Female (Number)		Total (Number)		
Age group	*S. aureus*	β lactam pos	*S. aureus*	β lactam pos	*S. aureus*	β lactam pos	%
0-9 yrs	2	1	0	0	2	1	50.0 %
10-19	10	7	10	7	20	14	70.0 %
20-29	19	15	28	22	47	37	78.7 %
30-39	10	8	4	3	14	11	78.6 %
40 & up	7	6	10	6	17	12	70.6 %
	48	37	52	38	100	75	75.0%