■ ***Original Article***

Antimicrobial susceptibility patterns of clinical isolates of *Staphylococcus aureus* in Eastern Nepal

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Abstract

Background: *Staphylococcus aureus*, versatile pathogen causes many serious and life threatening infections. Resistant *S aureus* has become a serious matter of concern. The antimicrobial susceptibility profile of local isolates is essential for the selection of appropriate therapy for the management of staphylococcal infections. **Objective:** To find out the current status of antimicrobial resistance among the clinical isolates in our set up. **Method:** *S aureus* isolated from the clinical specimens submitted to the microbiology unit of clinical laboratory services, BP Koirala Institute of Health Sciences (BPKIHS) hospital were studied. Isolation and identification of *S aureus* was done by standard microbiological technique. **Results:** A total 300 *S aureus* isolates were obtained from various clinical specimens. *S aureus* showed susceptible to chlorampenicol (95%), tetracycline (94.3%), cefotaxime (93.3%), erythromycin (89%), ciprofloxacin (88.35%) and gentamicin (78%).Sixty four percent of isolates were found to be resistant to co-trimoxazole and 26% were methicillin resistant (MRSA). Nearly half (52.66%) of *S aureus* showed resistance to penicillin. All the isolates were susceptible to vancomycin in disc diffusion method. It was isolated frequently from pus 223(74%) followed by blood (14%). Forty eight percent of isolates were from abscess, followed by sepsis (17%). **Conclusion:** Resistant *S aureus* is a common pathogen causing a wide spectrum of infections in our set up. Existence of MRSA among local isolates is a serious matter of concern. Although no isolate exhibited resistance to vancomycin, screening test and MIC determination are recommended in monitoring the response to therapy and for early detection of impeding resistance among local strains.

Keywords: staphylococcus aureus, antimicrobial susceptibility, BPKIHS

Introduction

Staphylococcus is commensal of human skin, nose and may be found in throat. It can cause a range of illness from minor skin infections and abscesses to life threatening diseases such as pneumonia, meningitis, endocarditis and septicemia. Antimicrobial- resistant *S aureus* strains are spreading among the patients, wards units and even hospitals, causing epidemic diseases. Increasing spread of polyresistant strains of *S aureus* is a problem of global extent. The emergence of methicillin- resistant *S aureus* (MRSA) with multi resistant has posed challenges in the treatments. The antimicrobial susceptibility profile of local isolates is essential for the selection of appropriate therapy for the management of staphylococcal infections. The present study was carried out to find out the current status of antimicrobial resistance among the clinical isolates in our set up.

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Methods

A total of 300 of *S aureus*, isolated during a period of one year from May 2007 to April 2008 from various clinical specimens comprising of urine, blood, pus, aspirates, wound swab, high vaginal swab, catheter tip, cerebrospinal fluid, peritoneal fluid, sputum, tissues and corneal scrapings coming to CLS for culture and sensitivity were studied. Gram positive cocci in clusters were identified on the basis of gram stain and colony morphology. Suspected smooth, densely opaque

colonies sometimes surrounded by a narrow zone of hemolysis with yellow pigment on blood agar plate, corresponding minute pink, lactose fermenting colonies on MacConkey, minute yellow lactose fermenting colony on CLED agar plate were further processed. Identification of *Staphylococcus aureus* was done on colony morphology, gram stain, catalase test and coagulase test.[1] Antimicrobial susceptibility testing was performed on Mueller Hinton agar (MHA) plates and interpreted according to CLSI guidelines against the following antibiotics.[2]

- Chloramphenicol 30mg
- Cotrimoxazole 23.75mg
- Ciprofloxacin 5mg
- Cefotaxime 30mg
- Erythromycin 15µg
- Gentamicin 10µg
- Nitrofurantoin 300µg
- Oxacillin 1µg
- PenicillinG 10units
- Tetracycline 30µg
- Vancomycin 30µg

Methicillin resistance was confirmed by agar screen test using Mueller-Hinton agar plate supplemented with 4% NaCl and oxacillin (6ìgm/ml) incubated at 35^{0}C for full 24 hour.[3] *S aureus* ATCC 25923 was used as control and tested daily along with the test strains.

Results

Twenty three percentage of isolates were obtained from the admitted patients from different wards and units where as 77% were from outdoor patients and gender distribution given in Fig1. Major groups were from age >20 years demonstrated in Fig 2. Majority were obtained from surgery department details are mentioned in Table 1.

It was isolated frequently from pus 223(74%) followed by blood (14%) the growth of *S aureus* were of various types descried in Fig 3. Sixty four percent of isolates

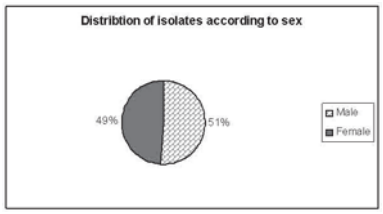


Figure 1: Distribution of isolates according to sex.

Table 1: Distribution of cases of inpatients and outpatients from various departments

Wards	Inpatient (n=231)	Outpatient (n= 69)	Total (n=300)
Surgery	112(48.48%)	24 (34.78%)	136(45.33%)
Orthopedics	31 (13.41%)	4 (5.79%)	35(11.66%)
Emergency medicine	7		7
Medicine	15 (6.49%)	3(4.34%)	18(6%)
Gynecology a) ANW b) PNW	19 (8.22%) (12) (7)	4 (5.72%)	23(7.66%)
Pediatrics a) Wards b) Nursery(NICU) c) PICU	22(9.52%) (14) (7) (1)	10 (14.49%)	32(10.66%)
ENT	23 (9.95%)	3 (4.34%)	26(8.66%)
ICU	7 (3.03%)	0	7
GOPD		13 (18.84%)	13
Dermatology	1	1	2
Ophthalmology	1	0	1
Total	231	69	300

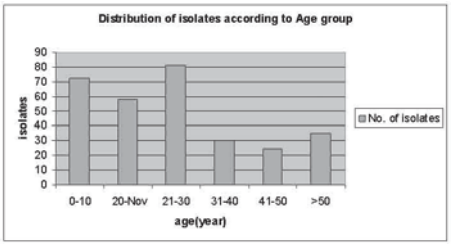


Figure 2: Distribution of Isolates according to age group

were found to be resistant to co-trimoxazole and 26% were methicillin resistant (MRSA). Nearly half (52.66%) of *S aureus* showed resistance to penicillin. The details of antimicrobial susceptibility patterns of *S aureus* demonstrated in Fig 4. All the isolates were susceptible to vancomycin in disc diffusion method. Forty eight percent of isolates were from abscess, followed by sepsis (17%), 10 cases from burn and 14 from diabetes mellitus associated infections and non-healing ulcer.

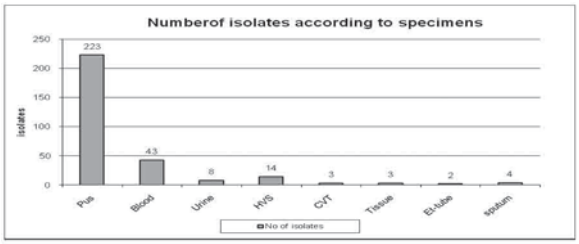


Figure3: Number of isolates from different specimens

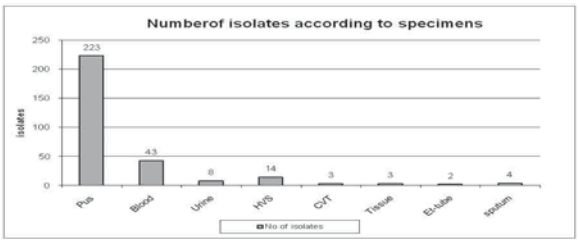
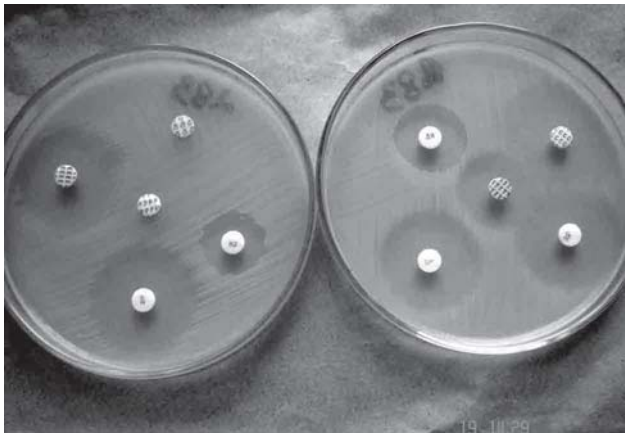


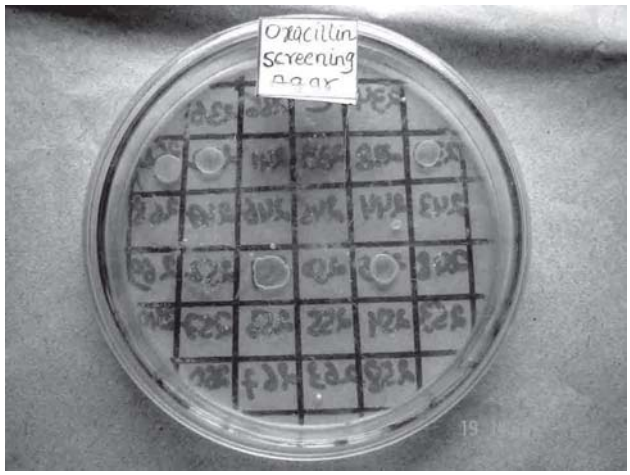
***Figure 4: Antimicrobial Susceptibility Patterns of* S aureus.**

Discussion

MRSA along with pseudomonas and acinetobacter are the common organisms associated hospital acquired worldwide .The prevalence of MRSA in the hospital was determined to be 26% which is lower as compared to varies studies conducted in India ranging(33%to 52.9%)[4-10], Pakistan (39% to 50%).[11,12] However, it s slightly lower than the rate of 15.4% and 20% in two different studies conducted in Nepal.[13,14] MRSA is usually introduced into an institution by an infected or colonized patient or by



Oxacilllin Screening Agar



Antibiotic Susceptibility test

colonized healthcare workers. It survives in the hospital environment and therefore is an important component in risk profile of the admitted patient.[15]

Seventy seven percentage of isolates were obtained from admitted patients in different wards and 23% were from patients attending outpatient department. The frequency of inpatient in our study was higher than the studies conducted in the tertiary referral hospital in South India in which admitted patients infected with *S aureus* formed 31.7%.[8]

S aureus gain access to the epidermis through cracks in the skin, abrasions, cuts, burns, surgical incisions and intravenous catheters causing wide spectrum of infections, from localized skin lesions such as abscesses, folliculitis to deep seated infections. In the present study too, 74% isolates were from pus. Similar finding was observed in a tertiary referral hospital in Eastern Uttar Pradesh where 70% of *S aureus* were isolated from pus.[7] *S aureus* is uncommon cause of

urinary tract infection. However, several studies have reported higher rate of isolation of this pathogen from patients with UTI. Eight isolates were from urine.

In the present study, second number of isolates was obtained from blood culture. A classic clinical scenario in the patient presenting with *S. aureus* bacteria in blood stream is demonstrable from primary site or secondary site of infection.

The isolates from blood culture in our studies could not be distinguished whether the organisms were limited to bacteremia or sepsis originated from distinct focus of infection. Various authors have reported different rate of isolates from blood culture.[15]

CV tips are the specimens responsible for harboring *S aureus*, perhaps from skin of the patients. In our study too, 3 isolates were from CV tips. Similar findings were reported in a study by Chaudhary et al.[8]

Isolation of *S. aureus* has also been reported from endotracheal tube. In a study by Chaudhary et al *S. aureus* from this source accounted for 13.2% of total 53 isolates [8]. We had two S. aureus from ET tube.

S. aureus is one of the common causes of bacteremia and hospital associated infection. Despite the availability of effective antibiotics, mortality in Staphylococcal septicemia ranges from 10- 15%.[15] In the present study, isolation of *S. aureus* from sepsis was 22(7.3%).

Penicillin was the antimicrobials of choice for staphylococcal infections. However the penicillin resistant staphylococci had already emerged by 1942s when the drug was available in market. The usage of penicillin decreased gradually due to the resistance developed in *S aureus* and allergy that occurred in many patients.

Most of studies documented the increased resistance to penicillin due to the destruction of the â lactam ring by the â lactamase produced by *S aureus*. In the current study, 52.66% *S. aureus* were resistant to penicillin which was reported as 87.1% to 96.1% in period of 2 years by Kalsoom et al [12], 54% by Supriya et al. [16] In our study no attempt was done to detect the production of â lactamase. Therefore it is difficult to comment on the nature of resistance to â lactam.

Erythromycin has been extensively used in the treatment of minor and serious staphylococcal infections. Moreover, the role of erythromycin in empirical treatment is further limited because of its resistance reported in most of the countries. In the current study 11% of isolates were resistant to erythromycin. This finding fully agrees with the result of the study conducted in Western Nepal (94.5%) by Subedi et al[13]and Chitra Rai et al.[14] Other studies in India and Pakistan noted resistance of (70.6%) [9] and 70.1% respectively.[12] In contrast developed countries have reported increased resistance i.e. 92.7% in USA, 75.3% Canada, 93% in Latin America, 82.6% in Europe and 94.7% in Latin America.[17]

Resistance to cefotaxime observed as 7.66% among *S. aureus* isolates, is less as compared to 27% detected by Kalsoom et al.[12]

Ciprofloxacin has been considered as potent antibiotic for effective management for staphylococcal infections. Wide use of ciprofloxacin resulted in a steadily increase in the incidence of fluroquinolones resistant Staphylococci species among clinical isolates.

In the present study 12% of *S. aureus* were resistant to ciprofloxacin, a finding which support the results from various studies in USA, Latin America, Europe and Western Pacific regions[12]. Where as 62% isolates included in a study in Pakistan were resistant.[12]

Trimethoprim–sulfamethoxazole (TMP-SMZ) is a good alternative for vancomycin for staphylococcal disease.TMP-SMZ was effective against the most of the strains of MRSA. However, emergence of resistance to this agent has been observed in MRSA as documented in many studies. This may be due to excessive use

of this drug for many other infections and over the counter availability of antimicrobials in the developing world for the treatment of many other infections.

In the present study 64% isolates had showed resistant to cotrimoxazole. Whether the resistance observed in tested isolates comes from their inherent genetic propensity to acquire resistance, or this is due to mere selection of antimicrobial resistance through monotherapy or under- dosage could not be classified as previous antibiotic intake data were not available.

In comparison to other studies our *S. aureus* isolates had increased susceptibility to gentamicin. Resistance of 11% is noted in the current study. Increased trend of resistance was noticed in USA (35.5%), Latin America (91.2%), Europe (71.2%) and Western Pacific regions (74%) [17]which were higher than our result.

Present study observed 2% resistance to chlorampenicol. Several authors have reported variable rate of resistance in *S aureus* to chloramphenicol such as 4.7% in USA, 4.9% in Canada and 9.4% in Europe.[17] Chloramphenicol is highly effective broad-spectrum antimicrobial agent with specific indications for use in seriously ill patients. Perhaps the usage of this drug is less because of its bone marrow toxicity which might be the reason that the bacterial resistance is low in this study.

Conclusion

Resistant S aureus is a common pathogen causing a wide spectrum of infections in our set up. Existence of MRSA among local isolates is a serious matter of concern. Although no isolate exhibited resistance to vancomycin, screening test and MIC determination are recommended in monitoring the response to therapy and for early detection of impeding resistance among local strains.

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