J Nepal Health Res Counc 2015 May - Aug;13(30): 126-32

Staphylococcus Aureus: Methicillin Resistance and Small Colony Variants from Pyogenic Infections of Skin, Soft Tissue and Bone

Ansari S,[1] Nepal HP,[1] Gautam R,[1] Shrestha S,[1] Chhetri MR,[2] Chapagain ML[1]

[1]Department of Microbiology, [2]Department of Community Medicine and Public Health, Chitwan Medical College, Bharatpur, Chitwan, Nepal.

ABSTRACT

Background: Staphylococcus aureus (S. aureus) colonizing the nares, is the leading cause of hospital as well as community acquired infections. The aim of this study was to identify the S. aureus from skin, soft tissue and bone related infections and typing them on the basis of antimicrobial susceptibility profile.

Methods: Clinical samples were collected from patients with skin, soft tissue and bone related infections from January to October 2013 at Chitwan Medical College Teaching Hospital, Bharatpur. The S. aureus, small colony variants and methicillin resistant strains were identified by standard microbiological methods recommended by American Society for Microbiology. Antimicrobial susceptibility testing was performed by modified Kirby-Bauer disk diffusion method.

Results: Among 333 samples processed, there was positive growth of S. aureus in 66 (19.8%) samples. Among the isolated S. aureus, 10 isolates were small colony variants of S. aureus and all the strains recovered were thymidine independent in this study. Amikacin, vancomycin and teicoplanin were found to be the most effective antibiotics. Methicillin resistance was found in 34.8% isolates of S. aureus.

Conclusions: It can be concluded that, S. aureus is one of the causative agent of pyogenic infections and the trend of antibiotic resistant is alarmingly high and also the rate of methicillin resistant S. aureus is comparably high in this study.

Keywords: Methicillin resistant Staphylococcus aureus (MRSA); small colony variants; staphylococcus aureus; thymidine dependent strains.

INTRODUCTION

A vast spectrum of acute and chronic community and hospital acquired infections are caused by Staphylococcus aureus and is one of the most common human pathogens.[1, 2] S. aureus is the most common causative agent of a large number of infections ranging from skin and soft-tissue infections (such as impetigo, furuncle, and abscess) to systemic infections (such as pneumonia and endocarditis).[3]

Small colony variants (SCVs) of S. aureus are a sub-population of bacteria, which cause recurrent and antibiotic refractory infections, probably because they may evade the host immune defense.[4] The ability of production of small colony and their intracellular survival are thought to posing difficulty in the treatment of infections caused by these strains.[5-7] Therefore, this study was designed to identify the methicillin resistant S. aureus (MRSA) strains and small colony variants from pyogenic infections.

METHODS

This is a hospital based cross-sectional study, which was carried out at Chitwan Medical College Teaching Hospital (a 600 bed teaching hospital), Chitwan, Nepal for bacteriological screenings of S. aureus from January to October 2013.

Correspondence: Shamshul Ansari, Department of Microbiology, Chitwan Medical College, Bharatpur, Chitwan, Nepal. Email: shamshulansari483@yahoo.com, Phone: +9779841455777.

During the study period, in total 333 clinical samples were collected from patients with skin, soft tissue and bone infections at the hospital. The clinical samples constituted swabs containing pus from soft tissue wounds. From closed abscesses and bone infections, the pus samples were collected using a disposable syringe after disinfecting the skin with 70% ethyl alcohol soaked in cotton wool swabs and from septic wounds and ulcers, they were first cleaned with sterile gauzes using aseptic technique and sterile cotton swabs were used to collect the pus samples from the deeper fresh part of the wounds. The samples were immediately processed in the laboratory after collection.

Isolation and identification of S. aureus was carried out by using several media brain heart infusion (BHI) broth, blood agar (BA), MacConkey agar (MA) without crystal violet (for isolation of thymidine independent strains), DNase agar and mannitol salt agar (HiMedia Laboratories Pvt. Limited, India) and the tests (catalase and coagulase) were used. The collected swab samples were gently rolled (for samples collected on swabs) on the blood agar (BA) and MacConkey agar (MA) plates and the samples collected in syringe or vials were inoculated by using a sterile loop to make primary inoculums on blood agar (BA) and MacConkey agar (MA) plates. The primary inoculums was spread using sterile inoculating loop to make streaks. All swab samples were also inoculated in brain heart infusion (BHI) broth for any possible organism by dipping and rubbing the swabs on inner wall of brain heart infusion (BHI) broth bottle. The inoculated culture plates and broths were incubated at 37°C for overnight. Sub-culture was made from each brain heart infusion (BHI) broths on blood agar (BA) and MacConkey agar (MA) plates and then it was incubated at 37°C for overnight. Identification of S. aureus was done following standard microbiological methods recommended by American Society for Microbiology (ASM).[8] A purity plate was employed to ensure that the inoculum used for the biochemical tests was pure.

All the isolates of S. aureus strains were cultured on blood agar, MacConkey agar (without crystal violet) and Mueller Hinton agar (MHA). The plates were incubated at 37° C for 24 hours and up to 48 hours. The small colony variants of S. aureus were grown as very small tiny colony on brain heart infusion(BA)as well as on MacConkey agar (MA) and Mueller Hinton agar (MHA) (if thymidine independent).

The strains growing on brain heart infusion (BA) as well as MacConkey agar (MA) and Mueller Hinton agar (MHA) were identified as thymidine independent strains and growth on only brain heart infusion (BA) were identified as thymidine dependent strains.

Antibiotic susceptibility tests were carried out on isolated and identified colonies of S. aureus isolates using commercially prepared antibiotic sensitivity disk (HiMedia Laboratories, Pvt. Limited, India) using modified Kirby-Bauer disk diffusion method in compliance with Clinical and Laboratory Standards Institute (CLSI) guidelines. The inhibition zone diameters were measured and susceptibility were considered from tables for interpretative zone diameters of Clinical and Laboratory Standards Institute (CLSI).[9] Antibiotics used were: penicillin G (10U), erythromycin (15µg), co-trimoxozole (25µg), gentamicin (10µg), amikacin (30µg), ceftriaxone 30µg), oxacillin (1µg), cefoxitin (30µg), vancomycin (30µg), clindamycin (2µg) and teicoplanin (30µg). Control strains of S. aureus ATCC 25923 was used for the standardization of the antibiotic sensitivity test.

The methicillin resistant S. aureus (MRSA) strains were identified by using cefoxitin disk (30µg). The diameter of the zone of inhibition (ZOI) of growth was recorded and interpreted as susceptible or resistant by the criteria of Clinical and Laboratory Standards Institute (CLSI). S. aureus isolates were deemed methicillin resistant when the zone of inhibition (ZOI) was ≤21 mm with the cefoxitin disk.[10]

The samples used in this study were from routine clinical specimens. This study was approved by the Institutional Review Committee of Chitwan Medical College (IRC-CMC), Bharatpur, Nepal.

Statistical analysis was performed using Epi-Info and SPSS-11.5 version. Association were assessed by using the Chi-square test. P values < 0.05 were considered as statistically significant.

RESULTS

During the study period, a total of 333 pus (pyomyositis, abscess, osteomyelitis and furuncle) samples were collected from inpatient and outpatient departments. Among total samples processed, there was growth of S. aureus strains in 66 (19.8%) samples. Out of 66 positive cases (male 34 and female 32), majority of the strains were isolated from abscess (53%) followed by pyomyositis (22.7%), furuncle (13.6%) and osteomyelitis (10.6%). Among the isolated S. aureus, 10 isolates were small colony variant (4 from abscess and 2 each from pyomyositis, osteomyelitis and furuncle) of S. aureus and all the strains recovered were thymidine dependent in this study.

There was wide variability in the susceptibility patterns

of the strains to the various antibiotics tested. Almost all of the isolates were resistant to the penicillin G and most of the isolates were resistant to various antibiotics (cotrimoxazole, ceftriaxone, gentamycin and erythromycin) (Figure 1). Almost all of the isolates originated from abscess (94.3%), pyomyositis (86.7%), furucle (89.0%) and osteomyelitis (100%) were resistant to penicillin G. More than 50% of the strains isolated from all the origins were resistant to cotrimoxazole and gentamycin. Amikacin, vancomycin and teicoplanin were found to be the most effective antibiotics against strains of all origins (Table 1). None of the strains isolated from pyomyositis, abscess, furuncle and osteomyelitis were found resistant to vancomycin and teicoplanin (Figure 1 and Table 1).

(P-penicillin, E-erithromycin, COT-cotrimoxazole, G-gentamicin, AK-amikacin, CTR-cefriaxone, OXA/FOX-oxacillin/cefoxitin, VAN-vancomycin, CD-clindamycin, TEI-teicoplanin.)

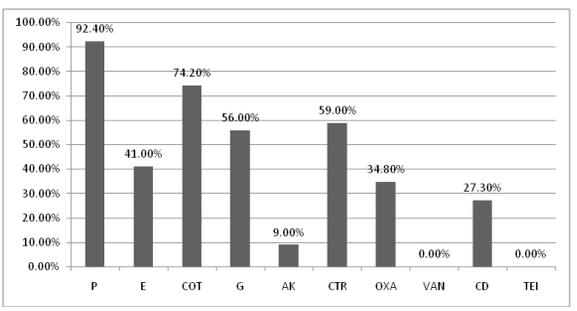

Figure 1. Overall resistance pattern of Staphylococcus aureus.

Table 1. Resistance pattern of Staphylococcus aureus to different antibiotics according to the origin.

Antibiotics tested	Resistant rates (%)			
	Abscess	Pyomyositis	Furuncle	Osteomyelitis
Penicillin	94.3	86.7	89.0	100
Erythromycin	37.1	60.0	33.3	28.6
Cotrimoxazole	74.3	73.3	77.8	71.4
Gentamycin	54.3	60.0	55.6	57.0
Amikacin	8.6	13.3	11.0	0
Ceftriaxone	65.7	73.3	44.4	14.3
Oxacillin/ cefoxitin	37.1	33.3	44.4	14.3
Vancomycin	0	0	0	0
Clindamycin	28.6	26.7	22.2	28.6
Teicoplanin	0	0	0	0

Among total isolates, 34.8% (37.1% strains isolated from abscess, 33.3% strains isolated from pyomyositis, 44.4% strains isolated from furuncle and 14.3% strains isolated from osteomyelitis) were resistant to oxacillin/ cefoxitin. Most of the antibiotics were found effective against small colony variants while 90% strains were resistant to penicillin G and 50% strains were resistant to cotrimoxazole and none of the strains were found resistant to amikacin, vancomycin and teicoplanin (Figure 2).

(P-penicillin, E-erithromycin, COT-cotrimoxazole, G-gentamicin, AK-amikacin, CTR-cefriaxone, OXA/FOX-oxacillin/cefoxitin, VAN-vancomycin, CD-clindamycin, TEI-teicoplanin.)

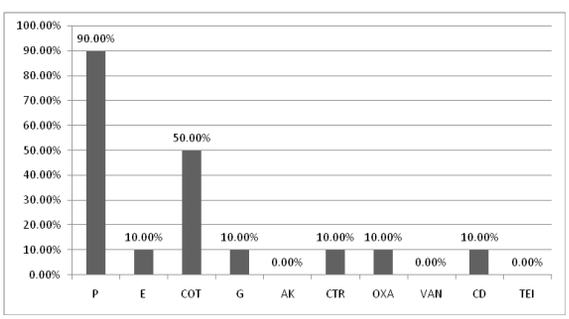

Figure 2. Resistance pattern of small colony variant of S. aureus.

For typing the isolates, numeric codes were attributed in agreement with the susceptibility to each antimicrobial tested, and then the isolates were grouped in agreement with the generated characteristic profile. The results of the antimicrobial susceptibility test grouped the 43 isolates Methicillin Sensitive Staphylococcus aureus (MSSA) in 25 different phenotypic profiles (Table 2) and the 23 isolates Methicillin resistant Staphylococcus aureus (MRSA) in 13 different phenotypes (Table 3).

Table 2. Antibiotyping of methicillin sensitive S. aureus (MSSA, n =43)

Antibiotypes	Resistance pattern								No. of isolates
	P	E	COT	G	AK	CTR	OXA/ FOX	CD	
1	+	+				+			1
2	+	+	+	+		+			4
3	+			+	+	+		+	1
4	+								5
5	+	+	+			+			2
6	+	+	+	+					1
7	+		+	+		+			1
8		+	+	+		+			1

9	+		+	+					4
10	+		+		+	+			2
11	+							+	1
12			+	+				+	1
13	+			+				+	2
14	+					+			1
15	+		+			+		+	2
16	+	+		+		+			2
17		+	+	+				+	1
18	+		+						4
19	+	+	+	+		+		+	1
20	+	+	+						1
21	+			+					1
22	+	+	+	+				+	1
23			+	+					1
24	+	+							1
25	+		+	+				+	1
MSSA									Total 43

(P-penicillin, E-erithromycin, COT-cotrimoxazole, G-gentamicin, AK-amikacin, CTR-cefriaxone, OXA-oxacillin, FOX-cefoxitin, CD-clindamycin.)

Table 3. Antibiotyping of methicillin resistant S. aureus (MRSA, N=23)

Antibiotypes	Resistance pattern								No. of isolates
	P	E	COT	G	AK	CTR	OXA/ FOX	CD	
1	+		+	+		+	+	+	4
2	+	+	+			+	+		2
3		+	+		+	+	+		1
4	+	+	+	+	+	+	+	+	1
5	+	+				+	+		1
6	+		+	+		+	+		4
7	+			+		+	+		1
8	+	+	+				+		1
9	+		+			+	+		2
10	+	+	+			+	+		2
11	+	+	+	+		+	+		2
12	+		+	+	+	+	+		1
13	+	+	+	+			+		1
MRSA									Total 23

(P-penicillin, E-erithromycin, COT-cotrimoxazole, G-gentamicin, AK-amikacin, CTR-cefriaxone, OXA-oxacillin, FOX-cefoxitin, CD-clindamycin.)

DISCUSSION

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, among total samples processed, there was positive growth of S. aureus strains in 66 (19.8%) samples. Similarly, 22.28% of positivity rate was also reported by Perveen et al in 2013 from Pakistan.[11] Unlike to our result, other study showed a higher rate of S. aureus from pus samples, 68.5% from Nepal,[12] 32% and 45.1% from India,[13,14] 46% from Pakistan.[15] These high rates may be because all the samples were collected from indoor patient (IPD) patients and in our study the samples were collected from both in door patient (IPD) and outdoor patient (OPD).

Out of 66 positive cases (male 34 and female 32), majority of the strains were isolated from abscess (53%, 35/66) followed by pyomyositis (22.7%, 15/66), furuncle (13.6%, 9/66) and osteomyelitis (10.6%, 7/66).In similar study by Sina et al in Benin (Nigeria) who reported almost 37% of Staphylococcus strains originated from abscess, 27% from pyomyositis, 14% from furuncle and 10% from osteomyelitis.[16]

Small colony variants have been recovered from patients with unusually persistent infections, particularly those patients with long disease-free intervals, and from patients who are chronically exposed to aminoglycosides and trimethoprim-sulfomethoxazole.[17]In our study, among the isolated S. aureus, 10 isolates were small colony variants of S. aureus and all the strains recovered were thymidine independent strains.

Development of resistance to antimicrobial agents by staphylococci is a major concern primarily because they are still frequently associated with hospital and community-acquired infections. The organisms exhibit remarkable versatility in their behavior towards antibiotics, with some strains having overcome most commonly used drugs.[18] Knowledge of the pattern of antibiotic resistance among isolates is very important both clinically and epidemiologically.[19]

A low proportion of isolates presented susceptibility to ß-lactam antibiotics. It was expected, since, currently, it has been recognized that only a small percentage of the S. aureus lineages from hospital origins do not produce ß-lactamases. In current study, almost all of the isolates originated from abscess (94.3%), pyomyositis (86.7%), furuncle (89.0%) and osteomyelitis (100%) were resistant to penicillin G and majority of the strains were resistant to ceftriaxone. This is consistent with previous report showing high rates of S. aureus resistance to benzyl

penicillin from Nepal[20]and 97% strains isolated from pus samples were found to be resistant to penicillin G in Benin (Nigeria)[21]suggesting that this antibiotic, one of the first to be introduced, is no longer effective against S. aureus. We attributed this to the presence of beta-lactamase producing S. aureus in hospital environment and 'selection pressure' due to the use of the beta-lactam antibiotics for the treatment, offering advantage for the selection colonization to more resistant beta-lactamase strains.

Present study revealed that more than 50% of the strains isolated from all the origins (abscess, pyomyositis, furuncle and osteomyelitis) were resistant to cotrimoxazole. Similar results also found by Mukhiya et al,[22]Tiwari et al,[23]Baral et al [24] from Nepal, Sina et al from Benin (Nigeria).[21] Cotrimoxazole historically had wide clinical application, is inexpensive, orally administered and available from diverse sources where they are sold without prescription in Nepal. It appears that misuse and overuse of these antibiotics could have contributed to this trend in Nepal.

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. Around quarter to half of all the strains from all the origins were found to be resistant to erythromycin in current study. Similar pattern of resistance to erythromycin was also found by other others, 18.2% by Singh et al[25] in 2006, 29.03% by Sanjana et al[20] in 2010 from Nepal and 32% by Jahan et al[13] in 2013 from India.

In our study, resistance to aminoglycoside was more in gentamicin (more than 50%) than amikacin (9.0%). The variability of resistant pattern of amikacin depends greatly on origin ranging from no resistant at all in osteomyelitis to 13.3% strains resistant in pyomyositis. Increased trend of resistance to gentamicin was also noticed in USA (35.5%), Latin America (91.2%), Europe (71.2%) and Western Pacific regions (74%).[26]Similarly, few resistant strains to amikacin have also been reported by Sanjana et al[20] in 2010 and Tiwari et al[23] in 2009 from Nepal.

For the past 50 years, S. aureus has been a dynamic human pathogen that has gained the deepest respect of clinician since the report of methicillin resistant S. aureus (MRSA) infection in US at a Boston city hospital in 1961. Since, then methicillin resistant S. aureus (MRSA) has become wide spread all over the world.[27] Methicillin resistant S. aureus (MRSA) is a major nosocomial pathogen causing serious morbidity and mortality in immune-suppressed patients.[28] The use of broad spectrum antibiotics in treating infections also increases the risk of acquiring methicillin resistant S.aureus (MRSA) and other resistant bacteria.[28, 29] Once the oxacillin was the most effective antibiotic for the S. aureus in Nepal.[25] But now the oxacillin resistance has reached the pinnacle. Even in our study, oxacillin/cefoxitin resistant methicillin resistant S. aureus (MRSA) was found in 34.8% of S. aureus strains. This proportion of resistant strains appears to be comparable with the recorded resistance rate of 26.67% in 2008 in Nepal,[30] 39.0% in India,[13]24.53% in Pakistan,[31]42.85% in Benin.[21]Comparably, based on the origin, the lower rate (14.3%) of methicillin resistant S. aureus (MRSA) strains were isolated from osteomyelitis in this study. Two different methods were employed for the detection of methicillin resistant S. aureus (MRSA). The cefoxitin disk method was comparable to oxacillin disk method, as both methods detected 34.8% (23/66) methicillin resistant S. aureus (MRSA) cases. According to CLSI, the cefoxitin disk test is comparable to the oxacillin disk test for the prediction of mecA-mediated resistance to oxacillin. The cefoxitin disk test is easier to read and thus is the preferred method. Besides, cefoxitin is an inducer of the mecA gene.[31]

Eeven though methicillin is the preferred antibiotic for treatment of staphylococcal infections. Vancomycin remains active against methicillin resistant strains of S. aureus. Fortunately, none of the strains were found to be resistant to vancomycin and teicoplanin and few strains were resistant to amikacin which seems to be the most effective antibiotics against strains of all origins corroborating with the result of a study conducted by Sanjana et al[20] from Nepal, Jahan et al[13] from India, Siddiqi et al[15] from Pakistan showing the amikacin and vancomycin is the most effective antibiotics. Vancomycin and teicoplanin seems to be the only antimicrobial agent which showed best sensitivity and so may be used as the drug of choice for treating multidrug-resistant methicillin resistant S. aureus (MRSA) infections. Despite to its high sensitivity, vancomycin is not a commonly prescribed drug, which is almost certainly due to the higher price of the antibiotic and its unavailability in many parts of the country.

Most of the antibiotics were found effective against small colony variants while 90% strains were resistant to penicillin G and 50% strains were resistant to cotrimoxazole and none of the strains were found resistant to amikacin, vancomycin and teicoplanin in our study.

Antibiotyping is valuable, especially in routine

laboratories, as a first-line screening method to determine strain relatedness. It may allow quick and early recognition of a previously defined epidemic strain in a particular hospital setting. In current study, antibiotic susceptibility profile of methicillin sensitive S. aureus (MSSA) categorized the strains in 25 different types of which the most prevalent was penicillin, cotrimoxazole and gentamicin accounting for 23.3%. Antibiotic susceptibility profile of methicillin resistant S. aureus (MRSA) divided the strains in 13 different types of which the most prevalent was penicillin, cotrimoxazole, ceftriaxone and oxacillin accounting for 78.3%.

CONCLUSIONS

The current study indicates that resistant S. aureus is a common pathogen causing wide spectrum of infections. Existence of methicillin resistant S. aures (MRSA) among local isolates is a serious matter of concern. There is a need for extensive surveillance of methicillin resistant S. aures (MRSA) and its antimicrobial profile. Although no isolate exhibited resistance to vancomycin and teicoplanin, screening test and the minimum inhibitory concentration (MIC) determination are recommended in monitoring the response to therapy and for early detection of impeding resistance among local strains.

ACKNOWLEDGEMENT

We are greatly indebted to the patients and the authors would like to thank the laboratory staffs of the bacteriology department of Chitwan Medical College Teaching Hospital (CMCTH) for their kind support in the laboratory tests during the study.

REFERENCES

1. von Eiff C, Becker K, Machka K, Stammer H, Peters G. Nasal carriage as a source of Staphylococcus aureus bacteremia. Study Group. N Engl J Med. 2001; 344(1):11–6.

2. Wertheim HF, Vos MC, Ott A, van Belkum A, Voss A, Kluytmans JA, van Keulen PH, Vandenbroucke-Grauls CM, Meester MH, Verbrugh HA. Risk and outcome of nosocomial Staphylococcus aureus bacteraemia in nasal carriers versus non-carriers. Lancet. 2004; 364(9435):703–5.

3. Holmes A, Ganner M, McGuane S, Pitt TL, Cookson BD, Kearns AM. Staphylococcus aureus isolates carrying panton-valentine genes in england and wales: frequency, characterization, and association with clinical desease. J ClinMicrobiol. 2005; 43(5):2384-90.

4. McNamara PJ, Proctor RA.Staphylococcus aureus small colony variants, electron transport and persistent infections.Int J Antimicrob Agents.2000; 14:117-22.

5. Proctor RA.Respiration and small-colony variants of Staphylococcus aureus, p. 434–442.In D. A. Fischetti, R. P. Novick, J. J. Ferrett, D. A. Portnoy, and R. P. Rood (ed.), Gram-positive pathogens, 2nd ed. ASM Press, Washington, DC, 2006.

6. Proctor RA, Van-Langevelde P, Kristjansson M, Maslow JN, Arbeit RD. Persistent and relapsing infections associated with small-colony variants of Staphylococcus aureus. Clin Infect Dis. 1995;20:95-102.

7. Proctor RA, Peters G, Small colony variants in staphylococcal infections: diagnostic and therapeutic implications. Clin Infect Dis. 1998;27:419-22.

8. Isenberg HD.Clinical Microbiology Procedures Handbook, 2nd edition. Washington DC: ASM press, 2004.

9. Clinical and Laboratory Standard Institute.Performance standards for antimicrobial susceptibility testing. USA:CLSI: M100-S16. Wayne, PA, 2006.

10. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing, 17th informational supplement. USA:CLSI: M100-S17. Wayne, PA, 2007.

11. Perveen I, Majid A, KnawalS, Naz I, Sehar S, Ahmed S, Raza MA. Prevalence and antimicrobial susceptibility pattern of methicillin-resistant Staphylococcus aureusand coagulase-negative staphylococci in Rawalpindi, Pakistan. BritishJ Medicine & Medical Res. 2013;3(1):198-09.

12. Upadhyay AK, Maharjan R, Shakya B. Multidrug resistance bacteria in different clinical samples in National Medical College and Teaching Hospital Birgunj, Nepal. Res J Pharm, BiolChemSci (RJPBCS). 2012;3(4):797-07.

13. Jahan A, Rahman M. Isolation and charcterization of Staphylococcus aureus isolated from pus sample of hospitalized patients in tertiary care hospital.International J Pharm Chem Sci. 2013:2(4):1743-45.

14. Verma P. Antibiotic sensitivity treatment for gram positive bacteria isolated from pus sample. Bull Environ Pharmacol Life Sci. 2012;1(10):03-06.

15. Siddiqi F, Masood M, Saba N, Samad A, Qayyum M,

Qazilbash AA. Antibiogram sensitivity pattern of methicillin-resistant Staphylococcus aureus isolates from pus samples. Pakistan J Biol Sci. 2002;5(4):491-93.

16. Sina H, Ahoyo TA, Moussaoui W, Keller D, Bankole HS, Barogui Y, Stienstra Y, Kotchoni SO, Prevost G, Baba-Moussa L. Variability of antibiotic susceptibility and toxin production of Staphylococcus aureus strains isolated from skin, soft tissue, and bone related infections. BMC Microbiology. 2013;13:188.

17. Proctor RA, Peters G.Small colony variants in staphylococcal infections: diagnostic andtherapeutic implications. ClinInfec Dis. 1998;27:419-23.

18. Grassi GG. Infections by Gram-positive bacteria: an overview. J Antimicrobiol Chem. 1988:21:1-7.

19. Ojulong J, Mwambu TP, Jalooba M, Bwanga F, Kaddu-Mulindwa DH.Relative prevalence Methicillin-Resistant Staphylococcus aureus and its susceptibility pattern in Mulago Hospital, Kampala, Uganda.Tanzania J Health Research. 2009;11(3):149-53.

20. SanjanaRK , Shah R, Chaudhary N, Singh YI.Prevalence and antimicrobial susceptibility pattern of methicillin-resistant Staphylococcus aureus(MRSA) in CMS-teaching hospital: a preliminary report. J Col Med Sci-Nepal. 2010;6(1):1-6.

21. Sina H, Baba-Moussa F, Ahoyo TA, Mousse W, Anagonou S, Gbenou JD. Antibiotic susceptibility and toxins production of Staphylococcus aureusisolated from clinical samples from Benin. African J Microbio Research.2011;5(18):2797-03.

22. Mukhiya K, Shrestha A, RaiSK, Pant K, Rai G.Methicillin-resistant Staphylococcus aureusin clinical samples of hospital located in Kathmandu valley, Nepal. Res J Pharm BiolChemSci (RJPBCS). 2013;4(2):617-21.

23. Tiwari HK, Das AK, Sapkota D, Sivarajan K, Pahwa VK.Methicillin resistant Staphylococcus aureus: prevalence and antibiogram in a tertiary care hospital in western Nepal. J Infect DevCtries. 2009;3(9):681-84.

24. Baral R, Khanal B, Acharya A. Antimicrobial susceptibility patterns of clinical isolates of Staphylococcus aureusin Eastern Nepal. Health Renaissance. 2011;9(2):78-82.

25. Singh A, Vaidya P, Tuladhar NR, Maharjan S.Drug sensitivity pattern of microorganisms of infected wounds at TUTH. Journal of Institute of Medicine.2006; 28(1).

26. Diekma JD, Pfaller AM, Schmitz JF, Smayevsky J, Bell J, Jones RN, Beach M, SENTRY Participants Group. Survey of infections due to Staphylococcus species: Frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe and the Western Pacific region for the SENTRY antimicrobial surveillance program, 1997-1999. ClinInfec Dis. 2001;32(Suppl 2):114-23.

27. Fernandez CJ, Fernandez LA, Colignon P, Australian Group on Antimicrobial Resistant.Cefoxitin resistant as a surrogate marker for the detection of MRSA.Journal of Antimicrobial Chemotherapy. 2005;55:506-10.

28. Sheff B. VRE and MRSA: Putting bad bags outof business.nursing findarticles.com. 1998.

29. Vidhani N, Mehndiratta PL, Mathur MD. Study of the Methicillin Resistant Staphylococcus aureus (MRSA) from high risk patients. Indian Med J Microbiol. 2001;19:13-16.

30. KumariN, Mohapatra TM, Singh YI.Prevalence of Methicillin-Resistant Staphylococcus aureus (MRSA) in a Tertiary-Care Hospital in Eastern Nepal.J Nepal Med Assoc. 2008;47(170):53-56.

31. Mahmood K, Tahir T, Jameel T, Ziauddin A, Aslam HF.Incidence of methicillin-resistant Staphylococcus aureus (MRSA) causing nosocomial infection in a tertiary care hospital. ANNALS. 2010;16(2):91-96.