Nepal Journal of Biotechnology. Dec. 2019 Vol. 7, No. 1: 82-89 DOI: https://doi.org/10.3126/njb.v7i1.26957

ORIGINAL RESEARCH ARTICLE

Screening of Methicillin Resistant *Staphylococcus aureus* (MRSA) from Wounds in Pediatric Patients Visiting Tertiary Care in Hospital

Ganesh Bhandari[1], Basant Pokhrel[1], Yogesh Oli[2], Archana Katuwal[2], Netra Lal Bhandari[1*]

[1]Department of Chemistry, Tri-Chandra Multiple Campus, Tribhuvan University, Kathmandu, Nepal
[2]Department of Microbiology, Tri-Chandra Multiple Campus, Tribhuvan University, Kathmandu, Nepal

Abstract

The extent of methicillin-resistant *Staphylococcus aureus* (MRSA) in children is still unknown. The collected wound pus samples were processed. Identification of *S. aureus* was done according to standard microbiological procedures as per the clinical laboratory standards institute (CLSI) guidelines (2016). The antibiogram of the isolates was carried out by the Kirby-Bauer disc diffusion technique. MRSA was determined by measuring the zone of inhibition (ZOI) surrounding to cefoxitin disc, with resistance defined as ZOI of ≤ 21 mm. Out of 357 bacterial culture-positive samples, 278 (77.87 %) were *S. aureus* isolates, among them 102 (36.69%) were found to be MRSA. The percentage of MRSA isolates was found high in male children and inpatients with 61.76 % and 73.52% respectively. All the MRSA isolates were susceptible to gentamicin (79.41%), whereas (91.17%) were resistant to penicillin. The distribution of MRSA in inpatients 75 (73.52%) is higher than that of outpatients 27 (26.74%). This study shows that the MRSA occurrence is prevalent in pediatric patients.

Keywords: *Staphylococcus aureus*, MRSA, Antibiotic susceptibility test, Disc-diffusion, Methicillin resistant

***Corresponding author**
Email: netra.tu.edu@gmail.com

Introduction

Staphylococcus aureus (*S. aureus*) is one of the important human pathogen known over the past several decades. It is the cause of hospital and community-acquired infections [1]. It is one of the versatile nosocomial pathogens worldwide causing skin infections to life-threatening systemic illnesses including pneumonia, osteomyelitis and endocarditis [2, 3]. *S. aureus* is one of the common and important Gram-positive hospital-acquired organisms. It can cause infection; most commonly at sites of lowered host resistance such as damaged skin or mucosal membranes, infections like pimples, boils and abscesses to severe systemic infections like bacteremia, endocarditis, pneumonia and toxic shock syndrome [4, 5].

S. aureus resistant to oxacillin, methicillin and a few others related antibiotics are all known under the generic term methicillin-resistant *S. aureus*. The penicillin-resistance *S. aureus* (PRSA) is typically coincides with the emergence of MRSA [6]. MRSA is a global public health problem, associated with considerable morbidity and mortality, causing both hospital and community-acquired infections [7]. Among the many species of antibiotic-resistant bacteria, MRSA is one of the most important causes of antibiotic treatment failure, increased morbidity and mortality. *S. aureus* has a remarkable ability to colonize the skin and mucous membranes. Hospitalized patients and health-care workers have higher colonization rates than the general population [8]. Studies revealed that nearly 6% hospital personnel and nearly 3% outpatients carry MRSA [9-12].

The prevalence of MRSA has varied from hospital to hospital in various countries. Several types of research and studies conducted in Nepal about *S. aureus* and their antibiotic susceptibility pattern suggest the gradual emergence of MRSA in hospitals. However, only a few reports related to MRSA are available in Nepal which is carried out in different hospitals at different periods. In a bacteriological study carried out at Tribhuvan University Teaching Hospital (TUTH), the prevalence of MRSA was found to be 23.5% [13]. A similar study carried out at Kanti Children's

Hospital showed 31.4% of MRSA isolates and 11.7% from TUTH [14]. Rajbhandari *et al.* (2002) reported 54.9% strains of MRSA [15]. Similarly, Kumari *et al.* (2008) reported 26.14% MRSA strains in a study carried out in a tertiary- care hospital in Eastern Nepal [16]. Sanjana *et al.* (2010) also reported 39.6% Methicillin-resistant *S. aureus* isolates at the College of Medical Sciences-Teaching Hospital, Chitwan. Tiwari *et al.* (2009) reported MRSA isolates were resistant to co-trimoxazole was 77% and 81.7% (Ansari *et al.* 2014). Pant *et al.* (2018) reported 82.6% MRSA resistance to chloramphenicol [17-20].

The prime focus of the study is the distribution and prevalence of *S. aureus* and MRSA in wound samples collected in the microbiology laboratory of International Friendship Children's Hospital as well as on its antibiotic sensitivity pattern. The study will also demonstrate the present scenario of MRSA in children and the sensitivity pattern of different antibiotics used against it. This is useful for future planning and policy making in health care centers and hospitals in order to combat the spreading of infectious diseases.

Materials and Methods

Sample collection and transportation

Clinical specimens were collected, stored, transported and processed in the laboratory immediately after collection by following standard microbiology guidelines [21]. The sterile swab was moved across the surface of wounds in a zigzag motion and pus aspirates were collected aseptically by puncturing the wound using a sterile syringe. The accurate, rapid microbiological diagnosis of MRSA and *S. aureus* infections begins with proper specimen collection and rapid transportation to the laboratory. To make sure the collection of the best possible specimen, the Health Care Workers (HCWs) must be properly trained, and the patient must be provided with clearly presented and fully understood instruction for sample collection.

Specimen processing

Pus samples received in the microbiology laboratory were processed as per routine standard microbiological procedures described by the established guideline for the isolation and identification of *S. aureus* [22].

Culture of specimen

All the pus samples were inoculated on blood agar (BA) and Mac-conkey agar (MA) plates and incubated aerobically at 37˚C for 24 to 48 hours in the incubator. If no growth was observed, it was reported as growth negative. The suspected *S. aureus* isolates (on the basis of haemolysis produced on BA) were inoculated in to mannitol salt agar (MSA) to check if the isolates ferment mannitol or not. Photograph of *S. aureus* culture on blood agar and MSA is pictured in **figure 1**.

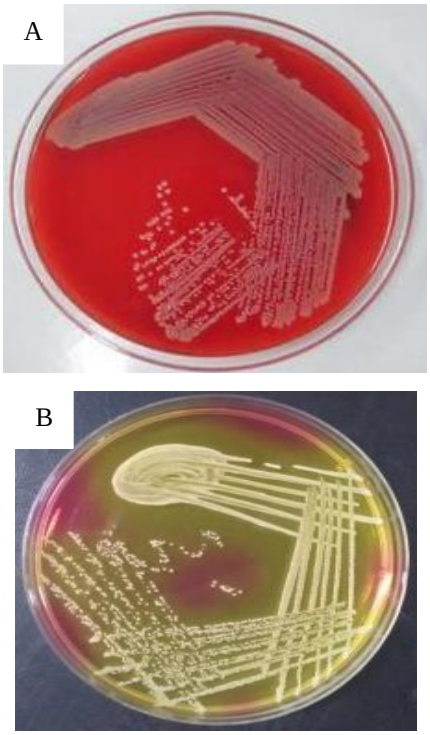

Figure 1. Photograph of *S. aureus* culture on (A) blood agar (B) mannitol salt agar (MSA)

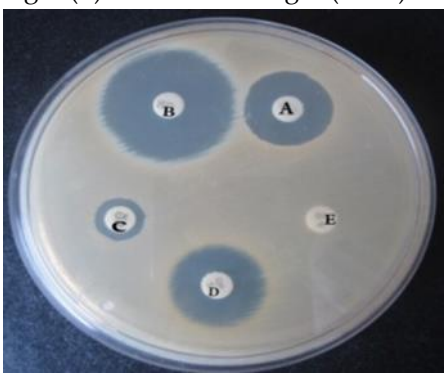

Figure 2. Photograph of antibiotic sensitivity test of S. aureus on MHA. A: Gentamicin (30mcg), B: Chloramphenicol (30mcg), C: Cefoxitin (30mcg), D: Ciprofloxacin (5mcg), E: Penicillin (10mcg)

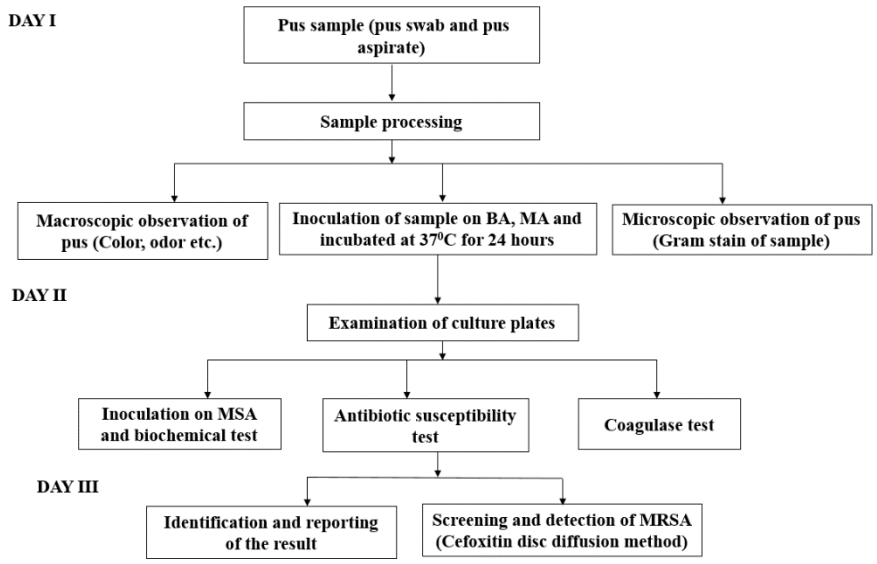

Figure 3. Flow chart representing isolation and identification of MRSA from pus samples

Identification of the isolates

The growth was identified as *S. aureus* by using conventional biochemical methods as per standard microbiological techniques described in Bergey's Manual of Determinative Bacteriology [23]. It comprises colonial morphology, staining reactions and various biochemical properties such as catalase-positive, coxidase negative, fermentative, Voges-Proskauer positive, etc.

Preservation of isolates

Isolates in pure culture were preserved in glycerol 20% (v/v) in brain heart infusion broth (BHI) at –20 ˚C to -70 ˚C and recovered by sub culturing in brain heart infusion broth at 37˚C for 24 hrs followed by further subculture on nutrient agar.

Antimicrobial susceptibility testing of *S. aureus*

Antibiotic susceptibility testing of isolates was assessed by the modified Kirby-Bauer disk diffusion method as recommended by the Clinical and Laboratory Standard Institute using Mueller Hinton Agar (MHA) [22]. Using a sterile wire loop, a single isolated colony of which the sensitivity pattern is to be determined was touched and inoculated into a nutrient broth (NB) tube and was incubated for 2-4 hours. After incubation in a good light source, the turbidity of the suspension was matched with the turbidity of the standard of Mac Farland 0.5 (Prepared by adding 0.6mL of 1% w/v barium chloride solution to 99.4mL of 1% v/v solution of sulphuric acid) [24]. Using a sterile swab, a plate of Mueller Hinton agar was inoculated with bacterial suspension using carpet culture technique and plates were taken for incubation at 37˚C for 18-24 hours. The result was interpreted as whether the organism was sensitive or resistant to the tested antimicrobial agents. Photograph of antibiotic sensitivity test of *S. aureus* on MHA is shown in **figure 2**.

Detection of MRSA

MRSA detection was done by the cefoxitin disc (30µg) diffusion test. As per CLSI (2015) guideline, a lawn culture was made on Mueller Hinton Agar (MHA) supplemented with 4% NaCl and cefoxitin disc from the suspension of turbidity equivalent to 0.5 Mc Farland standards from overnight growth in nutrient agar and incubated for 24 hours aerobically at 35˚C. After incubation, the plates were examined for zone of inhibition. Isolates exhibiting zone of inhibition ≥ 22 mm were considered as sensitive, whereas those showing zone of inhibition ≤ 21 mm were considered as resistant and were reported as MRSA.

Purity plate

The purity was used to ensure that the inoculation used for the biochemical tests was

Table 1. Bacterial growth in different pus samples

Types of sample	Growth (No. %)	No growth (No. %)	Total
Pus swab	303(55.09%)	247(44.91%)	550
Pus aspirate	54 (54%)	46 (46%)	100
Total	357 (54.92)	293 (45.08)	650(100)

Table 2. Gender wise distribution of patients (P > 0.05)

	Male	Female	Total
Culture positive	196(54.9)	161(45.09)	357(54.9)
Culture negative	162 (45.2)	131 (44.8)	293(45.1)
Total	358 (55.1)	292 (44.9)	650 (100)

pure culture and also to see whether the biochemical tests were performed in aseptic condition or not. Thus, while performing biochemical tests, in biochemical media, the same inoculum was sub-cultured on one half of Nutrient Agar (NA) medium before (pre purity) inoculation and another half after (post purity) inoculation. The maintenance of aseptic condition is indicated by the growth of the same organism in pure form in both pre and post halves of the medium. The Chart below represents the isolation and identification of MRSA from pus samples.

All the raw data collected in the microbiology laboratory were statistically analyzed by using computer-based software program SPSS version 20. The flow chart representing isolation and identification of MRSA from pus samples is shown in **figure 3**.

Results and Discussion

Bacterial growth in different pus samples and gender wise distribution of patients

Out of 650 total samples, 550 samples were pus swab and 100 samples were pus aspirate. Among total pus swabs 303 (55.09%) were culture positive and 247 (44.91%) showed no growth. Moreover, among total pus aspirates, 54 (54%) were culture positive and the remaining 46 (46%) showed no growth as shown in **Table 1**.

Out of total cases, 358 (55.07%) were male patients and 292 (44.92%) were female patients. The growth was found to be higher in male patients 196 (54.9%) than in female patients161 (45.09%). The association between gender and growth of bacteria was not statistically significant (p-value > 0.05) is presented in **Table 2**.

Among 650 pus samples, the majority i.e. 352 (54.15%) were In-patients who were admitted to the different departments in the hospital and 298 (45.23%) were from OPD. A total 221 (62.78%) of In- Patient Department (IPD) and 136 (45.36 %) of Out Patient Department (OPD) showed growth. Such distribution pattern of growth on the basis of the department was statistically significant (p-value <0.05) is as in **table 3**.

Distribution of *S. aureus* in pus sample

Among the culture-positive specimens, 278 (77.87%) *S. aureus* isolates were obtained while remaining 79 (22.12 %) were other bacterial isolates which include both gram-positive Coagulase Negative *Staphylococcus species* (CoNS) and gram-negative bacteria (*E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Enterobacter aerogens*) as shown in pie chart in **figure 4**.

Distribution of clinical isolates of *S. aureus* according to age and gender of the patient

Among 278 *S. aureus* isolates, a higher number of isolation was found in male 169 (60.79%) as compared to female 109 (39.20%) patients. In the male, the maximum number of *S. aureus* isolates (n=87, 51.47 %) was observed in the patient with an age group of 1-5 and in females, the maximum

Table 3. Distribution of bacterial growth on the basis of hospital Department

Organism	IPD {n (%)}	OPD {n (%)}	Total {n (%)}	p-value
Culture positive	221 (62.78)	136 (45.36)	357 (54.92)	
Culture negative	131 (37.21)	162 (54.36)	293 (45.07)	P < 0.05
Total	352 (54.15)	298 (45.84)	650 (100)	

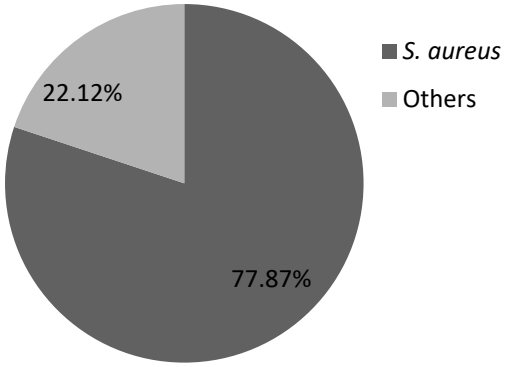

Figure 4. Distribution of S. aureus in pus sample.

number of *S. aureus* isolates (n=55, 50.45 %) was observed in the same 1-5 age group patients. Likewise, the least number of *S. aureus* isolates (n=12, 7.10%) was observed in 11-15 age group patient in male and female least number of *S. aureus* isolates (n=9, 8.25 %) was observed in the same 11-15 age group patients is shown in **Table 4.**

Table 4. Distribution of clinical isolates of S. aureus according to age and gender of the patient

Age group(in years)	Gender		Total	
	Male	Female	No.	%
Below 1	32	18	50	17.98
1-5	87	55	142	51.07
6-10	38	27	65	23.38
11-15	12	9	21	7.55
Total	169(60.79%)	109(39.2%)	278	100.0

Comparison of clinical isolates of *S. aureus* in inpatients and outpatients

Among 278 *S. aureus* isolates, 177 (63.66 %) isolates of *S. aureus* were from inpatients whereas 101 (36.33%) *S. aureus* were from outpatients. Similarly, among 79 growths other than *S. aureus* isolates, 47 (59.49 %) and 32 (40.50%) cases of cultures other than *S. aureus* were found from inpatients and outpatients respectively. A comparison of clinical isolates of *S. aureus* in inpatients and outpatients is pictured in **figure 5**.

Distribution of MRSA among *S. aureus* and in different age groups

Out of 357 culture-positive isolates, 278 (77.87 %) were *S. aureus*. Out of total *S. aureus;* 102 (36.69%) were MRSA and 176 (63.30 %) were MSSA (Methicillin-sensitive *Staphylococcus aureus*). Out of 169 (60.79%) *S. aureus* isolates among male patients, 63 (61.76%) were found to be MRSA. Likewise, among 109 (39.20 %) *S. aureus* isolates among female patients, 39 (38.23 %) were found to be MRSA. Such distribution of MRSA based on gender was not statistically significant (p-value; > 0.05) is given in the pie chart in **figure 6.**

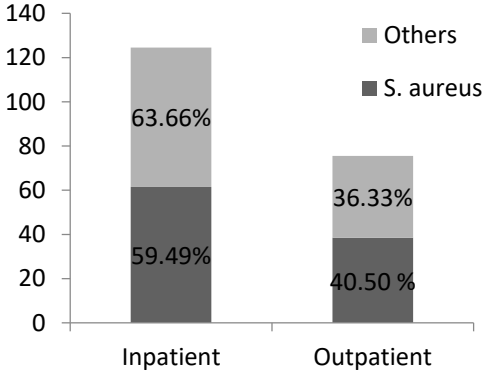

Figure 5. Comparison of clinical isolates of S. aureus in inpatients and outpatients

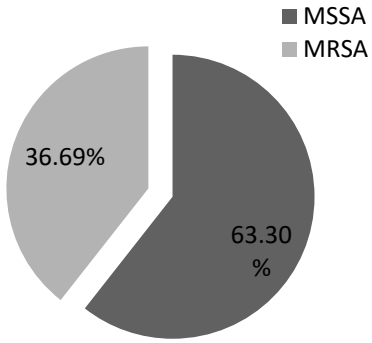

Figure 6. Distribution of MRSA among S. aureus

Out of 278 *S. aureus* isolates, altogether 102 (36.69%) and 176 (63.30%) were identified as MRSA and MSSA respectively by cefoxitin disc diffusion test which constituted 75 (73.52%) MRSA and 101 (57.38%) MSSA in admitted patients. Similarly, in outpatients, 27(26.47%) MRSA and 75(42.61%) MSSA were observed. The association in the MRSA occurrence and inpatients was statistically significant (p = 0.024) is in **Table 5**

Table 5. Distribution of MRSA in outpatients and inpatients

	Inpatients (No. %)	Outpatients (No. %)	Total (No. %)	P-value
MRSA	75(73.52%)	27 (26.47%)	102 (36.69%)	
MSSA	101 (57.38%)	75 (42.61 %)	176 (63.30%)	P < 0.05
Total	176	102	278 (100.0)	

Table 6. Antibiotic susceptibility pattern of S. aureus isolates

Antibiotics Used	Total No of *S. aureus* isolates =278			
	Sensitive	%	Resistant	%
Amikacin	236	84.89	42	15.10
Ciprofloxacin	100	35.97	178	64.02
Cotrimoxazole	125	44.96	153	55.04
Cefoxitin	176	63.30	102	36.69
Erythromycin	166	59.7	112	40.28
Cloxacillin	180	64.74	98	35.25
Cefotaxime	236	84.89	42	15.10
Penicillin	28	10.08	250	89.92
Ofloxacin	116	41.72	162	58.27
Chloramphenicol	255	91.72	23	8.27
Gentamicin	264	94.96	14	5.03

Antibiotic susceptibility pattern of *S. aureus* isolates

The antibiotic susceptibility pattern showed that the highest number of isolates were resistant to penicillin (n=250, 89.92%), ciprofloxacin (n=178, 64.02%), ofloxacin (n=162, 58.27%), cotrimoxazole (n=153, 55.04%), erythromycin (n=112, 40.28%), cefoxitin (n=102, 36.69%) and cloxacillin (n=98, 35.25%). The highest number of penicillin resistant S. aureus developed the ability to inactivate the β- lactam ring of penicillin by producing plasmid encoded β- lactamase [25]. Similarly, highest number of MRSA isolates were susceptible to gentamicin (n=264, 94.96%) is due to the combination β- lactam and amino glycosides which increases bacterial killing in-vitro and in animal model of endocarditis, followed by chloramphenicol (n=255, 91.72%), cefotaxime (n=236, 84.89%) and amikacin (n=236, 84.89%), cloxacillin (n=180, 64.74%), cefoxitin (n=176, 63.30%), erythromycin (n=166, 59.7%) is as shown in table 6.

Antibiotic susceptibility pattern of MRSA and MSSA isolates

The isolates of *S. aureus* were broadly categorized into two groups: MRSA and MSSA. Both the groups of *S. aureus* showed marked variation in the sensitivity pattern against common antibiotics.

It was observed that, for MRSA, the most effective antibiotic was cefotaxime (n=83, 81.37%) followed by gentamicin (n=81, 79.41%), amikacin (n=77, 75.49%), chloramphenicol (n=76, 74.50%). Similarly, highest resistance of MRSA was with penicillin (n=93, 91.17%) followed by ciprofloxacin (n=85, 83.3%) and cotrimoxazole (n=77, 75.49%) is in **table 7**.

Conclusion

MRSA has acquired resistant to previously effective antimicrobials including in the penicillin, methicillin in comparison over the past 50 years. In hospital infected patients and health carriers are also important source of nosocomial *S. aureus* [26, 27]. Out of 278 *S. aureus* isolates isolated from pus samples, 102 (36.69%) were methicillin-resistant (MRSA). From this study it can be concluded that MRSA infection in pediatric patients is still one of the most threatening infections in hospitals of Nepal. The rate of MRSA occurrence was found to be higher in inpatients compared to outpatients and the male patient than female patients. The highest number of MRSA isolates was found in 1-5 age

Table 7. Antibiotic susceptibility pattern of MRSA and MSSA isolates

Antibiotics Used	MSSA(n=176)				MRSA(n=102)			
	Sensitive	%	Resistant	%	Sensitive	%	Resistant	%
Amikacin	163	92.61	13	7.38	77	75.49	25	24.50
Ciprofloxacin	91	51.70	85	48.29	17	16.60	85	83.33
Cotrimoxazole	93	52.84	83	47.15	25	24.5	77	75.49
Cefoxitin	176	100.0	0	0.0	0	0.0	102	100.0
Erythromycin	114	64.77	62	35.22	59	57.84	43	42.15
Cloxacillin	151	85.79	25	14.20	76	74.50	26	25.49
Cefotaxime	161	91.47	15	8.52	83	81.37	19	18.62
Penicillin	35	19.88	141	80.11	9	8.82	93	91.17
Ofloxacin	88	50.00	88	50.00	26	25.49	76	74.50
Chloramphenicol	165	93.75	11	6.25	76	74.50	26	25.49
Gentamicin	167	94.88	9	5.11	81	79.41	21	20.59

group patients. The most effective antibiotic against MRSA in pediatric patients was found to be gentamicin. Similarly, chloramphenicol, cefotaxime were also found to be effective against MRSA infections whereas penicillin was found to be the least effective antibiotic to treat MRSA infections where 89.92 % of *S. aureus* isolates showed resistance towards this antibiotic.

Acknowledgements

Authors would like to thank Kanti Children's Hospital (KCH), Maharajgunj, Kathmandu, Nepal for laboratory facilities. Thanks also go to Department of Microbiology and Department of Chemistry, Tri-Chandra Multiple Campus, Tribhuvan University, Kathmandu, Nepal for a technical support.

References

1. Shittu AO, Jonson L: **Antimicrobial susceptibility patterns and characterization of clinical isolates of *Staphylococcus aureus*.** *BMC Infect Dis 6.* 2006, **125**: 2334-2336.
2. Rehm SJ: ***Staphylococcus aureus*: the new adventures of a legendary pathogen.** *Clev Clin J Med.* 2008 **75**(3): 177-180, 183-186, 190-192.
3. Mallick SK, Basak S: **MRSA-too many hurdle to overcome: a study from Central India.** *Trop Doct.* 2010 **40**(2): 108-110.
4. Foster TJ: **Colonization and infection of the human host by staphylococci: adhesion, survival and immune vasion.** *Vet Dermatol.* 2009 **20**(4-5): 456–470.
5. Otto M: **MRSA virulence and spread.** *Cell Microbiol.* 2012, **14**(10): 1513–1521.
6. Chambers HF: **The changing epidemiology of** *Staphylococcus aureus*. *Emerg Infect Dis.* 2001 **7**(2): 178-182.
7. Ray P, Gautam V, Singh R: **Methicillin-resistant *Staphylococcus aureus* (MRSA) in developing and developed countries: implications and solutions.** World Health Organization (WHO), Special issue on *Antimicrobial Resistance in South-East Asia. Regional Health Forum (WHO South-East Asia region)* 2011 **15**.
8. Sanford MD, Widmer AF, Bale MJ, Jones RN, Wenzel RP: **Efficient detection and long-term persistence of the carriage of methicillin-resistant *Staphylococcus aureus*.** *Clin Infect Dis.* 1994 **19**: 1123-1128.
9. Cesur S, Cokca F: **Nasal carriage of methicillin-resistant *Staphylococcus aureus* among hospital staff and outpatients.** *Infect Control Hosp Epidemiol.* 2004 **25**: 169-171.
10. Creechs CB, Kernodle DS, Alsentzer A, Wilson C, Edwards KM: **Increasing rates of nasal carriage of methicillin-resistant *Staphylococcus aureus* in healthy children.** *Pediatr Infect Dis J.* 2005 **24** (7): 617-621.
11. Eveillard M, Martin Y, Hidri N, Boussougant Y, Joly GM: **Carriage of methicillin-resistant *Staphylococcus aureus* among hospital employees: prevalence, duration, and transmission to households.** *Infect Control Hosp Epidemiol.* 2004 **25** (2): 114-120.
12. Kenner J, Connor OT, Piantanida N, Fishbain J, Eberly B, Viscount H, et al: **Rates of carriage of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* in an outpatient population.** *Infect Control Hosp Epidemiol.* 2003 **24** (6): 439–444.
13. Pokharel J, Rawal K: **Bacteriological study at T.U teaching Hospital, Kathmandu.** *Nepal J Institute Med.* 1993, **15**: 217-221.
14. Lamichhane R, Adhikari RP, Sherchand JB: **Study of methicillin-resistance *Staphylococcus aureus* (MRSA) isolated from different clinical samples.** A dissertation submitted to the Central Department of Microbiology, Tribhuvan University, Kirtipur, Nepal 1999.
15. Bhandari RR: **Prevalence and antibiotic sensitivity pattern of methicillin-resistance *Staphylococcus aureus* (MRSA) in Bir Hospital.** A dissertation submitted to the Central Department of Microbiology, Tribhuvan University, Kirtipur, Nepal 2002.
16. Kumari N, 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.
17. Sanjana RK, Shah R, Chaudhary N: **Prevalence and antimicrobial susceptibility pattern of methicillin-resistant *Staphylococcus aureus* (MRSA) in CMS- Teaching Hospital: a preliminary report.** *JCMS-Nepal.* 2010, **6**: 1-6.
18. Tiwari HK, Das AK, Sapkota D, Sivrajan K, Pahwa VK: **Methicillin-resistant *Staphylococcus aureus*: Prevalence and antibiogram in a tertiary care hospital in western Nepal.** *J Infect Dev Ctries.* 2009 **3**: 681-684.
19. Ansari S, Nepal HP, Gautam R, Rayamajhi N, Shrestha S, Upadhyay G, et al: **Threat of drug resistant *Staphylococcus aureus* to health in Nepal.** *BMC Infect Dis.* 2014 **14**: 157-162.
20. Pant MR, Shrestha D, Thapa S: **Antibiogram of bacterial species causing skin wound infections.** *NRMJ.* 2018 **2** (3): 53-60.
21. Isenberg H: **Clinical microbiology procedure handbook Vol I and II.** *American Society for Microbiology* (ASM) press, Washington DC, New York USA, 1992.
22. CLSI: **Performance standards for antimicrobial susceptibility testing.** 25th informational supplement, document M100–S25. Wayne, USA 2105, **35** (3).
23. Bergey DH, Buchanan RE, Gibbons NE: **Bergey's manual of determinative bacteriology.** *Williams and Wilkins* USA 1974.

24. Cheesbrough M: **Microbiological tests district laboratory practice in tropical countries, Part-II.** Low price edition, Cambridge University Press, India 2006, 01-267.
25. Lencastre DH, Oliveira D, Tomasz A: **Antibiotic resistant *Staphylococcus aureus:* a paradigm of adaptive power.** *Curr Opin Microbiol.* 2007 10(5): 428-435.
26. Lelievre H, Lina G, Jones ME: **Emergence and spread in French hospitals of methicillin-resistant *Staphylococcus aureus* with increasing susceptibility to gentamicin and other antibiotics.** *J Clin Microbiol.* 1999 **37:** 3452- 3457.
27. Pant J, Rai SK, Singh A, Lekhak B, Shakya B, Ghimire G: **Microbial study of hospital environment and carrier pattern study among staff in Nepal Medical College Teaching Hospital.** *NMCJ.* 2006 **8:** 194- 199.