Nepal Journal of Biotechnology
Publisher: Biotechnology Society of Nepal ISSN (Online): 2467-9313
Journal Homepage: www.nepjol.info/index.php/njb ISSN (Print): 2091-1130

Antibiotic Susceptibility Pattern of *Staphylococcus aureus* Isolated from Pus/Wound Swab from Children Attending International Friendship Children's Hospital

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Received: 04 Nov 2020; Revised: 05 Jul 2021; Accepted: 19 Jul 2021; Published online: 31 Jul 2021

Abstract
A wound gets infected when the organism gets invaded through the breached skin, proliferated and production of various enzymes, toxins, etc. In order to treat the wound infection, antibiotic susceptibility pattern of organism should be determined before the prescription of the medicine. The present study was conducted from September 2017 to March 2018 with an aim to determine antibiotic susceptibility pattern of *Staphylococcus aureus* identified from the pus/wound swab among the patients visiting the International Friendship Children's Hospital, Kathmandu, Nepal. Total 270 sample were processed, isolated and identified using standard microbiological procedure and biochemical test. Antibiotic susceptibility test was carried out by using Modified Kirby Bauer's Disc Diffusion Method. Out of total sample, 51.48% (139) showed growth. The growth distribution was found to be high in out-patient department 84.9% (118) than in-patient department 15.1% (21). Among 139 positive growth, 83.5% were gram positive and 16.5% were gram negative. All together 12 different organisms were identified, among which *S. aureus* was found to be predominant organism 105 (75.5%). *S. aureus* was found to be sensitive towards Linezolid followed by Doxycycline whereas it was found resistant towards Ciprofloxacin. Among *S. aureus* identified, 50% were Multidrug resistant (MDR) *S. aureus* and 55% were Methicillin resistance *S. aureus* (MRSA). MRSA was found to be sensitive towards Linezolid followed by Doxycycline and resistant towards Ciprofloxacin. The association between MDR and MRSA was found positively significant (i.e. p-value = 0.000). All strains of *S. aureus* were found to be sensitive towards Vancomycin. 22.86% were double disk diffusion test (D-test) positive. The prevalence of D-test was found to be high in MRSA (75%). The relationship between D-test and MRSA was found to be significantly correlated with each other (r = 0.39). Linezolid, Chloramphenicol, Vancomycin and Doxycycline is a drug of a choice for both *S. aureus* and MRSA infection.
Keywords: Pus/wound swab, *Staphylococcus aureus*, Antibiotic susceptibility test (AST), Multidrug resistance (MDR), Methicillin resistance *Staphylococcus aureus* (MRSA), D-test
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Introduction
Human skin acts as an excellent barrier to infection, protect underlying tissues, bones, organs, etc. and prevents the entry of microbes (i.e. potential pathogens) into our body unless the mechanism is breached due to injury, trauma or surgical intervention [1, 2, 3]. A break in the integrity of the skin or tissues which may be associated with disruption of the structure and compromises its protective function is called a wound [4]. A wound gets infected when proliferating microorganisms invade to a level that invokes a local or systemic response in the host [5]. During wound infection, the bacteria multiplies, healing is disrupted and wound tissues get damage and also spread to nearby tissues. The consequences of any tissue damage, wound infection or any internal tissue injury is pus [6]. Pus is defined as the accumulation of dead cells and microorganisms, together with accumulated fluid and various proteins [7].

Wound infection is a common problem during injury, mainly in the case of children [4, 8]. Injuries in the children may be due to falls followed by burns, cuts and animal bites which causes both financial and psychological strain on the family because it drags the patient to the health care facilities [9, 10]. Wound infection account for 70-80% mortality and also an important cause of morbidity among surgical patients and 75% of mortality following burn injuries [11, 12, 13]. The common organism responsible for pus formation or wound infection are: Coagulase negative *S. aureus* (CONS). *S. aureus*, *Bacillus* spp., *Clostridium* spp., *Peptostreptococcus* spp., *Actinomyces* spp., *E. coli*, *Proteus* spp., *Neisseria* spp., *Vibrio vulnificus*, *Candida* spp., etc. [14].

S. aureus is a versatile pathogen capable of causing a wide range of human diseases [15]. It is a significant human pathogen that causes wound infection, soft tissue infection and produces the pus [16, 17]. It belongs to the family Micrococcaceae, gram positive cocci having grape like cluster arrangement of 0.5-1.5 µm diameter, aerobic, facultatively anaerobic, β-hemolytic, fermentative, oxidase negative, non-sporing, non-motile, non-capsulated, yellow zone formation around the colonies on MSA and oil paint appearance on NA slopes [18, 19]. There has been a huge problem all over the world in the treatment of infectious disease due to increase in antibiotic resistant cases [20]. Multi-drug resistant (MDR) is defined as the non-susceptibility of organism to at least one agent in 3 or more antimicrobial categories, extremely drug resistance (XDR) is non-susceptibility to at least 1 agent in all but 2 or fewer antimicrobial categories and pan drug resistance (PDR) is non-susceptibility to all agents in all antimicrobial categories [21]. Methicillin resistant *S. aureus* (MRSA) has been identified as one of the major risk pathogens associated with the development of antimicrobial resistant [22]. MRSA is defined as a strain of *S. aureus* that is resistant to a large group of antibiotics called β-lactams, which include Penicillin and Cephalosporin [23]. In Nepal, various laboratories have reported the emergence of MRSA mainly community-associated MRSA (CAMRSA) which have been detected in the Lumbini medical college and teaching hospital while doing cross-sectional studies of prevalence of MRSA [24]. In another study, study carried out to assess the extent of MRSA in the Kathmandu Model Hospital Kathmandu, MRSA were more frequently isolated from pus samples and that too from hospitalized patients [23].

Vancomycin is a glycopeptide antibiotic that inhibits cell wall biosynthesis, remains a drug of choice for treatment of severe MRSA infections. *S. aureus* isolates with complete resistance to Vancomycin (MIC≥16µg/ml) are termed as Vancomycin resistant *S. aureus* (VRSA). VRSA was first reported in the U.S in 2002 [25]. In one of the studies conducted in the Manmohan Memorial College and Teaching Hospital, Kathmandu, Nepal, there all the MRSA identified was found to be susceptible towards the Vancomycin [26].

D-test is a simple disc diffusion test to study the macrolide lincosamide streptogramin B resistance (MLSB), both constitutive and inducible as well as macrolide streptogramin B resistance (MSB) in *S. aureus*. Macrolide group (Erythromycin, Azithromycin, Rokitamycin) is a drug used to treat of *S. aureus* infection and also used for those allergic to the Penicillin [24]. After a few years of drug's introduction in therapy, staphylococci developed resistance to Erythromycin in 1956. These resistant strains were found in France, U.K and in the U.S.A [27]. Lincosamide (Clindamycin) is used for the treatment of MRSA infection [28]. Since these both antibiotics have the same site of drug target, there is a high chance of cross resistant among these antibiotics due to modification of drug target [29]. This study helps to perceive the current status in prevalence of *S. aureus* in pus/wound swab, the antibiotic susceptibility pattern of the isolated *S. aureus* and also any presence of multidrug resistant strain among the isolates. It also helps to know the resistant towards commonly used antibiotic and aware the practitioner from misusing the antibiotic. Hence, the aim of the study was to assess the prevalence of *S. aureus* and the antibiotic susceptibility pattern of *S. aureus* isolated from the pus/wound swab from children attending International Friendship Children's Hospital (IFCH), Maharajgunj, Kathmandu.

Materials and Methods

Sample collection and identification of isolates

The research was conducted at the Microbiology Laboratory of International Friendship Children's Hospital, Maharajgunj, Kathmandu from September 2017 to March 2018. A hospital based cross-sectional study was carried out among the patients visiting to the hospital having wound infection below 16 years, requesting for culture and susceptibility testing.

In total, 270 pus/wound swab samples were collected using aseptic technique. Out of total sample, 228 samples were collected from out-patient department (OPD) and 42 samples were collected from in-patient department (IPD). Within IPD also, 12 samples were collected from general ward (GW), 4 samples from special ward (Sp. ward), 12 samples from surgical/burn ward (S/B ward), 6 samples from infant ICU (IICU), 3 samples from pediatric ICU (PICU), 4 samples surgical ICU (SICU) and 1 sample from neonates ICU (NICU). Here, 130 samples were of male and 140 samples were of female. 17 samples were of age group 0-1 month, 54 of 1 month-1year, 75 of 1-3 years, 54 of 4-6 years, 57 of 6-12 years and 13 of 12-15 years.

The specimens were well labelled and then transported to the laboratory and processed immediately. After macroscopic and microscopic observation, it was cultured on Blood Agar (BA) and Mac-Conkey Agar (MA) and incubated at 37°C for 24 hrs. The isolates were identified by colony morphology, gram staining and

various biochemical tests [30]. The gram-positive cocci in cluster observed under microscope was considered as *Staphylococcus* species and was subjected under different biochemical test for the confirmation of *S. aureus.* The *Staphylococcus* species showing catalase positive, oxidase negative, fermentative, yellow colony on mannitol salt agar (MSA), coagulase positive and DNase positive were confirmed as *S. aureus* [30]. For gram negative organism, different biochemical tests such as: catalase test, oxidase test, Sulphur Indole Motility (SIM) test, methyl red (MR) test, Voges-Proskauer (VP) test, citrate test, oxidative/fermentative (O/F) test, urease test and triple sugar iron (TSIA) test were performed for the identification of the organism.

Antibiotic susceptibility test

The antibiotic susceptibility testing was done by using modified Kirby-Bauer disc diffusion method [31] on Mueller Hinton agar using antibiotic discs of Hi-Media Laboratories Pvt. Ltd. The antibiotic used was selected by following Clinical and Laboratory Standards Institute (CLSI) 2017 guideline [31] for *S. aureus.* The antibiotics used were Cotrimoxazole (1.25/23 mcg), Chloramphenicol (30 mcg), Ciprofloxacin (5 mcg), Gentamycin (10 mcg), Doxycycline (30 mcg), Linezolid (30 mcg), Vancomycin (30 mcg), Azithromycin (15 mcg), Meropenem (10 mcg) and Piperacillin (100/10 mcg). Novobiocin (30 mcg) was used to identify *S. epidermidis* and *S. saprophyticus*. If the identified *S. aureus* was found to be resistant to at least one agent in three or more antimicrobial categories, then the organism was considered as multidrug resistant (MDR) and if the identified *S. aureus* was found to be resistant to at least 1 agent in all but 2 or fewer antimicrobial categories, then it was considered as extremely drug resistant (XDR) [21]. After screening MDR, the identified *S. aureus* was then screened for Methicillin resistant *S. aureus* (MRSA) using Cefoxitin disc (30 mcg).The organisms resistant i.e. ≤21 mm Zone of inhibition (ZOI) towards the Cefoxitin were confirmed as MRSA and those sensitive were confirmed as Methicillin sensitive *S. aureus* (MSSA) [25]. If the organism was found to be Vancomycin resistant in disc diffusion method, it was further processed for the confirmation of Vancomycin resistant *S. aureus* (VRSA) by using minimum inhibitory concentration (MIC) method [31]. *S. aureus* ATCC 25923 was used as control strain.

D-test

D-test was performed by using Erythromycin disc (15 mcg) and Clindamycin disc (2 mcg). The antibiotic discs were placed on a lawn cultured MHA plate at 15 mm apart and was incubated at 37° C at 18-24 hrs [24]. The organisms that showed flattening zone of Clindamycin adjacent to the Erythromycin disc were considered as D-test positive (MLSBi resistant, i.e. Inducible macrolide-lincosamide-streptogramin B resistance). If the organism was found to be resistant towards both discs then, it was taken as Constitutive MLSB (MLSBc) and if organism showed sensitive towards Clindamycin but resistant towards Erythromycin, then it was taken as D-test negative [24].

Data analysis

All the data was entered in Statistical Package for the Social Science (SPSS) version 16. Most of the data was analysed by using SPSS version 16 (SPSS for Windows, Chicago, SPSS Inc). The association between MDR and MRSA was determined by performing chi-square test analysed by SPSS version 16 whereas the correlation coefficient between D-test and MRSA was calculated by using statistical method, i.e. Karl Pearson's correlation coefficient. In the study, we used Pearson's chi-square test to test whether MRSA influences the increase in MDR cases or not whereas Karl Pearson's correlation coefficient test was used to test whether there is significant correlation between MRSA and D-test.

Results

Growth pattern of culture and distribution of culture positive within the departments

Out of 270 pus/wound swab samples, 139 (51.48%) were found to be culture positive while remaining 131 (48.52%) showed no growth. OPD showed highest positive culture 118 (85%) compared to that of the IPD 21 (15%). Within the hospital department, highest growth was seen in the department of Neonates ICU (NICU) 100% (1/1) followed by Surgical ICU (SICU)75% (3/4), Infant ICU (IICU) 66.67% (4/6), OPD 51.75% (118/228), Surgical/Burn ward (S/B ward) 50% (6/12), General ward (GW) 41.67% (5/12), Pediatric ICU (PICU) 33.33% (1/3) and lowest in Special ward (Sp. ward) 25% (1/4) **(Figure 1).**

Bacteriological profile of pus/wound swab

In the study, 116 (83.5%) out of 139 were gram positive and 23 (16.5%) were gram negative. Out of total 139 culture positive cases, *S. aureus* 105 (75.5%) was found to be common isolates followed by *Escherichia coli* 7 (5.04%) and *Staphylococcus epidermidis* 7 (5.04%); *Pseudomonas aeruginosa* 6 (4.3%); unidentified organism 4 (2.9%); *Enterococcus* 2 (1.45%), *Proteus mirabilis* 2 (1.45%) and *Staphylococcus saprophyticus* 2 (1.45%); *Salmonella* Typhi 1

(0.72%), *Klebsiella oxytoca* 1 (0.72%), *Klebsiella pneumoniae* 1 (0.72%) and *Citrobacter* species 1 (0.72%) **(Table 1)**.

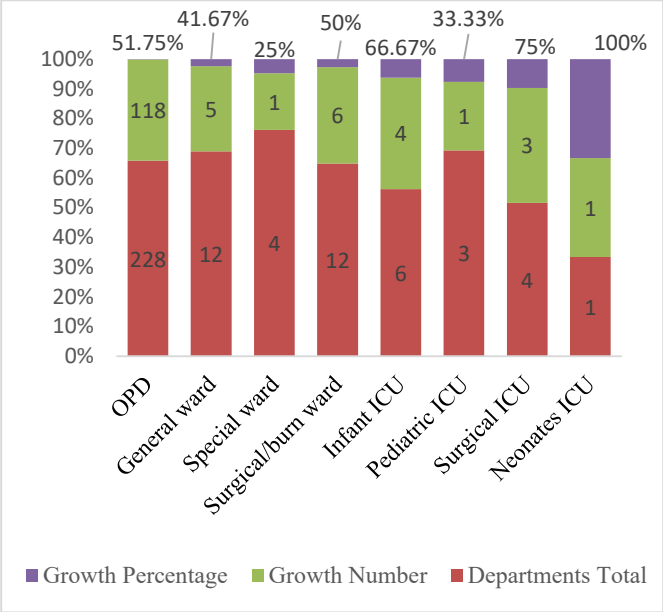

Figure 1. Distribution of culture positive cases within the departments.

Table 1. Bacteriological profile of pus/wound swab

Microorganism identified	Number	Percentage
S. aureus	105	75.5%
E. coli	7	5.04%
Citrobacter spp.	1	0.72%
P. aeruginosa	6	4.3%
Enterococcus	2	1.45%
S. saprophyticus	2	1.45%
S. epidermidis	7	5.04%
S. Typhi	1	0.72%
P. mirabilis	2	1.45%
K. oxytoca	1	0.72%
K. pneumoniae	1	0.72%
Unidentified	4	2.8%
Total	139	100.00%

Distribution of *S. aureus* according to the gender, age and within the hospital departments

Among 105 positive sample showing *S. aureus*, 51% (54) were found to be female patient and 49% (51/105) were male patient. The highest prevalence of *S. aureus* was found among age group 12-15 yrs. 87.5% (7/8) followed by the age group 1-3 yrs. 84.21% (32/38), age group 1 month-1 yrs. 76.92% (20/26), age group 6-12 yrs. 75% (24/32), 0-1 month 71.42% (10/14) and age group 4-6 yrs. 57.14% (12/21) **(Figure 2)**. Most of the *S. aureus* was highly isolated from IICU department 100% (4/4), PICU 100% (1/1), NICU 100% (1/1) followed by GW 80% (4/5), OPD 78.81% (93/118), S/B ward 33.33% (2/6) and no *S. aureus* were isolated from Sp. ward) 0% (0/1) and SICU 0% (0/3) **(Table 2)**.

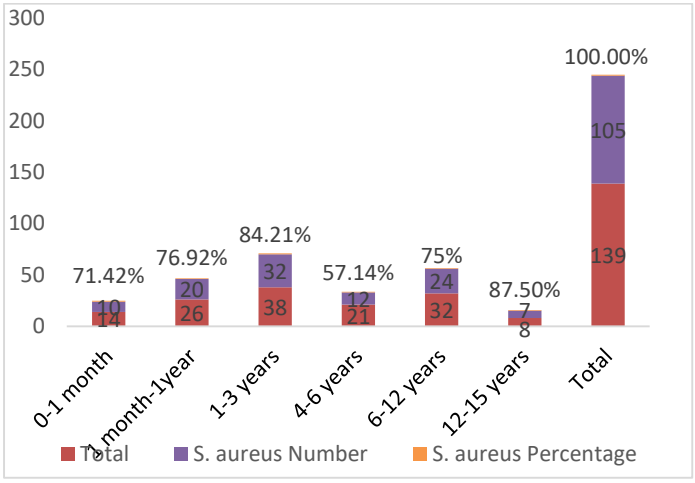

Figure 2. Distribution of *S. aureus* within the age group of the patient

Table 2. Distribution of *S. aureus* within hospital departments

Departments	*S. aureus*		
	Total	Number	Percentage
OPD	118	93	78.81%
General ward	5	4	80%
Special ward	1	0	0%
Surgical / burn ward	6	2	33.33%
Infant ICU	4	4	100%
Pediatric ICU	1	1	100%
Surgical ICU	3	0	0%
Neonates ICU	1	1	100%
Total	139	105	75.5%

Table 3. Antibiotic susceptible pattern of S. aureus (N= 105)

Antibiotic used	Antibiotic Susceptible Pattern		
	Resistant	Intermediate	Sensitive
Gentamycin	11(10%)	9(9%)	85(81%)
Ciprofloxacin	71(68%)	10(10%)	24(23%)
Chloramphenicol	4(4%)	4(4%)	97(92%)
Cotrimoxazole	26(25%)	8(8%)	71(68%)
Cefoxitin	58(55%)	0(0%)	47(45%)
Erythromycin	58(55%)	18(17%)	29(28%)
Clindamycin	28(27%)	2(2%)	75(71%)
Piperacillin	4(4%)	5(5%)	96(91%)
Meropenem	1(1%)	2(2%)	102(97%)
Azithromycin	54(51%)	7(7%)	44(42%)
Doxycycline	0(0%)	2(2%)	103(98%)
Linezolid	1(1%)	0(0%)	104(99%)
Vancomycin	9(9%)	0(0%)	96(91%)

Antibiotic susceptibility pattern of *S. aureus* and Multidrug resistant (MDR) *S. aureus*

While performing antibiotic susceptibility test **(Figure 3)**, out of 105 *S. aureus*, 104 (99%) were found to be sensitive towards Linezolid followed by Doxycycline 103 (98%), Meropenem 102 (97%), Chloramphenicol 97 (92%) and Vancomycin 96 (91%). The organism was found to be

Table 5. Correlation between MRSA and D-test

	D-test positive	D-test negative	Constitutive resistant	Sensitive	Total	r value
MRSA	18 (75%)	8(38.1%)	20 (69%)	12 (38.71%)	58	0.39
MSSA	6 (25%)	13 (61.9%)	9 (31%)	19 (61.29%)	47	
Total	24	21	29	31	105 (100%)	

resistant towards Ciprofloxacin 71 (68%) followed by Cefoxitin 58 (55%) and Erythromycin 58 (55%) respectively **(Table 3)**. In our study, 50% (52) were found to be multidrug resistant. Among multidrug resistant also, one strain was found to be resistant to all the antimicrobial agent used to be tested, i.e. extremely drug resistant (XDR).

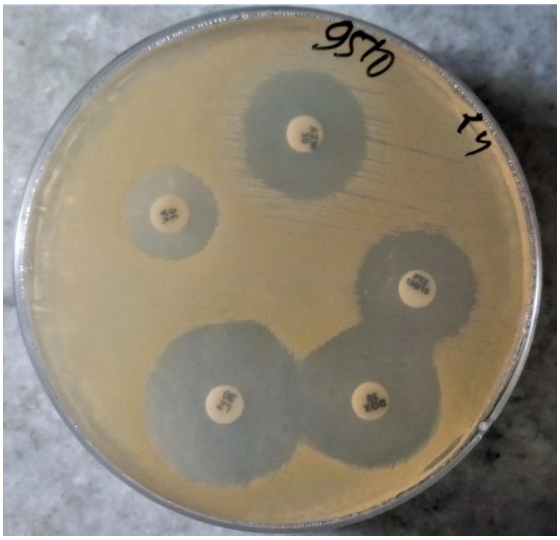

Figure 3. Antibiotic susceptibility test of *Staphylococcus aureus* on MHA. (VA = Vancomycin, AZM = Azithromycin, PTZ = Piperacillin, DOX = Doxycycline and LZ = Linezolid).

Distribution of MRSA among *S. aureus* positive sample and its antibiotic susceptibility pattern

In the study, 55% (58) were found to be Cefoxitin resistant showing Methicillin resistant strains (MRSA) whereas 45% (47) were found to be Cefoxitin sensitive showing Methicillin sensitive strains (MSSA). All the resistant strains were further tested for Vancomycin susceptible test.

Table 4. Antibiotic Susceptibility Pattern of MRSA (N=58)

Antibiotics	Resistant	Intermediate	Sensitive
Cotrimoxazole	18(31.04%)	3(5.17%)	37(63.79%)
Chloramphenicol	3(5.17%)	3(5.17%)	52(89.66%)
Gentamycin	6(10.35%)	7(12.07%)	45(77.58%)
Ciprofloxacin	51(87.93%)	6(10.35%)	1(1.72%)
Clindamycin	21(36.21%)	0(0%)	37(63.79%)
Erythromycin	38(65.53%)	8(13.79%)	12(20.68%)
Piperacillin	4(6.9%)	4(6.9%)	50(86.20%)
Meropenem	1(1.72%)	1(1.72%)	56(96.56%)
Azithromycin	43(74.13%)	5(8.62%)	10(17.25%)
Linezolid	1(1.72%)	0(0%)	57(98.28%)
Doxycycline	0(0%)	2(3.44%)	56(96.56%)
Vancomycin	9(15.52%)	-	49(84.48%)

Here, MRSA was found sensitive towards Linezolid 98.28% (57) followed by Doxycycline 96.56% (56), Meropenem 96.56% (56), Chloramphenicol 89.66% (52), Piperacillin 86.20% (50), Vancomycin 84.48% (49), Gentamycin 77.58% (45), Cotrimoxazole 63.79% (37) and Clindamycin 63.79% (37). MRSA was found to be resistant towards Ciprofloxacin 87.93% (51) followed by Azithromycin 74.13% (43), Erythromycin 65.53% (38) and was found to be zero resistant towards Doxycycline. **(Table 4)**.

VRSA and MIC

Among isolated *S. aureus*, 9 were found to be resistant towards the Vancomycin disc. While performing minimum inhibitory concentration test, all positive strains were found to be sensitive towards Vancomycin in a very low concentration, i.e. 0.25 µg/ml and minimum bactericidal concentration was found to be 0.25 µg/ml.

Association between MDR and MRSA

In the study, 44 (84.61%) MRSA were found to be MDR and 14 (26.42%) MRSA were found to be MDR negative. By analyzing the data of MDR and MRSA using chi-square test, the value was found to be chi-square (1, N=105) =35.958, p<.01. Therefore, MDR was found to be statistically significant associated with MRSA.

D-test of *S. aureus* and Correlation between D-test positive and MRSA

In D-test (**Figure 4**), out of total 105 *S. aureus* identified, 24 (22.86%) were found to be D-test positive, 21 (20.0%) were D-test negative, 31 (29.52%) were sensitive to both Erythromycin and Clindamycin and 29 (27.62%) were constitutive resistant (**Table 5**).

Figure 4. Double disk diffusion test (D-test) on MHA medium showing sensitive (K) and positive result (L). CD = Clindamycin and E = Erythromycin)

Here, 18 (75%) MRSA were D-test positive, 8 (38.1%) MRSA were D-test negative, 20 (69%) MRSA were constitutive resistant and 12 (38.71%) MRSA were sensitive as shown in **Table 5**. The correlation coefficient (r) between D-test and MRSA was found to be 0.39 (r = .313, p<.01), i.e. D-test and MRSA was found to be positive but lowly correlated with each other.

Discussion

Out of total sample, 139 (51.48%) showed growth and 131 (48.52%) showed no growth. The growth result was found nearly similar to the study conducted by Hanumanthappa et al, where they found 56% growth rate [32]. The result was lower to the study conducted by Rai et al [10] 58.6%; Khan et al [33] 65.2% and Patil et al [34] 86%. The lower growth might be due to difficult-to-grow fastidious organisms, inappropriate methods of collection and transportation of specimens or the administration of antibiotics prior to specimen collection. Among 139 positive growth result, 118 (85.65%) were found to be positive from out-patient department and 21 (14.4 %) from in-patient department. High prevalence of growth in OPD might be due to increase in community acquired infection. Higher positive growth in OPD was also found in the study carried by KC et al [35] and found contrary to the study carried out by Pant et al, where they found 63.1% from IPD and 56.2% from OPD [36].

Out of total growth 139, 116 (83.5%) were found to be Gram-positive and 23 (16.5%) were found to be gram negative. The high prevalence of Gram-positive organism might be due to the presence of Gram-positive bacteria as a normal flora of the human body. The result was found to be similar to the research conducted by KC et al [35]; Devi et al [37] and Pant et al [36]. The result was found contrast to the study conducted by Patil et al (2019)78% gram negative and 22% Gram-positive bacteria [34].

The predominance of *S. aureus* (75.5%) in the study might be due to *S. aureus* being normal flora of skin, glands, nails, etc. and having various virulence factors. The result was seems to be related to the study conducted by Sultana et al (2015) 40.45% *S. aureus* followed by *E. coli* 28.18% [38]; Barakoti et al (2017) 41.45% *S. aureus* followed by *E. coli* 22.79% [39]; Bankar et al (2018) 34.21% *S. aureus* followed by *E. coli* 23.02%[40] and Shahi et al (2018)70.6% *S. aureus* [41]. However Mahat et al [42] and Patil et al [34] *Pseudomonas* spp. as predominant organism.

The infection in the age group 12-15 yrs. might be due to the various activities performed in the school, environment, involved in fight, their playmates and contact with various object. The children under 5 years are also prone to get pus/wound infection because of unintentional falls, burns, etc. and not only that these group also has low immune power to overcome any kind of infection, therefore it is likely to get infected. The outcomes were found to be contrast with the study conducted in 2017 by Pokhrel et al, where they got higher prevalence of *S. aureus* in age group 1-3yrs [43]; Rai et al in age less than 1 year [10] and Pant et al in age group 1-5yrs [36].

The distribution of *S. aureus* among gender was found to be high in female patient 54 (51%) than the male patient 51 (49%). The finding resembled with the research conducted by Muluye et al [20] and Bhatt et al [44]. The result obtained from our study was contrast to the research conducted by Shrestha et al [28] and Garoy et al [45].

The high prevalence of *S. aureus* in ICU departments might be due to the colonization of *S. aureus* from patient's own flora, transmission through staff hands, air, procedure of surgery, inanimate object, longer period of hospital stays, etc. Similar study was carried out by Bhatta et al. (2014) who had reported higher prevalence of *S. aureus* in hospital setting accounting [44].

S. aureus was found to be highly sensitive towards Linezolid (99%) followed by Doxycycline (98%). The outcome was found similar to the study conducted by Nirmala et al [2] of 100% sensitive towards Linezolid and Vancomycin and by Khan et al [33]. It was found to be a bit different from the research carried out in 2018 by Tadesse et al [46] in which they found 100% sensitive towards Ampicillin.

From the study, out of 105 isolates, 52 (50%) were found to be MDR. Among MDR also one strain was found to be resistant to all the antimicrobial agents to be tested (Extremely drug-resistant). MDR cases may be due to accumulation of multiple genes, expression of genes that code for multidrug efflux pumps, extruding a wide range of drugs, mutational alteration of the target protein, enzymatic inactivation of drugs, etc. [47].

Here, 44 (84.61%) MDR were found to be MRSA and 8 (15.39%) MDR were found be MSSA. The increase in MDR in MRSA may be due to a distinctive feature of MRSA, i.e. their resistance to β-lactam antibiotics. Therefore, once the *S. aureus* is resistant to Methicillin, it may also show resistance towards other antibiotic classes like: aminoglycosides, macrolides, tetracycline, chloramphenicol and lincosamide. Our result was lower in comparison to Upreti et al [48] with 68.2% MDR, Pahadi et al [49] with 86.41% were MDR; Tadesse et al

[46] 82.3% MDR; whereas higher than he study conducted by Kadariya et al [50] with 44.2% were MDR and Mama et al with 27.8% were MDR [51].

The strong association between the MDR and MRSA was found (p<.01) while performing Pearson chi-square test. Hence, we can say that the prevalence of MDR increases as the prevalence of MRSA increased. The data obtained from the research was found to be similar to the study conducted by Joachim et al in which 21.3% were MDR, out of which 72.7% of MRSA strains were MDR showing statistically significant association between MRSA and MDR among *S. aureus* isolates (p=0.001) [52].

The prevalence of MRSA was found to be 58 (55%) and methicillin sensitive *S. aureus* (MSSA) was found to be 47 (45%). The study resembles to the study carried out by Devi et al (2017), where 50.79% were MRSA and 49.21% were MSSA [37]. However, the study was in contradiction to the study carried by Kayastha et al (2010) 8.92% MRSA [23]; Ansari et al (2014) 43.1% MRSA [53]; Jaiswal et al (2016) 72% MRSA [54] and Adhikari et al (2017) 35.50% MRSA [55].

Since our research was conducted from September 2017 to March 2018, the prevalence of MRSA seems to be increasing in Nepal as well [56, 53, 55, 57, 48, 45, 41]. The development of resistance of *S. aureus* towards Methicillin may be due to the acquisition of staphylococcal chromosome cassette mec (SCC mec) in its chromosome, which carries a mec A gene facilitating resistance to Methicillin via Penicillin binding protein (PBP-2a). Although the acquisition of the mecA gene, the organism cannot exhibit resistant towards Methicillin unless the gene is activated.

MRSA was found to be sensitive towards Linezolid 98.28% (57) followed by Doxycycline 96.56% (56) and resistant towards Ciprofloxacin 87.93% (51) followed by Azithromycin 74.13%. Similar sensitive pattern in MRSA was found in the study carried out by Choudhury et al (2016) in which organism was found sensitive towards Linezolid (99.3%), Vancomycin (99.3%) and resistant towards Cefuroxime (59.50%) [58].

In our study, Vancomycin resistant was found to be 9% (9/105) from disc diffusion method but while performing the MIC, *S. aureus* was found to be 100% sensitive towards Vancomycin, i.e. 0.25 µg/ml and MBC was found to be 0.25 µg/ml. Hence, isolated *S. aureus* was found to be 100% susceptibility towards Vancomycin. Therefore, we need to perform MIC for the confirmation of Vancomycin resistant strain. The cause of Vancomycin resistance may be due to the activation of van A and van B gene. The finding was found to be similar to the research conducted by Kshetry et al, where organism was found sensitive towards Vancomycin while performing MIC test [59] and study by Bamigboye et al showed 1.4% VRSA but found to be van A and van B gene negative [25].

From the study, only 22.86% (24) were found to be D-test positive, 20% (21) were found to be D-test negative, 29.52% (31) were found to be susceptible to both Erythromycin and Clindamycin and 27.62% (29) were found to be constitutive resistant. The resistance of the Erythromycin and Clindamycin may be due to the resistance encoded in Erythromycin methylase (*erm*) genes. The constitutive expression may be due to the organism being resistant to all macrolides, lincosamides and type B streptogramin antibiotics. The study resembled to the study carried out by Mama et al [51] with 24.1% D-test positive, 1% D-test negative, 2% constitutive D-test and 60.85% sensitive towards Erythromycin and Clindamycin.

In this study, D-test positive was also seen high in MRSA 75% (18/24) compare to the MSSA 25% (6/24). Similar result was obtained in research conducted by Pal et al [60]. The correlation (r) between D-test and MRSA was found to be 0.39 which means D-test and MRSA are positively but lowly correlated, i.e. D-test cases may increase as increase in MRSA cases. The result obtained was contrast with the study carried out by Gosh et al [61].

The increase in reported inducible Clindamycin resistant shows the increase in prevalence of inducible Clindamycin resistance along with constitutive resistant among the clinical isolates of *S. aureus*. Hence, the screening of inducible Clindamycin resistant should be done in every clinical laboratory.

Conclusion

Prevalence of wound infection was found to be high (51.48%) in our study. The growth rate was found high in OPD patient than IPD. *S. aureus* was predominant organism followed by *E. coli* and *S. epidermidis*. The prevalence of *S. aureus* was seen high in the age group of 12-15 years. The cases were also seen high in the department of IICU, PICU, and NICU. High prevalence of MRSA was observed in this study. The isolates were sensitive mainly towards Linezolid, Doxycycline, Meropenem, and Chloramphenicol, Vancomycin. The organism was found highly resistant towards Ciprofloxacin. 50% of isolates were found to be MDR. Among MDR, one strain was found to be XDR. MDR was mainly found in MRSA than MSSA strain. Hence, all MRSA are considered as MDR. D-test positive cases was found higher in MRSA cases. Since, inducible D-test has

been reported, it is necessary to screen the inducible Clindamycin resistance before the prescription of the medication for the effective treatment of infection. Vancomycin, Linezolid, Doxycycline, Meropenem, and Chloramphenicol were effective drug for *S. aureus* and MRSA.

Author's contribution

BM conducted laboratory experiments, data analysis, interpretation and manuscript writing; STK designed the research conception, reviewed the manuscript; RM designed the research, contributed in data analysis, manuscript writing, reviewing and editing. All authors read and approved the final manuscript.

Competing interests

We have read Nepal journal of biotechnology policy on declaration of competing interest and declare that we have no competing interests.

Funding

The author(s) declared that no grants were involved in supporting this work.

Acknowledgements

We are very beholden for the support provided by International Friendship Children's Hospital and the St. Xavier's College, Kathmandu, Nepal.

Ethical approval and Consent

This research was approved by Nepal Health Research Council (NHRC), Kathmandu, Nepal (Ref. no.-2610), International Friendship Children's Hospital, Maharajgunj, Nepal and the St. Xavier's College, Kathmandu, Nepal. Informed consent was obtained from parents of participants before their participation.

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