Prevalence of Methicillin Resistant *Staphylococcus aureus* among Dumpsite Workers in Kathmandu Valley

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ABSTRACT

Objectives: The main objective of this study was to determine prevalence of methicillin resistance *Staphylococcus aureus* among dumpsite workers.

Methods: Total 60 nasal swab samples were collected. Conventional microbiological methods were used to isolate and identify *S. aureus.* Antibiotic susceptibility test was performed by Kriby Bauer disc diffusion method. MRSA was confirmed by using Cefoxitin disc. The organism showing resistance against three or more class were considered as MDRSA.

Results: The prevalence rate of *S. aureus* and MRSA was found to be 46.67% (28/60) and 6.67% (4/60) in total population. The nasal carriage rate of *S. aureus* was found to be higher in age group 28-37 (70.06%, 12/17), gender male (47.5%, 19/40), district Kathmandu (70%, 14/20) and dumpsite workers (50%, 15/30). The isolated *S. aureus* were resistant to Penicillin (35.7%), Erythromycin (35.7%), Cefoxitin (14.3%) and Oxacillin (10.7%). *S. aureus* was susceptible (100%) to Tetracycline, Gentamycin, Ciprofloxacin, Co-trimoxazole, Chloramphenicol, and Vancomycin. Multidrug resistant *S. aureus* was not found in community of Kathmandu Valley.

Conclusion: The study shows prevalence of MRSA strains of *S. aureus* in Kathmandu Valley. The occurrence of MRSA indicates development of Community acquired-antibiotic resistant bacteria.

Keywords: *S. aureus,* MRSA, dumpsite workers, multidrug resistant *S. aureus* (MDRSA)

INTRODUCTION

S. aureus, a commensal normal flora of skin and anterior nares of human body, has been arising as a potentially serious pathogen. Over a period, it became able to adapt to the selective pressure of antibiotics such as Penicillin followed by Methicillin resulting in the emergence and spread of (MRSA) Methicillin Resistant *Staphylococcus aureus* (Deurenberg et al 2008)*.* Its resistivity has been seen in different classes of antibiotic including various other β-lactam antibiotics, aminoglycosides, tetracycline etc. harboring a multi-drug resistant (MDR) strains (Chen and Huang 2014).

S. aureus is alleged for inducing human infections in both the hospital as well as community setting (Uhlemann et al2014; Bonesso et al 2014) with a plethora of infections ranging from simple soft skin tissue infection to invasive life scaring bacteremia and septicemia (Bhatta et al 2015). In present days, MRSA strains are found to be responsible for causing infection such as minor skin and soft tissue infections(SSTIs) in community setting, so called Community acquired Methicillin Resistant *Staphylococcus aureus* (CA-MRSA) (Kawada-Matsuo et al 2012).

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About 5-10% of CA-MRSA infection are invasive and live scaring (Uhlemann et al 2014). The origination of CA-MRSA is still a subject of debate and are presumed to be feral descendant of hospital isolates and horizontal transfer of methicillin resistant determinant. Besides, different studies also showed that contamination in the environment could be the source of CA-MRSA infections (Boyce 2007)

Recent studies have shown that the prevalence rate of MRSA in different parts of Nepal ranges from 39% to 69% and the burden of MRSA infection is high in Asia (Bhatta et al 2015; Subedi and Brahmadathan 2005). The study on prevalence of MRSA colonization in the community, in case of low-income countries, are missing. Dumpsite workers are continuously exposed to different type of wastes, so are more prone to *S. aureus* colonization causing CA-MRSA infection and can serve as asymptomatic carriers disseminating infections in the community. Recent studies have reported the increasing infection by CA-MRSA and the emergence of MDR strains leading to a serious public health threat (Boswihi and Udo 2018). Therefore, the prevalence of CA-MRSA colonization and infection needs to be better explained. Thus, this type of study would be helpful for promoting the public health by increasing awareness in maintaining personal and public hygiene, reducing the over exploitation of antimicrobials and the incidence of resistant gene.

METHODS

Study design, site and period

The study was conducted at Microbiology laboratory of St. Xavier's College (Maitighar, Kathmandu) to gain knowledge regarding the prevalence of methicillin resistance *S. aureus* among dumpsite workers in Kathmandu Valley and covered the total period of five months from August 2018 to January 2019.

Study population and sample size

Total 60 nasal swab samples were collected from different places of Kathmandu valley after receiving the ethical approval from Nepal Health Research Council (NHRC) **(Reg. no.:584/2018)**. Among 60 samples, 30 samples were obtained from dumpsite workers (10 samples each from Kathmandu, Lalitpur and Bhaktapur district) and 30 samples from public were taken as control. Informed consent was taken before the sample collection. The

samples of dumpsite workers were collected from their attendance office i.e. Kathmandu (Ward no. 11 office, Thapathali), Lalitpur (Lalitpur metropolitan office, Balkumari) and Bhaktapur (Suryabinayak Municipality Office). Similarly, for control group, the samples of residents visiting the laboratory area from each districts were collected.

Isolation

Nasal swabs were collected and transported to laboratory in peptone broth within 2 hours by maintaining 4°C in the icebox (Higgins 2008). The sample was cultured on Mannitol Salt Agar (MSA) and *S. aureus* were identified colonies showing yellow colonies on MSA, Gram positive cocci in cluster, catalase positive, oxidase negative, coagulase positive and fermentative on O/F test. All the reagents and media were used of HiMedia Company.

Susceptibility testing

All the *S. aureus* isolates obtained were subjected to in vitro antibiotics susceptibility testing by Kirby- Bauer disc diffusion method as recommended by CLSI (2014)**.** The screening of Methicillin Resistant *Staphylococcus aureus* (MRSA) was done by using Cefoxitin disc (30μg). The organism showing resistance against three or more class were considered as MDRSA (Magiorakos et al 2011).

Data analysis

Raw data obtained from laboratory investigation were tabulated and presented in defined tables and graphs to explore the findings. The data were analyzed using Excel and IBM SPSS statistics 21.0 version software.

RESULTS

Growth profile analysis

Out of 60 nasal swab sample received, 44/60 (73.33%) samples showed growth in MSA plates while 16 /60 (26.67%) samples showed no growth. 63.63% (28/44) were found to be *S. aureus* and 36.36% (16/44) were found to be other bacteria. (Figure 1).

Antibiotic sensitivity pattern for *Staphylococcus aureus*

Among 28 *S. aureus* isolates, 35.4%, 14.3%, 10.7% and 35.7%, were resistant to Penicillin, Cefoxitin, Oxacillin and Erythromycin respectively while all the isolates were sensitive towards Tetracycline, Gentamicin, Ciprofloxacin and Cotrimoxazole. Out of 28 isolates, four isolates were resistant to Cefoxitin and were assumed MRSA and 3 isolates were resistant to Oxacillin and hence, were

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assumed to be ORSA (Oxacillin Resistant *Staphylococcus aureus*). (Table 1)

Distribution of *Staphylococcus aureus* and MRSA on different basis

S. aureus was found in 47.5% [19/40] male and 45% [9/20] female participants. Similarly, 7.5% [3/40] and 5% [1/20] of male and female subjects respectively harbor MRSA strains.

This study includes participants of the age between 18-58 years old. Colonization with MRSA was highest 11.76% [2/17] among the age group 28-38 years, followed by the age group of 18-37 years [10.53%, 2/19].

This study includes participants from three districts i.e. Kathmandu, Bhaktapur and Lalitpur. The colonization

MRSA was higher in Lalitpur [15%, 3/20], followed by Kathmandu [5%, 1/20] and no colonization in Bhaktapur. The study showed that the colonization of MRSA was higher in Dumpsite worker [10%, 3/30] than in community people [3.33%, 1/30]. However, there was no significant association between the bacterial load (MRSA) and Population type. (p= 0.557) (Table 2).

Antibiotic sensitivity pattern of MRSA isolates

About 25% (1/4), MRSA isolate was found to be resistant against Erythromycin and all 4 isolates were susceptible to Tetracycline, Gentamicin, Ciprofloxacin, Vancomycin and Chloramphenicol. There was no prevalence of MDR strains of *S. aureus*. (Table 3)
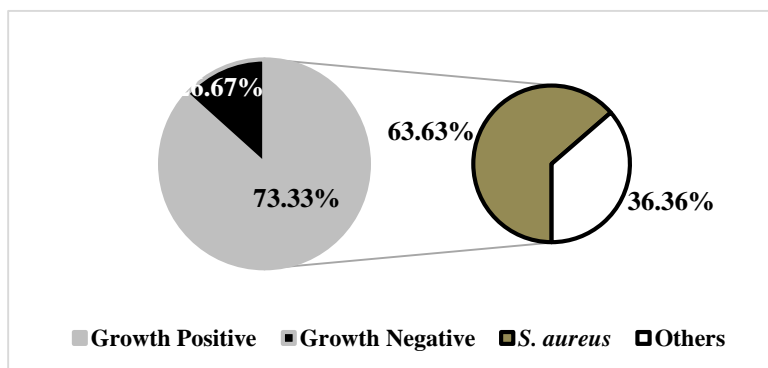

Figure 1: Pie Chart showing growth profile in nasal swab sample

Table 1: Antibiotic Sensitivity Pattern for *Staphylococcus aureus*

Antibiotic Class	Antibiotic (mcg)	Total *S. aureus* isolates(N= 28)		
		Sensitive No. (%)	Intermediate No. (%)	Resistant No. (%)
β-lactam	Penicillin(10)	18(64.3)	-	10(35.7)
	Cefoxitin(30)	24(85.7)	-	4(14.3)
	Oxacillin(1)	25(89.2)	-	3(10.7)
Macrolide	Erythromycin(15)	16(57.1)	2(7.14)	10(35.7)
Tetracycline	Tetracycline(30)	28(100)	-	-
Aminoglycoside	Gentamicin(30)	28(100)	-	-
Floroquinolones	Ciprofloxacin(5)	28(100)	-	-
Sulphonamide	Cotrimoxazole(25)	28(100)	-	-
Miscellaneous	Chloramphenicol(30)	28(100)	-	-

Table 2: Distribution of *Staphylococcus aureus* and MRSA into different categories.

Category	Division	No. of Samples	*Staphylococcus aureus*		MRSA	
			Number	Percentage (%)	Number	Percentage (%)
Gender	Male	40	19	47.5	3	7.5
	Female	20	9	45	1	5
Age group	18-28	19	8	42.11	2	10.53
	28-38	17	12	70.06	2	11.76
	38-48	16	7	43.75	0	0
	48-58	8	1	12.5	0	0
Districts	Lalitpur	20	10	50	3	15
	Kathmandu	20	14	70	1	5
	Bhaktapur	20	4	20	0	0
Population Type	Dump site workers (DSW)	30	15	50	3	10
	Community Peoples (CPs)	30	13	43.33	1	3.33

Table 3: Antibiotic Sensitivity Pattern of MRSA isolates (Determination of MDR strains)

Antibiotic Class	Antibiotics (mcg)	Total MRSA isolates	
		Sensitive No. (%)	Resistant No. (%)
Macrolide	Erythromycin(15)	3(75)	1(25)
Tetracycline	Tetracycline(30)	4(100)	-
Aminoglycoside	Gentamicin(30)	4(100)	-
Floroquinolones	Ciprofloxacin(5)	4(100)	-
Sulphonamide	Cotrimoxazole(25)	4(100)	-
Glycopeptides	Vancomycin(30)	4(100)	-
Miscellaneous	Chloramphenicol(30)	4(100)	-

DISCUSSION

S. aureus, a commensal of the human microbiota, can act as an opportunistic pathogen causing infections. With the emergence of resistance against various antibiotic, *S. aureus* infection in both the hospital and community setting, has become a global health threat (Yilmaz and Aslantas 2017). This study was conducted with the concern to archive the status of prevalence of methicillin resistant *S. aureus* among dumpsite workers in Kathmandu Valley.

The prevalence rate *S. aureus* was 46.67% (28/60) in the community setting of Kathmandu Valley. The nasal carriage was higher than studies carried out by Shrestha et al (2010), Bhatt et al (2014) and Khatri et al (2017) which was 27.1%, 30.4% and 18.3% receptively. This indicates nasal carrier rate of *S. aureus* is higher in community setting as compared to previous studies conducted in a hospital setting of Nepal. Similarly, our results of colonization of *S. aureus* is comparable to study of Joachim et al (2018), Kejela & Bacha (2013), and Akerele et al (2015) having prevalence rate 41.1%, 47.74%, and 49.5% respectively. In contrary, our result showed low prevalence than the studies of Bonesso et al (2014), Hasan et al (2016) and Kumar et al 2011 having prevalence 56.9%, 60.6% and 72.5% respectively. The nasal colonization of *S. aureus* was found to be higher in age group 28-37 (70.06%, 12/17), gender male (47.5%, 19/40), district Kathmandu (70%, 14/20) and dumpsite workers (50%, 15/30). The prevalence rate also depends upon the type of study population, geographical locations and socio-economic conditions. The variation might be due to frequent exposure to polluted environment, poor hygienic condition etc. impairing the immune status of individual and making them (DSWs and CPs) more susceptible to be colonized by *S. aureus.* (Sangvik et al 2011).

The isolated *S. aureus* were found to be sensitive against Tetracycline, Gentamicin, Ciprofloxacin, Co-trimoxazole, and Chloramphenicol making it effective for treatment. Whereas, about 35.7%, 14.3%, 10.7%, 35.7% of *S. aureus* isolates were resistant to Penicillin, Cefoxitin, Oxacillin, and Erythromycin respectively. Similar results were observed in the studies of Naimi et al (2003), Wang et al (2018) and Baggett et al (2003) showing Vancomycin 100% susceptible. Yilmaz and Aslantas (2017) and Early et al (2012) also showed 100% susceptible to chloramphenicol. Resistant pattern of Erythromycin was comparable to studies of Wang et al (2018) (46.2%), and Naimi et al (2003) (44%). Ciprofloxacin was also 100% susceptible in study of Baggett et al (2003).

MRSA predominance rate was found to be 6.67% (4/60) in Kathmandu Valley, which was higher than Shrestha et al (2010) i.e. 2.3%.

The MRSA colonization rate was 15.4%, 19%, 34.75%, 21.1% in hospital based study conducted by Subedi and Brahmadathan (2005); Bhatt et al (2014); Bhatta et al (2015) and Khanal et al (2018) in context of Nepal that is higher than our study. However, studies in community acquired MRSA are less reported and on correlating with the above studies, it suggests that MRSA prevalence in community setting is lower than in hospital setting of Nepal. Similarly, Joachim et al (2018) noted MRSA predominance to be 37.6% in Tanzania and Bonesso et al (2014) stated it to be 10.6% in Brazil. This indicates that MRSA prevalence in Nepal is lower in contrast to Tanzania and higher as compared to Brazil. The variable result is due to the variable distribution of MRSA strains depending upon geographical location, study population and different laboratory technique used. (Sollid et al 2014).

The study showed the MRSA nasal carrier rate to be higher among early (18-28) and mid age (28-38) group population i.e. 3.33% (2/60) in each group and was absent as age increases (<40). Similar reports were highlighted by McMullen et al (2009) i.e. 56% (high) MRSA in mid age group people. The higher MRSA ubiquity among mid and early age group people may be due to improper intake of antibiotics (Sollid et al 2014). The prevalence rate of MRSA was found to be higher in male (5%, 3/60) in this study. Similar results were seen in investigations by McMullen et al (2009) (52.2%), Shrestha et al (2010) (28.6%) and Khanal and Jha (2010) (75%) showing high prevalence in male than in female. This suggests that male are more prone to MRSA colonization. This might be due to differences in hygienic habits, hormonal difference (estrogen level affects the expression of virulence factor of *S. aureus)* and other factors like occupation, frequent exposure to contact supports, socio-economic status, obesity may also trigger the MRSA carrier rate among genders whose further research need to be done. (Humphreys et al 2015). In this study, the male: female ratio was 2:1 and MRSA isolated from 3 males were DSWs, who are more frequently exposed to polluted sites and belongs to low socioeconomic background while 1 MRSA isolated from female belong to community people as a result of which MRSA carrier rate was found higher in male than in female.

The higher prevalence of MRSA was among DSWs i.e. 5% (3/60). According to CDC (2006), CA-MRSA colonization and infection is more frequently occurring in individual with poor hygiene and cleanliness, adults from low socio-economic condition, participation in activities resulting in compromised skin surface, limited access to health, frequent antibiotic exposure etc. The high prevalence of MRSA in DSWs may be due to the repeated exposure to different types of wastage and pollution, contact with the contaminated

inanimate materials, lack of hygienic condition, low socio economic status etc. making them more susceptible to get colonized by CA-MRS. (McMullen et al 2009). Hence, serve as a carrier and reservoir of *S. aureus infection* in the community.

The study on ubiquity of MRSA in community setting of Kathmandu valley has not been described, although studies on hospital setting has been recorded. This study showed the prevalence rate of CA-MRSA to be higher in Lalitpur districts (5.00%). Distribution pattern of CA-MRSA varies with geographical location, genotypic strains of species organism and type of sample population (Sollid et al 2014). None of the *S. aureus* isolates showed resistivity against at least one agents of any three antimicrobial class indicating absence of multi drug resistant *S. aureus* in the community. However, according to Magiorakos et al, 2011; in case of *S. aureus*, all MRSA isolates are defined as MDR because resistance to oxacillin or cefoxitin predicts non susceptible to all categories of beta- lactam with exception of anti-MRSA cephalosporins. This considers that all the MRSA strain to be MDR strains of *S. aureus*. This warrants an attention about threat and presence of MDR strains *S. aureus.* These findings do not signify the complete absence of CA-MRSA or MDRSA infection. Nevertheless, it widens the proficiency of the status on prevalence of *S. aureus,* CA-MRSA and MDRSA in community setting of Kathmandu Valley of Nepal.

CONCLUSION

The prevalence of MRSA strains of *S. aureus* in Kathmandu Valley was observed. Resistant to Cefoxitin predicts non susceptible to all categories of beta- lactam indicating development of Community acquired Multidrug resistant organism. This alarms the presence of increasing trends of antibiotic resistant *S. aureus.* Future spread and outbreak of strains can be prevented by routine surveillance.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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