Detection of Methicillin Resistant *Staphylococcus aureus* in Public Transportation of Kathmandu Valley, Nepal

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ABSTRACT

Objectives: The purpose of this study was to assess microbial load and Methicillin Resistant *Staphylococcus aureus* from surfaces of public transport vehicle.

Methods: The surfaces of public transport vehicle were sampled by swabbing. A total of 56 samples from 28 different vehicles operating in Kathmandu valley were collected and processed according to the standard methodology. The isolates were identified by culture, biochemical tests and subjected to antimicrobial susceptibility testing by modified Kirby-Bauer disk diffusion method following CLSI 2013 guidelines. Methicillin resistant species of *Staphylococcus* were detected by the virtue of cefoxitin resistance.

Results: All 56 samples from the 28 different vehicles were found to have bacterial growth with average bacterial load of $2.47 \pm 1.22 \times 10^5$ CFU/cm^2. The gas vehicles were found to be the most contaminated. Out of 56 samples, 35 (25.9%) were found to be *S. aureus* growth positive 11 (31.4%) of them being MRSA.

Conclusion: The high flow of people with different health conditions in public transport makes the exchange of microorganism more significant. High bacterial load along with MRSA indicates the threats of transmission of infection among travelers. This is of a great public health concern as the mass population of different health condition is in direct exposure and is prone to get infected.

Key words: Public transport, antibiotic susceptibility testing, MRSA.

INTRODUCTION

Microbes in public area such as public transport, restaurants, schools, daycare centers can be a critical issue in public health, since they can bring a large number of people together which facilitate the transmission of microbes (Stepanovic et al. 2006; Kassem et al. 2007). This becomes a subject of prime concern when microbes are drug resistant and pathogenic. Therefore, increased attention has been paid to environmental microbes, to the numbers and strains of bacteria found in public places (Reynolds et al. 2005; Kassem et al. 2007; Otter and French, 2009).

The public transportation such as three-wheelers, mini/micro buses, buses, trolleybuses, trams, trains and ferries is mainly available for use by anyone (Scott and Bloomfield, 1990) and generally operates on fixed routes. Public transportation system has become increasingly important in urban areas due to mass transit and increased awareness to energy-saving methods of transportation (EPA, 1973; Barrero, 2008). During the travel, various components of the vehicle such as seats, handle, door handle are frequently encountered and may act as the important reservoir for transmission of different pathogenic and non-pathogenic microbes (Oranusi et al. 2016).

Staphylococcus aureus is an opportunistic pathogen often found on the skin, which causes a wide range of infections such as skin lesions, abscesses, endocarditis, septicemia, and toxic shock syndrome. It has now been a pathogen of concern due to the existence of methicillin resistant strains. Also the strains resistant to vancomycin, a drug often referred to as the "drug of

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last resort", have been reported (Henriques Normark et al. 2001; Jarraud et al. 2002). Methicillin-resistant Staphylococcus aureus (MRSA) is one of the major human pathogens responsible for mild to severe life threatening infections worldwide (de Lencastre and Tomasz, 2011; ECDPC, 2012). Since the mid-1990s, MRSA has also been identified as the etiological agent of infections acquired in the community (CA-MRSA) (Naimi et al. 2003; Patel, 2009; Graves et al. 2010). Within the last 5 years, MRSA has moved from being primarily a nosocomial pathogen to one that is also found in community areas and public places (Carleton et al. 2004; Popovich et al. 2008).

Microbes in the public transportation can be significant as public health is concerned because of the cases of transfer of organisms either from individual to individual directly or by indirect means which includes transfer from individual to inanimate objects like seats, handles bar etc. and then to other individuals thereby causing infection (Ehrenkranz, 1964; Yeh et al. 2011). This study was performed to screen MRSA from the surfaces of public transport system of Kathmandu, Nepal.

MATERIALS AND METHODS

In this study, a total of 56 samples from 28 different public vehicles (Handle and seat surface of each vehicle) operating in Kathmandu valley were collected by wet swab methods as described by Yeh et al. (2011) from June to August, 2017. The area of sampling (2cm x 4cm) was marked and swabbed with sterile moistened cotton swab and was kept in sterile container having sterile buffered peptone water (1ml). Similarly, another moistened swabbed was used in the same way and then kept in a dry sterile container simultaneously. Then, it was transported to microbiological laboratory as soon as possible in cold chain condition.

The enumeration of bacteria was performed as described in Isenberg HD (2004). The sample (in buffered peptone water) was serially diluted in sterile normal saline up to 10^{-6} and enumerated by spread plate technique incubating overnight at 37ºC. Another swab was cultured on selective and differential media (Mannitol Salt Agar and Blood Agar, HI-media, India), and incubated at 37°C for up to 48 hours. The isolated colonies from these media were then identified as '*Staphylococcus aureus*' with morphology, Gram staining, and catalase, oxidase, coagulase, DNase tests after subculture on NA.

Modified Kirby-Bauer disk diffusion test based on the guidelines of Clinical and Laboratory Standard Institute (CLSI, 2013) method was used to evaluate the antimicrobial susceptibility pattern of the isolates to a set of antibiotics and determination of methicillin resistance. The antimicrobial agents tested were: Chloramphenicol (C, 30µg), Gentamicin (GEN, 10µg), Erythromycin (E, 15µg), Penicillin (PEN, 10 units) and Cefoxitin (CX, 30µg) supplied from Hi-media. Methicillin resistant species of staphylococci were detected by the virtue of cefoxitin resistance on MHA as per CLSI M100-S23 guidelines (CLSI 2013).

RESULTS

All the 56 surface samples were found to be contaminated with an average bacterial load of 2.47 ± 1.22 x10^5 CFU/cm^2. The samples from the bus were found to be more contaminated (2.57 ±0.88 x10^5 CFU/cm^2) while that of the tempo were found to be the least contaminated (2.35 ± 1.84 x10^5 CFU/cm^2). The average bacterial load was found to be higher on the seat surfaces (2.50 ± 1.51 x10^5 CFU/cm^2) as compared to the handles (2.41 ± 1.93 x10^5 CFU/cm^2).

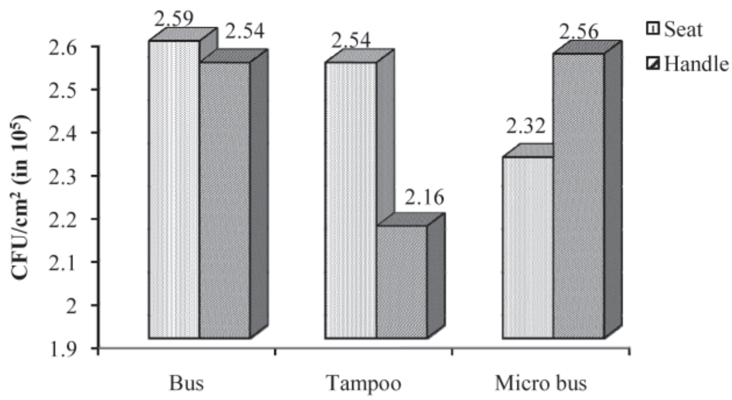

Figure 1: Average bacterial load in vehicles

The surfaces of the gas vehicles were found to be heavily contaminated (3.14 ± 1.75 x 10^5 CFU/cm^2) while that of the electric vehicles were found to be least contaminated (1.48 ± 0.64 x 10^5 CFU/cm^2). Sample-wise, the seat of the gas vehicle was found to be heavily contaminated (3.14 ± 1.75 x10^5 CFU/cm^2) while that of the electric vehicle was found to be least contaminated (1.17 ± 0.79 x10^5 CFU/cm^2).

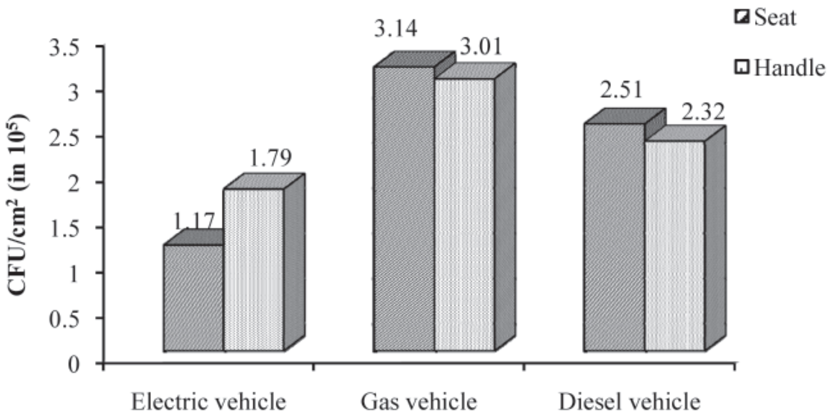

Figure 2: Average bacterial load in the surface of vehicles with respect to fuels

All the vehicles were found to harbor a diverse group of bacteria irrespective of the sample differences. A total of 135 bacteria were isolated from the study with the highest being from the surfaces of the bus 57 (42.2%). Of them, 35 (25.9%) were identified to be *Staphylococcus aureus* and 18 (13.3%) Coagulase negative staphylococci (CONS).

Table 1: Vehicle wise distribution of the bacterial isolates

S.N.	Vehicle	Number of sample	Bacterial isolates	Number	Percent
1	Tampoo	16	*S. aureus*	11	29.7
			Gram positive bacilli	16	43.2
			CONS	7	18.9
			Gram negative bacilli	3	8.1
	Sub total			37	
3	Bus	24	*S. aureus*	13	22.8
			Gram positive bacilli	28	49.1
			CONS	8	14.0
			Gram negative bacilli	8	14.0
	Sub total			57	
5	Micro bus	16	*S. aureus*	11	26.8
			Gram positive bacilli	14	34.1
			CONS	3	7.3
			Gram negative bacilli	14	34.1
	Sub total			41	
	Total			**135**	

A total of 11 (31.4%) *S. aureus* were found to be methicillin resistant (MRSA) and 34 (68.5%) isolates were methicillin susceptible strains (MSSA). Chloramphenicol was found to be the most effective antibiotic in vitro for both MRSA (11, 100%) and MSSA (34, 100%). Penicillin was found to be least effective antibiotic in vitro for both MRSA (11,100%) and MSSA (29, 85.2%).

Table 2: Antimicrobial susceptibility pattern of MSSA and MRSA

S.N.	Antibiotics	MSSA (n=34)		MRSA (n=11)	
		S (%)	R (%)	S (%)	R (%)
1	Penicillin	5(14.7)	29 (85.2)	0(0)	11 (100)
2	Erythromycin	7(20.5)	27(79.4)	3(27.2)	8(72.7)
3	Gentamycin	32(94.1)	2(5.8)	11 (100)	0(0)
4	Chloramphenicol	34(100)	0(0)	11 (100)	0(0)

DISCUSSION

As part of daily activities, many common spaces are shared with other people. This makes it possible to spread diverse microorganisms that can lead to infections. People who use public transport can pass the etiological agents of different infections to other apparently healthy people (Rusin et al. 2002). Due to the regular high flow of mass and the environmental condition of operation, public vehicles have a powerful impact on health of the consumers and the influence is growing globally. Nepal is an under-developed nation and there is no provision of systematic public transport in spite of the mass mobility (UN 2018). Public transport service was started in Kathmandu valley from 1959 and now, different- capacity vehicles such as Tempo, Micro bus, Mini bus, and large bus are in operation in Valley's road and increasing day by day (Pokharel and Acharya, 2015). The public transportation serves people of different health conditions, and become colonized with different pathogenic and non-pathogenic microbes thereby serving as the source of infection to the travelers (Dora et al. 2011). To the best of knowledge, paper regarding the microbiological condition of public transport in Nepal is not available yet. This study was aimed to assess the bacterial load, *S. aureus* and MRSA in public transport vehicle of Kathmandu Nepal.

All the vehicles were found to be colonized with different microbes with the average bacterial load of $2.47 \pm 1.22 \times 10^5$ CFU/cm^2 which indicates poor hygienic condition according to the surface hygiene guideline (BC Centre for Disease Control 2010). The physical contact of the travelling population or their clothing to the surfaces of the bus and the generation of droplets while talking, coughing or sneezing may be primarily responsible for the colonization of the microbes on the solid surface of vehicles (Chowdhury et al. 2016). Similarly, the dust generated in the streets, especially of Kathmandu Valley is also considered to be equally responsible for the microbial colonization (Gautam 2010; Sattar 2016).

The load was in harmony with the reports of Turkey (Tan and Erdoğdu, 2017) but lower than that of Chittagong, Bangladesh 3.1-23.9x10^5 CFU/4cm^2 (Chowdhury et al. 2016). The variation in the results may be attributed to the geographical diversity and the corresponding socio-economic and anthropogenic status of the population leading to crowding and ill hygienic practices (Chowdhury et al. 2016).

A total of 35 (25.9%) of *S. aureus* was detected in the study with 31.4% being methicillin resistant. A number of studies have reported varying degrees of MRSA isolates from the hand-touch surface in the public vehicles worldwide (CS et al. 2018; Iwao et al. 2012; Lutz et al. 2014; Otter and French 2009; Peng et al. 2015). The difference in pattern may be due to the geographical, health status of the living population and socio-economic differences between the sample sites (Bogomolova and Kirtsideli 2009; Jones and Harrison 2004).

Chloramphenicol and Gentamicin were found to be effective against MRSA but the increasing trends of antibiotic resistance among MRSA strains is a matter of concern since the public vehicles can act as a potential point source of infection to the apparently healthy population that may ultimately lead to serious epidemics and potential therapeutic failure (Rusin et al. 2002; Eguia and Chambers 2003; Kramer et al. 2006; Tan and Erdoğdu, 2017). Hence, it is suggestive to consider the proper measures for the sanitation and cleansing of the vehicle that may be primitive detergent washing or recent breath-safe system that may decrease the load of some pathogenic organisms (Lukasik 2009).

CONCLUSION

The presence of high bacterial load, *S. aureus* and MRSA on the surfaces of different parts of public vehicles impose the possibility of transmission of serious infection with antibiotics resistant microorganisms.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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