Recovery of Methicillin Resistant Staphylococci from Public Vehicles in Kathmandu

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ABSTRACT

Objectives: The purpose of this study was to determine the extent of staphylococcal contamination in public vehicles in Kathmandu and to determine the antibiotic susceptibility pattern of the isolates.

Methods: This cross-sectional study was conducted from December 2022 to May 2023 in the Microbiological laboratory of Padma Kanya Multiple Campus, Kathmandu. A total of 120 swab (sterile cotton swab moistened with sterile normal saline) samples were collected by gently rubbing the surfaces from 19 different micro-buses during the time of heavy passenger flow (office time) and transported to the laboratory maintaining a cold chain. *Staphylococcus aureus* and Coagulase negative Staphylococci (CoNS) were identified by their characteristic yellow pigmented colonies on Mannitol Salt Agar (MSA) and the ability of *S. aureus* to produce coagulase enzyme. Kirby-Bauer disc diffusion method was employed to test the antibiotic susceptibility pattern of isolates. MRSA were identified on the basis of the zone of inhibition (<21 mm diameter) against Cefoxitin 30mcg disc.

Results: Out of 41 Staphylococcal isolates, 46.36% were *S. aureus*, among which 84.21% exhibited methicillin resistance (MRSA). All Coagulase Negative Staphylococci (CoNS) isolates (n=22) were resistant to methicillin (MRCoNS). MRSA and MRCoNS isolates were also resistant to antibiotics like Penicillin and Erythromycin.

Conclusion: This study reports relatively high occurrence of MRSA in environmental samples, predominantly in areas frequently touched by people indicating the possibility of easy spread of these organisms and subsequent opportunistic infections.

Keywords: Methicillin, MRCoNS, MRSA, public, Staphylococci

INTRODUCTION

Staphylococci are a group of major human pathogen, Gram positive in nature, and are responsible for causing different types of infectious diseases in humans like minor skin infections and major infections like meningitis, endocarditis and toxic shock syndrome (TSS) (Lalaouna et al. 2018). About 33% of human beings have *Staphylococcus aureus* colonized in their nares persistently. The secreted and surface proteins possessed by these bacteria help in colonization and in invading immune responses ultimately paving the way for adhesion to tissue components and then entering the hosts. Methicillin resistant *Staphylococcus aureus* (MRSA) have shown resistance to β-lactam antibiotics and are the major cause of hospital acquired infections (Algammal et al. 2020). Hypervirulent community acquired MRSA has also found to be increasing (Foster et al. 2015).

In addition to coagulase positive Staphylococci, known as *S. aureus*, there are more than 50 species of Staphylococci which are coagulase negative (CoNS) like *S. epidermidis*, *S. saprophyticus*, *S. hominis, S. haemolyticus* etc. MRSA and MRCoNS are regarded to be responsible for major health problem. Community-acquired MRSA (CA-MRSA) have been reported to be on the rise in recent studies. Transfer of community-acquired infections caused by CoNS has not been reported but there are reports of gene transfer from MRCoNS to MRSA (Seng et al. 2017, Otto 2013).

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S. aureus is susceptible to many antibiotics naturally. Due to the transfer of genes horizontally from intrinsically resistant coagulase negative staphylococci (CoNS), they confer *S. aureus* the resistance to different antibiotics. Such genes are usually present on plasmids or cassettes, the mobile genetic elements (MGEs). Resistance to β-lactam antibiotics and methicillin is due to the acquisition of methicillin resistance gene, mecA which is carried on MGEs known as the staphylococcal chromosome cassette *mec* (SCC*mec*) (Basset et al. 2011).

This study helps to provide some insight into the environmental surfaces that might be potential reservoirs for Staphylococcal species, in particular MRSA and MR-CoNS. The identification of such reservoirs will help to provide guidelines to reduce the occurrence of these organisms and help in controlling the transmission and infection in the community.

MATERIALS AND METHODS

Study Design, Sample Size and Study Site

The study was quantitative, and primary data collection was done from December 2022 to May 2023. The study variables were occurrence of *S. aureus*, Coagulase Negative Staphylococci (CoNS) and the susceptibility pattern towards Methicillin and other antibiotics. It was a cross-sectional study that included both field and laboratory procedures. Collection of swab samples (n=120) was done from the frequently touched parts of the public vehicles, in particular micro-buses, that operate from Kapan to Jamal, Kathmandu during office time when there was heavy flow of the passengers.

Sample collection and transportation

Sterile normal saline was used to moisten the standard sterile cotton swabs which were employed to collect samples from the frequently touched surfaces of the micro-buses by gentle spreading the swab. Nineteen swab samples each were collected from front seat, back seat, door, window, front handle, back handle and 6 samples were collected from the looking glass. The collected swabs were kept in glass vials containing little amount of sterile normal saline, screw capped and clearly labelled. The collected swab samples were transported to the microbiology laboratory of Padma Kanya Multiple Campus as soon as possible maintaining a cold chain. In case of delay, they were kept in refrigerator at 4^0C.

Isolation and identification of *S. aureus* and CoNS

Primary inoculation of the swab samples was done on Mannitol Salt Agar (MSA). Initial identification of Staphylococcal colonies was done on the basis of yellow-coloured colonies (Figure 1). Colony characteristics were noted and further phenotypic identification was done by microscopic morphology revealed from Gram staining and biochemical tests like catalase, oxidase and oxidative/fermentative (O/F) tests. *Staphylococcus aureus* was distinguished from other Staphylococcus species (Coagulase Negative Staphylococci, CoNS) by coagulase test.

Antibiotic Susceptibility Test and Detection of Methicillin Resistant isolates

Kirby Bauer Disc Diffusion Technique (modified) was followed to determine antibiotic susceptibility pattern following CLSI guidelines (2018) for the choice of antibiotics and the interpretation of sensitivity or resistivity. All the isolates of *Staphylococcus* species were tested using Cefoxitin disc of 30mcg. *S. aureus* isolates which showed inhibition zone diameter of ≤ 21 mm were considered to be methicillin resistant and those showing ≥ 22 mm as methicillin susceptible (MSSA). Similarly, CoNS isolates having zone of inhibition diameter ≤ 24 mm were methicillin resistant and ≥ 25 were susceptible to methicillin (MSCoNS).

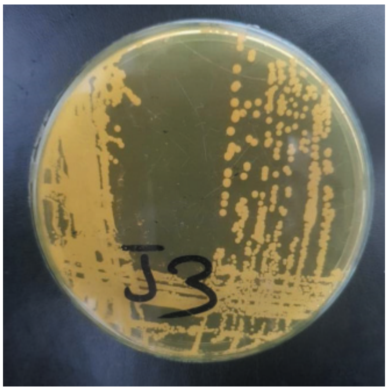

Figure 1: Yellow colonies of *S. aureus* in Mannitol Salt Agar

Further confirmatory tests were done after sub-culturing in Nutrient Broth (NB)/ Nutrient Agar (NA) for 4 hours at 30^{0}C.

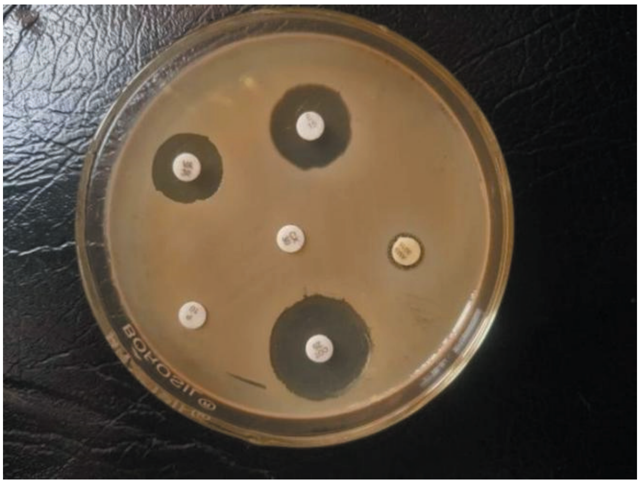

Figure 2: Antibiotic Susceptibility test of *Staphylococcus* species showing sensitivity to Cotrimoxazole and Erythromycin

RESULTS

Recovery of *S. aureus* and CoNS from micro-buses

Among the 120 samples collected from different parts of 19 public vehicles, i.e. micro-buses, 41(34.16%) showed growth of *Staphylococcus* species among which 19(46.34%) were found to be *S. aureus* and 22(53.65%) were Coagulase Negative Staphylococci (CoNS) (Fig. 3).

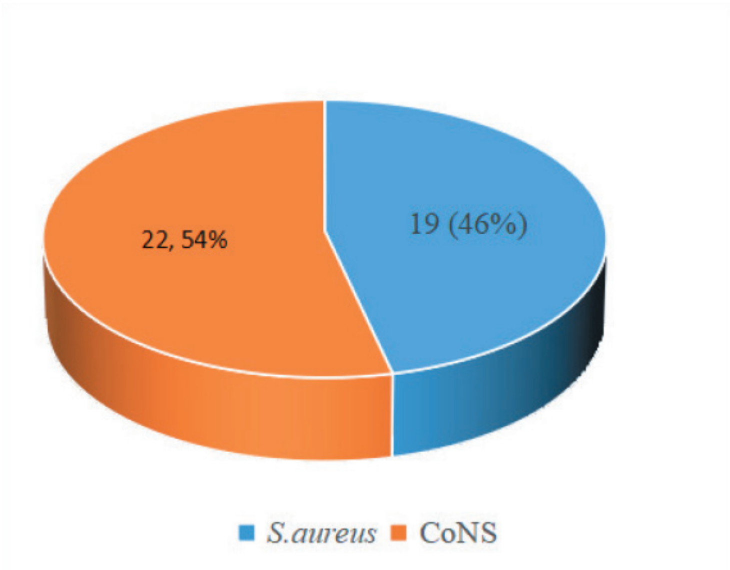

Figure 3: Prevalence of *S. aureus* and CoNS in public vehicles (micro-buses)

Recovery of MRSA/MRCoNS from micro-buses

Out of the 19 *S. aureus* isolated, 16(84.42%) were found to be resistant to methicillin. Likewise, resistance to methicillin was detected in all the 22 isolates of Coagulase Negative Staphylococci (CoNS).

Site-wise occurrence of *S. aureus* and CoNS

Majority of *S. aureus* and CoNS were found in front of the seat and behind the seat, whereas lesser number of isolates were from window, front handle and back handle. Meanwhile, there were no isolates of *S. aureus* or CoNS from the samples taken from the looking glass (Table 1).

Table 1: ***S. aureus*** **and CoNS detection in different parts of the vehicles**

Sites of Sample collection	Sample size	No. of *S. aureus* isolates (%)	No. of CoNS isolates (%)
Seat front	19	7 (36.84)	4 (21.05)
Seat back	19	4 (21.05)	8 (42.10)
Door	19	3 (15.78)	5 (26.31)
Window	19	2 (10.52)	2 (10.52)
Handle front	19	2 (10.52)	2 (10.52)
Handle back	19	1 (5.26)	1 (5.26)
Looking glass	6	0	0
Total	120	19	22

Distribution of MRSA and MRCoNS in different parts of micro-buses

Among the different parts of the micro-buses, majority of the MRSA were isolated from Seat front (n=5; 26.31%) which is located near the door and frequently touched by people for support to enter inside or stand. Lesser number of MRSA were found in Seat back, door (15.78% each), window and handle front (10.52% each). Higher number of MRCoNS was isolated from Seat back (n=8; 36.36%), door (n=5; 22.72%) and Seat front (n=4; 18.18%). *S. aureus* and CoNS were not detected from looking glass (Figure 4).

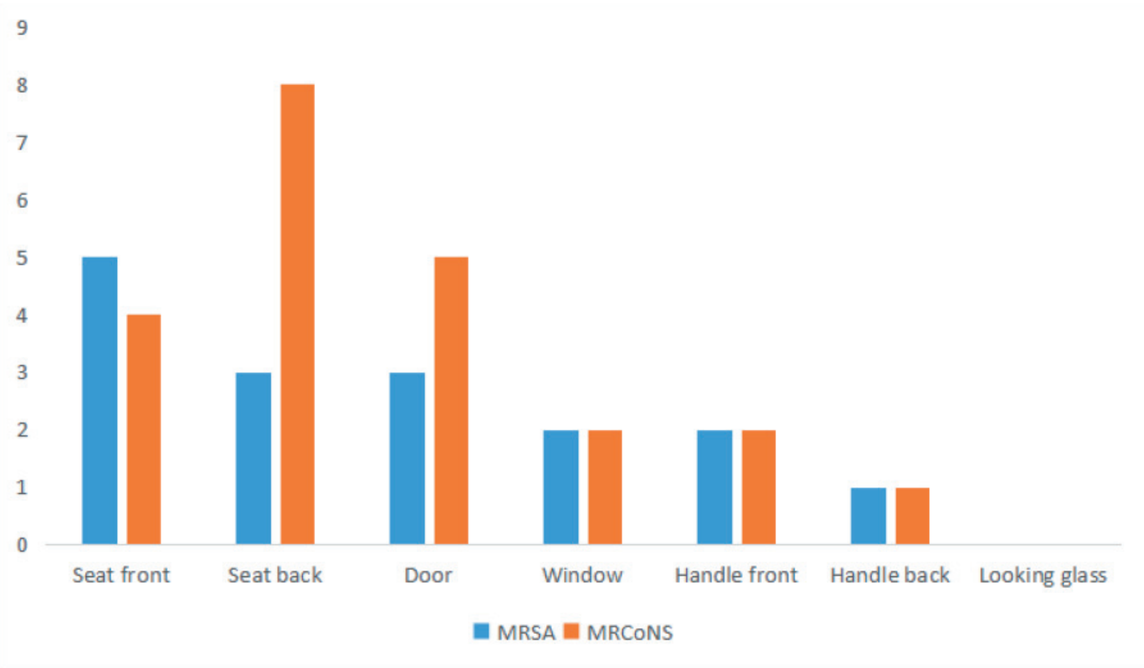

Figure 4: Distribution of MRSA and MRCoNS in different part of vehicles

Antibiotic Susceptibility pattern of *S. aureus*

Antibiotic susceptibility profile of *S. aureus* isolates is shown in Table 2. Antibiotic resistance profile shown by *S. aureus* is as follows: Cefoxitin (n=16; 84.21%), Erythromycin (n=9; 47.36%), Gentamicin (*n* = 8; 42.10%), Ciprofloxacin (n=3; 15.78%), Chloramphenicol (n=3; 15.78%), and Vancomycin (n=1; 5.26%). All the isolates were found to be resistant to Penicillin. Two isolates each were intermediately resistant to Erythromycin and Chloramphenicol (n=2; 10.52%).

Table 2: Antibiotic Susceptibility profile of *S. aureus*

Antibiotics (µg)	Susceptibility Pattern of *S. aureus* (n=19)		
	Sensitive (%)	Intermediate (%)	Resistant (%)
Vancomycin (30)	18 (94.73)	-	1 (5.26)
Cefoxitin (30)	3 (15.78)	-	16 (84.21)
Erythromycin (15)	8 (42.10)	2 (10.52)	9 (47.36)
Ciprofloxacin (5)	16 (84.21)	-	3 (15.78)
Chloramphenicol (30)	14 (73.67)	2 (10.52)	3 (15.78)
Gentamicin (10)	11 (57. 89)		8 (42.10)
Penicillin (10)	-	-	19 (100)

Antibiotic Susceptibility pattern of CoNS

All the CoNS isolated were completely resistant (100%) to Cefoxitin and Penicillin. They also showed resistance to Erythromycin (n=9; 40.90%), Vancomycin (n=7; 31.81%), Ciprofloxacin (n=5; 22.72%) and Co-trimoxazole (n=2; 9.09%). Furthermore, the pattern of intermediate resistance of CoNS were observed for Erythromycin (n=4; 18.18%), and Co-Trimoxazole (n=1; 4.5%) (Table 3).

Table 3: Antibiotic Susceptibility profile of CoNS

Antibiotics (µg)	Susceptibility Profile of CoNS (n=22)		
	Sensitive (%)	Intermediate (%)	Resistant (%)
Vancomycin (30)	15 (68.18)	-	7 (31.81)
Cefoxitin (30)	-	-	22 (100)
Erythromycin (15)	9 (40.90)	4 (18.18)	9 (40.90)
Ciprofloxacin (5)	17 (77.27)	-	5 (22.72)
Co-Trimoxazole (30)	19 (86.36)	1 (4.54)	2 (9.09)
Penicillin (10)	-	-	22 (100)

Multidrug resistant pattern of MRSA

Distribution percentage of Multi Drug Resistant (MDR) *S. aureus* is shown in Table 4. A total of 17 MDR was detected, more from seat front, seat back and door (6, 3 and 3 respectively). The occurrence of MDR is almost the same as the occurrence of MRSA.

Table 4: MDR pattern of MRSA isolates

Sampling Sites	No of MRSA (% of *S. aureus* isolates in individual sites)	MDR (% of *S. aureus* isolates in individual sites)
Seat front	5 (71.42)	6 (85.71)
Seat back	3 (75)	3 (75)
Door	3 (100)	3 (100)
Window	2 (100)	2 (100)
Handle front	2 (100)	2 (100)
Handle back	1 (100)	1 (100)

DISCUSSION

Among 120 swab samples collected from different surfaces of micro-buses, a common means of public transportation in Kathmandu, and analyzed, about 67% of the samples showed no growth of *Staphylococcus* species which might be due to the relative absence of the organism in those surfaces. This indicates that these surfaces were clean and free of the common normal flora present in human skin and nares and the cleanliness of such surfaces is well maintained.

The aim of this study was to identify *Staphylococcus aureus* and Coagulase Negative Staphylococci (CoNS) present in surfaces of public transportation and to find the pattern of susceptibility of these isolates against different antibiotics. Relatively a smaller number of samples were processed in comparison to the similar studies conducted in clinical setting.

Previous studies performed in the shrines of Kathmandu valley had shown higher presence of environmental *S. aureus* as they had isolated 17.5% of *S. aureus* out of the same sample number (n=120) (Arjyal et al. 2020). Similar study was conducted in Kathmandu valley a year later in different public places like temples, ATM booths, campus areas, market areas and cafes in which slightly higher recovery of *S. aureus* (20.3%) and CoNS (43.1%) was reported (Arjyal et al.

2021). The variance in the number of isolates might be due to the diverse location of sample collection in these studies as compared to the present study where sample collection was confined to the surfaces of micro-buses.

A slightly earlier study conducted in 59 saliva samples from wild monkeys residing in the temples in Kathmandu have reported 6.8% of macaque MRSA (Roberts et al. 2018) whereas our study showed 84% of MRSA out of 19 *S. aureus* isolates. All the CoNS isolated in this study were detected as Methicillin Resistant (MRCoNS). Even though the number of isolates seem to be less, the frequency of methicillin resistance is significantly high in our study. Very similar high incidences of MRSA (82.6%) and MRCoNS (96.8%) were reported in another study done in a tertiary hospital in which isolates were obtained from clinical samples in northern region of Thailand (Kitti et al. 2018).

The occurrence of CoNS was assessed from environmental samples in a hospital in Tunisia and 63 out of 150 samples (41.3%) from inanimate surfaces and 83 out of 200 (41.5%) tested samples were CoNS. This was the first study on CoNS from environmental samples reported in Tunisia (Dziri et al. 2016).

The variation in the number of MRSA and MRCoNS is highly attributed by the number of *S. aureus* and CoNS recovered from the different parts of the micro-buses. It is clearly evident that the surfaces frequently touched by the passengers had higher load of both *S. aureus* and CoNS; there were no isolates detected from the looking glass of any of the 19 vehicles from which the samples were collected. The handles of the micro-buses do not serve similar purpose as the handles of bigger buses so lesser isolates were recovered from the samples from the handles which in general is expected to have higher bacterial population. The standing passengers usually cling on to the back of the seats during their travel which is demonstrated by the highest number of isolates from this part of the micro-buses. The front part of the seat is also heavily touched by most passengers in order to get on to the micro bus and the area near it is used by standing passengers, owing to the fact that second highest number of isolates was from the front side of the seat. The next site of the micro bus with more occurrence of the Staphylococci was door, followed by windows and handles. Xu et al. (2022) have reported the presence of Methicillin Resistant Staphylococci from the shared public bicycles in 12 cities from 11 provinces in China. They recovered 146 Staphylococci belonging to 13 different species.

Different antimicrobial agents (antibiotics) were used to test the susceptibility of the Staphylococcal isolates, both *S. aureus* and CoNS. They were found to be resistant to other antibiotics in addition to Methicillin, as exemplified by very little or no zone of inhibition shown when tested against 30mcg of Cefoxitin. *S. aureus* showed the following antibiotic resistance pattern: Cefoxitin (n=16; 84.21%), Erythromycin (n=9; 47.36%), Gentamicin (*n* = 8; 42.10%), Ciprofloxacin (n=3; 15.78%), Chloramphenicol (n=3; 15.78%), and Vancomycin (n=1; 5.26%). All the isolates showed resistance to Penicillin. Two isolates each were intermediately resistant to Erythromycin and Chloramphenicol (n=2; 10.52%). Vancomycin, Ciprofloxacin and Chloramphenicol were found to be comparatively more effective antibiotics.

All the CoNS isolated were completely resistant (100%) to Cefoxitin and Penicillin. CoNS also showed resistance to Erythromycin (n=9; 40.90%), Vancomycin (n=7; 31.81%), Ciprofloxacin (n=5; 22.72%) and Co-Trimoxazole (n=2; 9.09%). Intermediate resistance patterns of CoNS were observed for Erythromycin (n=4; 18.18%), and Co-Trimoxazole (n=1; 4.54%). Vancomycin, Ciprofloxacin and Cotrimoxazole were detected to be more effective for CoNS than other antibiotics.

Multidrug resistance was found to be present in about 89% *S. aureus* isolates; the number of such MDR isolates were in congruence with the prevalence of *S. aureus* in different parts of the vehicles. The vehicle part in which there was higher count of *S. aureus* was the one from which a greater number of MDR isolates were detected and less MDR isolates were detected from the parts with lower number of *S. aureus* isolates.

A study conducted at a large Mid-Western University Campus to assess the level of environmental contamination with *S. aureus* resonates with our finding, the antibiotics resistivity pattern was detected as follows: erythromycin (n=12, 35.3%); oxacillin (n=9, 26.5%); clindamycin (n=5, 14.7%); tetracycline (n=2, 5.9%); ciprofloxacin (n=3, 8.8%); levofloxacin (n=2; 5.9%) and minocycline (n=1, 2.9%). Twelve isolates (35.3%) were MDR-*S. aureus* against which the antibiotics showed similar resistance pattern (Thapaliya et al. 2017).

Similar study was conducted in university environment in Thailand for finding the prevalence of coagulase negative Staphylococci (CoNS) which reported that 20.5% samples (41/200) showed positive results for CoNS and all the isolates were methicillin resistant (MR-CoNS). The occurrence was found to be highest (43.3% of the samples tested) in library (Seng et al. 2017) which is pretty expected in the sense that it is a closed space frequently visited by many students, who also stay there for longer time.

Our study was conducted after the restrictions on public movement imposed by CoVID-19 pandemic was lifted and the use of masks was not mandatory. It can be speculated that if the habit of using masks and hand sanitizers was established as a culture among general public, a smaller number of Staphylococci would have been isolated in our study. Such comparative studies could be conducted in future to assess the use of simple hygienic practices and its impact on the recovery of Staphylococci which are more serious threat when they exhibit methicillin resistance or multi-drug resistance.

Future studies can also be directed towards expanding the range of samples from other means of public transportation and including wider geographic locations. Lack of phylogenetic analysis, molecular detection can be considered as the limitations of this study. The findings from environmental samples cannot be considered for clinical settings which can be taken as a drawback and emphasize the need for studies that encompass both environmental and clinical settings.

CONCLUSIONS

Relatively high occurrence of MRSA and MRCoNS was detected in the samples from micro-buses, one of the common means of transportation in Kathmandu, predominantly in areas frequently touched by people. This is an indication of the easy spread of these organisms and subsequent opportunistic infections. Future studies could be directed towards establishing a connection between the humans and the environmental surfaces of public places that might share these organisms.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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