Research Article

Methicillin-Resistant Staphyloccocus aureus Contamination in Hospital Door Handles of Pokhara, Nepal

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ABSTRACT

The presence of contaminants on the door handles surfaces of hospitals may be a common means of transfer of potentially pathogenic bacteria among users and can act as vehicles of diseases transmission. The aim of this study was to isolate, identify and evaluate the presence of bacterial contaminants and Methicillin Resistant Staphylococci (MRSA) with their antimicrobial susceptibility pattern present on the door handles of selected hospitals of Pokhara Metropolitan City, Western Nepal to take the necessary remedial measures. Isolation, identification and antimicrobial susceptibility test of bacteria were done using standard microbiological procedures. Further screening and confirmation of MRSA were done according to Clinical Laboratory Standard Institutes (CLSI) guidelines.

Out of the 100 swab samples cultured, 96 (96%) showed bacterial growth. 62.86% (88/140) of the isolates were found to be Gram positive. Percentage distribution of the isolates showed that the most prevalent Gram-positive bacteria identified was Staphylococcus aureus (43.18%), followed by Coagulase negative Staphylococci (15.91%), Bacillus spp (11.36%), Diptheroides (10.23%) but considerable number of Micrococci (7.95%), Streptococcus pneumoniae (6.82%), Enterococci (4.55%). Prevalence of MRSA in this study was 39.47%. Significant difference in antibiotic resistance pattern was found among MRSA and MSSA isolates ($P<0.05$) reflecting increased ability of MRSA to develop resistance against various antimicrobials. Drugs like clindamycin, cefazoline and amikacin were found quite effective

against MRSA in the present study would be better options for the management of such infections.

MRSA strains' contaminating on the door handles of hospitals may cause threat of infections to patients, hospital staffs, visitors to hospitals and people in the community. Thus, regular surveillance and disinfection with appropriate agent at regular interval would minimize the settlement and transmission of various pathogens including MRSA.

Keywords*:* Contaminants; disinfection; door handles; hospital; MRSA; pathogen

INTRODUCTION

Door handles provide a suitable environment for bacteria to spread easily, this makes it a hotspot for bacteria to gather and create colonies. Much more focus is placed on the door handles and their contribution to the spread of hospital acquired infections (HAIs)/ nosocomial infections and as the most commonly touched surfaces in any health care facility; they should always refer to an as "critical contract point" (McDonadh, 2015). Nosocomial infections are a major challenge to the health care system and are associated with significant mortality, morbidity and high economic burden (WHO, 2011).

Indeed, bacterial resistance is an increasing threat to the successful treatment of such hospital acquired infection. Emergence of MDR strains among the common clinical microbes causes treatment failure, prolonged hospital stays along with increased mortality and morbidity. It has been shown that hard, non-porous surfaces, such as door handles, have the highest bacterial transfer rates to hands (Rusin et al., 2002). Door handles might be contaminated with improperly washed contaminated hands that might lead to increased prevalence of the bacterial infectious disease due to contaminants. Despite the use of variety of cleaning and sterilization techniques of environmental surfaces, they might still act an important vehicle of transmission of drug resistant pathogens like Vancomycin Resistant Enterococci (VRE), Methicillin Resistant Staphylococci (MRSA), *Acinetobacter spp*, *Clostridium difficile*, and many others. There has been much debate over the infection risk to patients from contaminated health care surfaces (Dancer, 2009).

MRSA are important cause of nosocomial infections worldwide and are often multidrug resistant. The treatment options for MRSA are limited due to its resistance to all Beta-lactam antibiotics, that include penicillins and cephalosporins. Vancomycin remains an acceptable therapeutic option for MRSA infection. However, there is higher likelihood of mortality or

treatment failure among patients infected with MRSA due to the irrational use of vancomycin (Chen et al., 2014). MRSA and Methicillin Susceptible *S. aureus* (MSSA) increasingly develop resistance to various antibiotics making it very challenging to treat the range of infections it causes (Rayner & Munckhof, 2005).

MRSA strains were believed to be limited within the healthcare setting (Dulon et al., 2011) but recent evidence suggests noticeable community prevalence of MRSA on a global scale (Kong et al., 2016). MRSA strains' contaminating on the door handles of hospitals may cause threat of infections to patients, hospital staffs, visitors to hospitals and people in the community. The delay in detection and reporting multidrug resistant pathogens may lead to complications, prolonged hospital stays of patients, increased health care cost and augmented morbidity and mortality (Lutenbach et al., 2001) and hence can be considered an economic burden to society more in context of developing country like Nepal. Therefore, regular evaluation and disinfection of door handles of hospitals are essential for infection control. Contaminated door handles may increase the risk of spreading infections that often results from contact with restroom door handles which might contaminated by those who don't wash their hands, sick people, gloves and the possibility of cross contamination of many subjects and objects in hospital setting (Dancer, 2009).

Door handles of public restrooms and offices were investigated for possible bacterial contamination (Nworie et al., 2012). To our knowledge, limited published data were available on the possible contamination of door handles with MRSA of the hospitals in Pokhara Metropolitancity, Nepal till date. This study is expected to increase the awareness of people about a source of bacterial contamination. Indeed, the findings of this study shall explore whether door handles could play a role in the spread of bacterial pathogens associated with nosocomial infections and to offer possible control or preventive measures that could be instituted to avoid this likely vehicle of infection.

DATA AND METHODS

Sample Collection and Processing

A total of 100 swab samples were collected from the door handles of selected two hospitals of Pokhara Metropolitan City, Nepal by simple random sampling method and these sample were processed in the microbiology laboratory of Janapriya Multiple Campus (JMC), Pokhara, Nepal. Permission was granted by both hospitals to conduct this research. Door handles/knobs

were rubbed on using separate sterile swabs moistened with sterile normal saline. Each swab was placed in test tube containing Brain Heart Infusion (BHI) broth, labelled, held below 4^0C and transported to the laboratory of JMC for analysis within two hrs. After pre-enrichment at 37^0C for 24 hours, the turbid BHI broth was sub cultured on Blood agar and Mannitol salt agar plates following incubation at 37^0C. The isolated bacteria were identified using standard microbiological tests (Cheesbrough, 2006). Pure cultures of identified organisms were plated onto nutrient agar prior to all antibiotic susceptibility tests that was performed as instructed by the Clinical and Laboratory Institute Standard (CLSI) guidelines (CLSI, 2015).

MRSA Screening and Confirmation

Methicillin resistance was determined by testing resistance to 10 microgram oxacillin and 30 microgram cefoxitin disc. If the inhibition zone for oxacillin was ≤ 10 mm and for cefoxitin was ≤21 mm on MHA after overnight incubation at 35^0C, the cultures were considered as MRSA. Similarly, CONS which showed a zone of inhibition ≤ 17mm with oxacillin (10 μg) and zone size of inhibition ≤ 24mm with cefoxitin (30 μg) on MHA after overnight incubation at 35^0C, were considered as MR-CONS (CLSI, 2014).

All the data obtained were organized in the computer sheets and were analysed by using GraphPad Prism software for window (version 6). Data were presented in appropriate table, figures by calculating percentage, rate etc. Appropriate statistics were applied wherever applicable.

RESULTS AND DISCUSSION

A total of 100 door handles/knob swabs were collected from doors in different hospitals in Pokhara. Cultivation of these 96 swabs yielded bacterial growth while rest, 4 swabs showed no bacterial growth out of total 100 samples. From 96 culture positive samples, 62.86% (88/140) of the bacteria were Gram positive and 37.14% (52/140) of the isolates were Gram negative.

Distribution of Gram-Positive Bacteria Isolated from Various Door Handles/Knobs

The total numbers of Gram-positive bacteria isolated was 88/140 (62.86%). Among Gram-positive isolates, the most prevalent bacteria identified was *S. aureus*, followed by Coagulase negative Staphylococci, *Bacillus* spp, Diptheroides but considerable number of Micrococci, Streptococci and Enterococci were also recovered (Table 1).

Table 1

Distribution of Gram-positive Bacteria Isolated from Various Door Handles/Knobs.

Organism Identified	Number	Percentage
Staphylococcus aureus	38	43.18
Coagulase Negative Staphylococci (CONS)	14	15.91
Bacillus spp	10	11.36
Diptheroids	9	10.23
Micrococci	7	7.95
Streptococcus pneumoniae	6	6.82
Enterococci	4	4.552
Total	88	100

Antibiotic Sensitivity Tests of the Isolated Gram-positive Bacteria

Kirby Bauer disc diffusion method was adopted to determine the antibiotic susceptibility pattern. *Staphylococcus aureus* was found to be 100% resistant to Penicillin. No Vancomycin resistant *S. aureus* was reported. The effective antibiotics were Tetracycline (97.3%), Cefazoline (86.8%), Amikacin (84.2%), Clindamycin (86.8%), Cefoxitin (60.53%), Oxacillin (60.53%), Ciprofloxacin (52.63%), Cotrimoxazole (42.1%) and Erythromycin (26.3%) (Table 4). However, Coagulase Negative Staphylococci showed 100% sensitive to Vancomycin, Amikacin and Tetracycline followed by Clindamycin (92.8%), Cefazoline (85.7%), Gentamycin (78.5%), Oxacillin (71.4%), Cefoxitin (71.42), Ciprofloxacin (64.2%), Cotrimoxazole (64.2%), Amoxicillin+ Clavulanate (35.7%) and Erythromycin (31.2%). Isolated all CONS showed 100% resistant to Penicillin (Table 2).

*Streptococcus pneumonia*e 100% sensitive to Vancomycin followed by Amikacin (83.3%), Cefazoline (83.3%), Tetracycline (83.3%), Gentamycin (66.6%), Amoxicillin + Clavulanate (50%), Ciprofloxacin (50%), Cotrimoxazole (50%), Clindamycin (33.3%), erythromycin (16.6%) and Penicillin (16.6%) (Table 2).

No vancomycin resistant Enterococci were observed in this study. The isolated 75% Enterococci were sensitive to Amikacin, Tetracycline, Amoxicillin+ Clavulanate, 50% sensitive to Clindamycin, Cotrimoxazole, Ciprofloxacin, Gentamycin and 25% sensitive to Penicillin and Erythromycin (Table 2).

Table 2

Antibiotic Susceptibility Pattern of the Isolated Gram-positive Bacteria.

	Total no. of Isolates	No. (%) of isolates sensitive to												
		OX	CX	GEN	P	AMC	CPL	COT	CI	TE	ERY	V	AK	CZ

Staphylococcus aureus	38	23 (60.5)	23 (60.5)	33 (86.8)	0 (0)	18 (47.3)	20 (52.6)	16 (42.1)	33 (86.8)	37 (97.3)	10 (26.3)	38 (100)	32 (84.2)	33 (86.8)
Coagulase negative Staphylococci	14	10 (71.4)	10 (71.4)	11 (78.5)	0 (0)	5 (35.7)	9 (64.2)	7 (50)	13 (92.8)	14 (100)	(31.2)	14 (100)	14 (100)	12 (85.7)
Streptococcus pneumoniae	6	-		4 (66.5)	1 (16.6)	3 (50)	3 (50)	3 (50)	2 (33.3)	5 (83.3)	1 (16.6)	6 (100)	5 (83.3)	5 (83.3)
Enterococci ssp	4	-		1 (25)	1 (25)	3 (75)	2 (50)	2 (50)	2 (50)	3 (75)	1 (25)	4 (100)	3 (75)	2 (50)

Ox, Oxacillin; Cs, Cefoxitin; Gen, Gentamycin; P, Penicillin; AMC, Amoxicillin+Clavulanate; CPL, Ciprofloxacin; COT, Contrimoxzole; Cl, Clindamycin; TE, Tetracycline; ERY, Erythromycin; V, Vancomycin; AK, Amikacin, CZ, Cefazoline.

Prevalence of Methicillin Resistant *Staphylococcus aureus* (MRSA) among the Isolated *Staphylococcus aureus* and Coagulase Negative Staphylococci (CONS)

Out of total 38 *Staphylococcus aureus* isolated, 15 (39.47%) were Methicillin Resistant *Staphylococcus aureus* (MRSA) and 23 (60.53%) were Methicillin Sensitive *Staphylococcus aureus* (MSSA) (Figure 1). Whereas, out of total 14 CONS isolated 10 (71.42%) were Methicillin sensitive CONS (MS-CONS) and 4 (28.57%) were Methicillin resistant CONS (MR-CONS) (Figure 2).

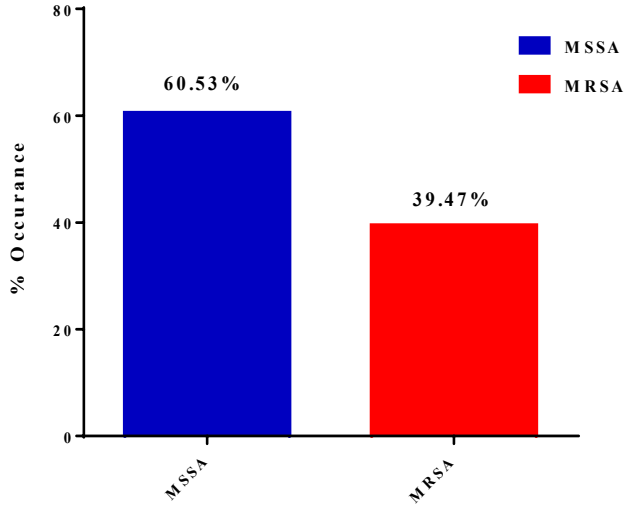

Figure 1

Percentage Occurrence of MRSA Recovered among the Isolated Staphylococcus Aureus

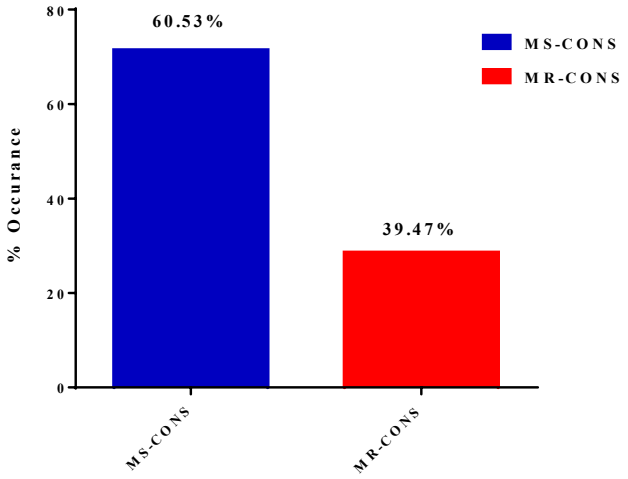

Figure 2

Percentage Occurrence of MR-CONS Recovered among the Isolated Coagulase Negative Staphylococci

Antibiotic Resistance Pattern of Isolated MSSA and MRSA Isolates

Cent percent MSSA and MRSA isolates were found resistant to penicillin. Significant difference in antibiotic resistance pattern was found among MRSA and MSSA isolates (*P=<0.05*) reflecting increased ability of MRSA to develop resistance against various antimicrobials as shown in Table 3. The majority of MSSA strains were sensitive to antibiotics like ciprofloxacin, erythromycin, cotrimoxazole, Amoxycillin+Clavulanate, Gentamycin Amikacin, Tetracycline, Clindamycin, Cefazoline except Penicillin (100% resistant). In contrary majority of MRSA were resistant to Erythromycin, Amoxycillin+Clavulanate, Ciprofloxacin, Cotrimoxazole and sensitive to Gentamycin, Clindamycin, Cefazoline and Amikacin. Vancomycin was the only drug to which 100% isolates were susceptible (Table 3).

Table 3

Antibiotic Resistance Pattern of Isolated MSSA and MRSA Isolates

Antibiotic	Resistant isolates (%) (n=38)	Resistant MSSA isolates (%) (n=23)	Resistant MRSA isolates (%) (n=15)	*Chi square (p value)
Gentamicin	5(13.1%)	2 (8.6%)	3 (20.5%)	85.206 (<0.001)
Penicillin	38 (100%)	23 (100%)	15 (100%)	14.02 (<0.001)

Amoxycillin-clavulanic acid	20(52.6%)	7 (30.43%)	13 (86.6%)	133.504 (<0.001)
Ciprofloxacin	18 (47.3%)	7 (30.4%)	11 (73.3%)	90.568 (<0.001)
Cotrimoxazole	22 (57.8%)	10 (43.4%)	12 (80.0%)	45.364 (<0.001)
Clindamycin	3 (7.8%)	1 (4.3%)	2 (13.3%)	22.400 (<0.001)
Tetracycline	1 (2.6%)	0	1 (6.6%)	7.001 (0.008)
Erythromycin	20 (52.6%)	10 (43.47%)	10 (66.6%)	35.524 (<0.001)
Vancomycin	0	0	0	--
Amikacin	1 (2.6%)	0	1 (6.6%)	23.589 (<0.001)
Cefazolin	4(10.5%)	1 (4.3%)	3 (20.0%)	51.257(<0.001)

Door handles are important reservoir of microorganisms. Out of 100 door handle samples processed, 96(96%) showed bacterial contamination. This was slightly higher than the reports of some researchers who observed 86.7% bacterial contamination (Nworie et al., 2012). This variation in the number of positive samples from one place to the other may be due to difference in the source of swabs; the study of Nworie and his colleagues (2012) investigated swabs taken from door handle of toilet and bathroom, while the present study sampled a door handles of hospitals, while that provide environment help in the more exposure of bacteria as well as the common knowledge that large pathogenic microorganisms are found in a hospital setting compared to community. This study demonstrated the significant bacterial contamination of the hospitals' door handles with Gram- positive and Gram-negative bacteria. However, Gram positive bacteria were found to occur more in comparison to Gram- negative bacteria. Gram positive bacteria are most of the skin normal flora, that would account for their predominance on door handles.

The level of contamination was much in the swabs obtained from door handles/knobs of Outpatient Departments (OPDs) following Emergency, Laboratory, General wards and Toilets in comparison to door handles/knobs of Staff rooms, Radiology Room, Intensive Care Unit (ICU) and Operation Theatre (OT). Here, the contamination in door handles/knobs of ICU and OT were lower and could be ascribed to the fact that they are less frequently used as other places studied. This finding is in agreement with the findings of other researches who reported that traffic, exposure, environment and frequency of movement might vary the level of contamination of door handles (Boone & Gerba, 2010; Nworie et al., 2012; Hedieh et al., 2012). The present study showed that *Staphylococcus aureus* was the most prevalent organism (43.18%) among isolated Gram-positive organisms which was in consistent with that reported by Kennedy et al., (2005) who found that the most common bacterial contaminant was *S. aureus* (30.1%).

Higher percentage of *Bacillus* spp was isolated in this research that was in agreement with the findings of Brooks et al., (2007) that also reported that *Bacillus* spp as predominant organism isolated from door handles. Samy et al., (2012) also reported the isolation of *Bacillus* spp from environmental sites in Mecca city. *Micrococcus* spp were also isolated in this study and was in compliance with the work of Opere et al., (2013) who reported the isolation of Micrococcus spp from public toilets. Coagulase negative Staphylococci (CONS) and Enterococci were also reported in significant numbers here in this study which was in agreement with the finding of study done by Opere et al., (2013) who also reported the isolation of *Bacillus* spp, *S. aureus*, *S. epidermidis*, Micrococcus, Pseudomonas and *Enterococcus faecalis* from public toilets.

S. aureus and *Bacillus spp* are major normal flora of skin that probably justifies their higher prevalence, as these can easily be discharged by several human actions and they may be passed from person to person by direct contact or via surfaces, including door handles and may cause variety of disease due to their high drug resistance such as boils, pimples food poisoning, abscesses, wound infections etc if entered the body and could lead to bacteraemia, sepsis, pneumonia, meningitis and osteomyelitis. This finding was in agreement with the observations of other researchers (Nworie et al., 2012; Brooks et al., 2007).

In this study, 100% *S. aureus* and CONS were found to be resistant to Penicillin. No Vancomycin resistant *S. aureus* was reported. The effective antibiotics were Tetracycline (97.3%), Cefazoline (86.8%), Amikacin (84.2%), Clindamycin (86.8%). However, Coagulase Negative Staphylococci were sensitive to Vancomycin, Amikacin and Tetracycline followed by Clindamycin (92.8%), Cefazoline (85.7%), Gentamycin (78.5%). *Streptococcus pneumoniae* 100% sensitive to Vancomycin followed by Amikacin (83.3%), Cefazoline (83.3%), Tetracycline (83.3%), Gentamycin (66.6%), Amoxicillin + Clavulanate (50%), Ciprofloxacin (50%), Cotrimoxazole (50%), Clindamycin (33.3%), erythromycin (16.6%) and Penicillin (16.6%). No vancomycin resistant Enterococci were observed in this study. The isolated 75% Enterococci were sensitive to Amikacin, Tetracycline, Amoxicillin+ Clavulanate. The data obtained for antibiotic susceptibility tests were in lieu with findings of other studies. Among gram positive bacteria, *S. aureus* is notorious for resistance against various antimicrobial agents. Drug resistance among Staphylococci is a serious worldwide problem. Methicillin resistant *Staphylococcus aureus* (MRSA) have become widespread in hospitals and intensive care units (ICUs) than it was first identified in 1961 (Jevons et al., 1998). Global scenario of MRSA showed its increasing prevalence (Grundmann et al., 2006; Lescure et al., 2006). Earlier reports of MRSA from Nepal reported prevalence of 15.4%-29% (Subedi & Brahmadathan,

2005; Kumari et al., 2008). Recent studies from various hospitals of Nepal reported higher prevalence of 39.6%-69% (Sanjana et al., 2010; Tiwari et al., 2009). Prevalence of MRSA in our study was 39.47% which is comparable with study from Chitwan, Nepal who had observed the MRSA prevalence of 39.6%. The burden of MRSA infections in Asia is high, and approximately 13% cases of nosocomial pneumonia in Asia are caused by MRSA (Cao et al., 2015). Early detection of MRSA and preparation of effective antibiotic policy in hospitals are of great significance as health care workers and hospital instruments are likely to be colonized by MRSA.

Significant difference in antibiotic resistance pattern was found among MRSA and MSSA isolates ($P<0.05$) reflecting increased capability of MRSA to develop antibiotic resistance pattern against various antibiotics. The majority of MSSA isolated were susceptible to ciprofloxacin, erythromycin, cotrimoxazole, and amikacin but not with penicillin. 100% isolates were susceptible to Vancomycin. Clindamycin, cefazoline and amikacin which were found quite effective against MRSA in the present study would be better options for the management of such infections. *S. aureus* were resistant to ciprofloxacin, erythromycin, co-trimoxazole and amoxicillin-clavulanic acid. These antibiotics being not expensive and easy to administer and hence extensively used in past might be the reasons for their higher drug resistance pattern. Minimal use of injectable and expensive antibiotics like amikacin and gentamicin reflects on higher percentage of sensitive isolates. Being much expensive and very minimal use in the past, Clindamycin also showed quite high sensitivity against MRSA isolates. Random use of antibiotics and delay in seeking medical treatment could be additional reason for higher rate of antibiotic resistance to variety of antimicrobials in different hospitals of Western region of Nepal. Isolation of drug resistant MRSA from door handles/knobs of the hospital is worrisome. MRSA strains' contaminating on the door handles of hospitals may cause threat of infections to patients, hospital staffs, visitors to hospitals and people in the community. Thus, regular surveillance and disinfection with appropriate agent at regular interval would minimize the settlement and transmission of various pathogens including MRSA.

CONCLUSIONS

Thus, the outcomes of this study have identified a significant presence of drug resistant pathogenic bacteria including MRSA with antibiotic susceptibility patterns found on door handles of hospitals which might offer possible control or preventive measures that could be

instituted to avoid this likely vehicle of infection. Indeed, hospital staffs, inpatients, outpatients and visitors should adopt the habit of hand washing practice after using the door handles and routine surface disinfection of the hospital door handles might prevent cross contamination.

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