Antibiotic resistance pattern of Methicillin-resistant *Staphylococcus aureus* in asymptomatic prisoners of Kathmandu valley

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ABSTRACT

Objectives: The purpose of this study was to determine the prevalence of methicillin-resistant *Staphylococcus aureus* and its antimicrobial resistance among the prisoners within the prisons of Kathmandu valley.

Methods: A total of 320 nasal samples were collected from the prisoners. *S. aureus* was confirmed by using standard microbiological techniques including culture and biochemical tests. MRSA was confirmed using cefoxitin disc and antimicrobial susceptibility testing was performed by Kirby-Bauer disc diffusion method results were interpreted according to the guidelines of Clinical Laboratory Standard Institute (CLSI).

Results: Out of 320 samples, 41 (12.82%) isolates were confirmed *S. aureus* and 8 (19.51%) of them were screened as MRSA positive. Highest percentage of MRSA was found in the samples collected from Nakhu prison 6 (75%) followed by Central prison 1 (12.5%) and Juvenile Correctional Home 1 (12.5%). Majority of the of MRSA isolates (62.5%) were also found to be multiple drug-resistant strains. All of the MRSA (100%) were sensitive towards linezolid and resistant towards ciprofloxacin.

Conclusion: The presence of methicillin-resistant *S. aureus* among the prisoners indicates the emergence of drug resistance in a vulnerable population which may lead to hazardous disease breakouts in the prison and even in the community after their release. Regular monitoring and its minimization should be done in order to control its dissemination.

Keywords: Methicillin-resistant *S. aureus*, Antibiotic Susceptibility pattern, prisoners, multiple drug-resistance, Nepal

INTRODUCTION

Staphylococcus aureus (*S. aureus*) is an opportunist pathogen capable of causing a wide range of infections from mild to fatal in its host (humans and animals). It is one of the most important pathogens worldwide and has emerged as a prominent organism infecting critically ill individuals. The impact of *S. aureus* infection on human health has dramatically increased as a result of its remarkable ability to become resistant to antibiotics (Vestergaard, Frees, & Ingmer, 2019). Because of its primary habitat in moist squamous epithelium of the anterior nares, most invasive *S. aureus* infections are assumed to arise from nasal carriage (Mehraj et al., 2016).

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S. aureus infections potentially ranges from minor skin infection, osteomyelitis, food poisoning, pneumonia, and toxic shock syndrome to post-operative wound infection, infections associated with foreign bodies, bacteremia, and necrotizing pneumonia (Cheung, Bae, & Otto, 2021).

S. aureus can adapt rapidly to the selective pressure of antibiotics, and this has resulted in the emergence and spread of methicillin resistant- *Staphylococcus aureus* (MRSA). The difference between MRSA and methicillin-susceptible *Staphylococcus aureus* (MSSA) is resistance to β-lactam antibiotics which include penicillins and cephalosporins. MRSA has become resistant to β-lactam drugs through modification of the target, not allowing the antibiotic to bind to and weaken the bacterial cell wall.The *mecA* gene is located on a mobile genetic element known as the Staphylococcal Cassette Chromosomal mec element (SCCmec) .This is often associated with resistance to multiple other antibiotics, which limits the therapeutic options (Liu et al., 2016).

Various categories of MRSA based on epidemiologic characteristics are commonly used and include Hospital Acquired MRSA (HA-MRSA), Community-Acquired MRSA (CA-MRSA) and Livestock-Associated MRSA (LA-MRSA). HA-MRSA and CA-MRSA are most frequent cases of hospital and community-acquired infections and constitute a major burden on society world-wide (DeLeo, Otto, Kreiswirth, & Chambers, 2010). CA- MRSA differs in several ways from health-care-associated infections. CA- MRSA is not associated with known risk factors such as comorbidities and long-term antibiotic use and is several times more likely than health-care associated strains to cause skin and soft tissues infections. Many CA- MRSA strains differ from hospital-associated strains in their mobile genetic element, carrying staphylococcal chromosomal cassette mec (SCCmec) type IV.This genotype is often less resistant to non-beta- lactam antibiotics, but more likely to carry Panton-Valentine leukocidin (PVL), a virulence factor that may be responsible for the increased morbidity and mortality associated with community associated MRSA infections (He & Wunderink, 2020)

The US Centers for Disease Control and Prevention (CDC) estimates that about 12% of MRSA infections are now community-acquired, however, this percentage can vary in different communities and patient population due to recent antibiotic use, sharing contaminated items, having active skin diseases or injuries, poor hygiene and living in crowded settings (David & Daum, 2010). The transmission of MRSA is largely from people with active MRSA skin infections and mostly spread by direct physical contact except through the air and indirect contact by touching objects such as towels, bedsheets, wound dressings, clothes, workout areas, sports equipment contaminated by the infected skin of a person with MRSA (Grundmann, Aires-de-Sousa, Boyce, & Tiemersma, 2006).

In the 1990s a change in the epidemiology of methicillin resistant Staphylococcus aureus (MRSA) was noted when serious, sometimes even fatal infections began to occur among healthy members of the community without the traditional risk factors associated with exposure to the hospital setting. MRSA has now been reported among healthy children, urban poor/homeless, military personnel, prisoners, injection drug users, institutionalized adults with developmental disabilities, and members of athletic teams (Aiello, Lowy, Wright, & Larson, 2006).

Many individuals serve as a carrier to MRSA unknowingly and hence raises global concern (Baillargeon et al., 2004). In addition to risk factors for carriage of MRSA, factors that may enhance risk of infection in the community setting include living or working in crowded conditions, skin diseases, and immunosuppression. Before incarceration and after release from prison, prisoners may serve as an important reservoir of resistant organisms that can then be transmitted to the community (Aiello et al., 2006)

So, this study was aimed to determine the prevalence of the MRSA and their antibiotic resistance pattern within the prison inmates that are mostly isolated from general society.

MATERIALS AND METHODS

Study site, duration and study population

This study comprised a total of 320 nasal samples obtained from asymptomatic prisoners held in four different prisons of Kathmandu valley. The samples were collected from January to April 2018.

Sample collection and culture of specimen

The Nasal swabs were collected following strict aseptic

conditions. Prisoners were provided with an informational questionnaire and interested inmates were selected for the sampling. Using the sterile cotton swab (Hi-Media, India) dipped in normal saline, inmates were asked for the samples from their nasal cavity from both the nares aseptically. Those prisoners who were unable to do so by themselves were assisted by our sampling team. The collected samples were transferred to the sterile screw-capped tube containing m- Staphylococcus broth (Becton, Dickinson and Company, USA) enriched with the final concentration of 75 mg/L of polymyxin B, 0.01% potassium tellurite and 12.5 mg/L nystatin (Sigma-Aldrich, USA).

The collected samples in the enrichment media were incubated at 37^0C for 48 hours in microaerobic condition. After incubation, the samples with black precipitate were further inoculated in Mannitol Salt Agar (Hi-Media). The agar plates were incubated at 37^0C for 24 hours. Next day the colonies exhibiting the yellow coloration were subcultured in Nutrient Agar (NA) and Nutrient broth (NB) (Hi-Media, India) for biochemical tests and antibiotic susceptibility test.

Isolation and identification of isolates

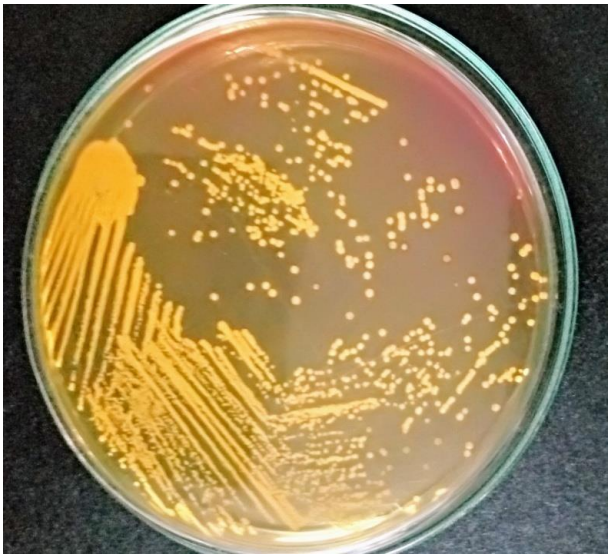
The yellow colonies on MSA were considered as *S. aureus* (Photograph 1). Standard Gram staining was followed to identify the morphology and arrangement of bacterial cells. The Gram-positive cocci in clusters were further processed for biochemical tests. Various biochemical tests were performed by following standard protocol and those with catalase positive, oxidase negative, coagulase positive and fermentative were confirmed as *S. aureus.*

Antibiotic susceptibility test

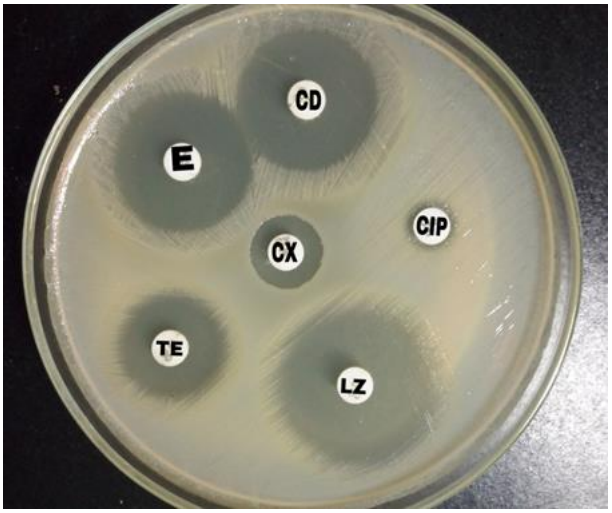
All the organisms identified as *S. aureus* were subjected to in-vitro antibiotic susceptibility test following Kirby-Bauer disc diffusion method as recommended by CLSI guidelines (CLSI 2014) (Photograph 2). The antibiotics used for the tests were erythromycin (15µg), clindamycin (2µg), co-trimoxazole (25µg), cefoxitin (30µg), tetracycline (30µg), ciprofloxacin (15µg), and linezolid (30µg). The inoculums were prepared by inoculating 2-3 identical colonies on sterile normal saline from NA. The inoculums were matched with the turbidity of 0.5 McFarland Standard. Lawn cultures of inoculums were done on Mueller-Hinton Agar (Hi-Media, India) with the help of sterile cotton swab dipped into prepared inoculums. Using sterile forceps all the antibiotic discs were subjected to the culture plate maintaining the distance. All the agar plates were incubated at 37^0C for 24 hours. After incubation, following the guideline suggested by CLSI the zone of inhibition around the antibiotic disc was measured (CLSI 2014). Isolates resistant to cefoxitin (30µg) were noted as MRSA and isolates susceptible to cefoxitin (30µg) were noted as MSSA.

D-test of MRSA

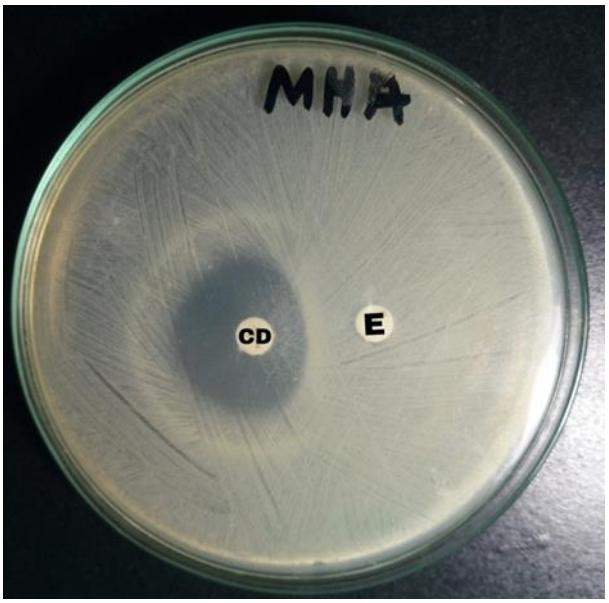
In this test, isolated MRSA was inoculated over an MHA media through lawn culture and erythromycin (macrolide) and clindamycin (lincosamide) derivative of the antibiotic disc were placed adjacent to each other 15 mm. apart. The organism showing the growth flattering end at a site facing towards erythromyc was interpreted as D-test positive organism (Photograph 4).



Photograph 1: Growth of *S. aureus* on MSA



Photograph 2: Antibiotic susceptibility pattern of MRSA



Photograph 3: Inducible Clindamycin Resistance Test (D-test)

RESULTS

A total number of 320 nasal samples were collected from prisoners of four prison. Among prisons, highest number of samples collected from Nakhu 110(34.4%) and least from Dillibazar 52(16.2%) as shown in table 1.

Table 1: Sample distribution among different prisons

Prison	Number of samples		Total (%)
	Male	Female	
Central	75	16	91(28.4)
Dillibazar	52	-	52(16.2)
Nakhu	110	-	110(34.4)
Juvenile correctional home	67	-	67(21)
Total	304(95%)	16(5%)	320 (100)

Among 320 nasal swabs samples processed, 41 (12.8%) showed S. aureus growth positive. Of 41 S. aureus isolates, highest number was from Nakhu prison 13 (31.7%) followed by Juvenile correction home 12 (29.3%), Central prison 9 (22.0%) and Dillibazar prison 7 (17.0%).

The high prevalence of MRSA was observed in Nakhu prison 6 (75%) followed by single isolates of MRSA from Central prison and Juvenile correction home, and none from Dillibazar prison.

As shown in figure 2, the highest number of samples were collected from 21-30 years 97 (30.3%) age group and least from 50 and more than 50 years (6.9%). Highest percent of *S.aureus* isolated from 11-20 years (16%) followed by 31-40 (15.9%) and 41-50 years (15.8%) group. But highest MRSA percent was derived from 31-40 years (3.4%) followed by 21-30 years (3.1%) in respective group. None observed in 50 and more age group.

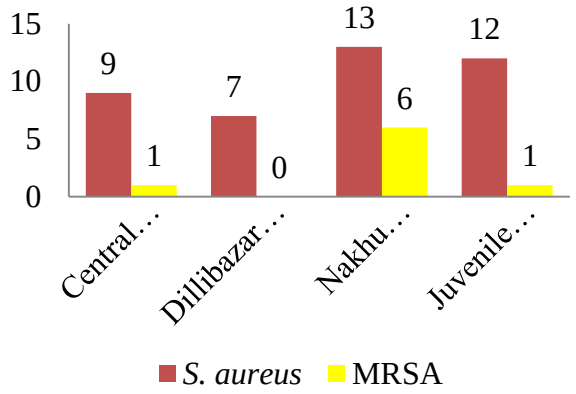


Figure 1: Distribution of S. aureus and MRSA among different prisons

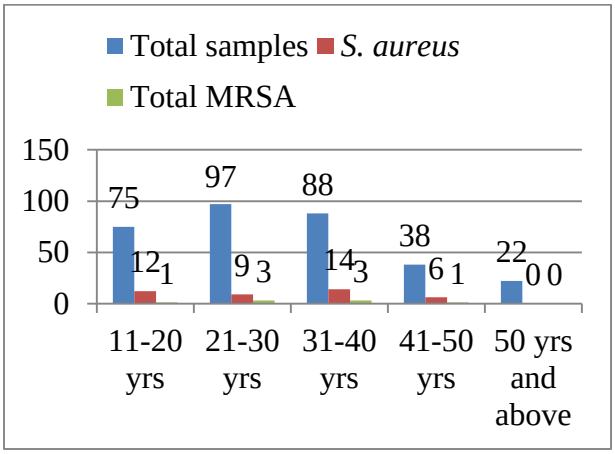


Figure 2: Distribution of *S. aureus* and MRSA among prisoners according to age group

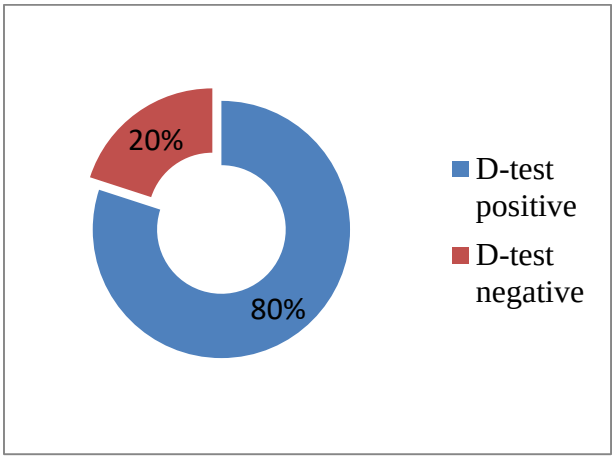


Figure 3: Inducible clindamycin resistance in MRSA strain

Antibiotic susceptibility pattern in Table 2 showed all of the MSSA isolates were sensitive to clindamycin and linezolid and 39.4% resistant to ciprofloxacin. In case of MRSA, 100% resistant towards ciprofloxacin followed by erythromycin (62.5%) and 100% sensitive to linezolid followed by clindamycin (62.5%).Among 5 isolated MRSA that showed erythromycin resistance and clindamycin sensitive, 4 (80%) strains showed Inducible clindamycin resistance pattern (CLSI 2014) where remaining 1 (20%) isolate didn't show ICR pattern shown in Figure 3. Out of 8 MRSA isolates 5 (62.5%) isolate showed Multiple Drug Resistance (MDR) and rest 3 (37.5%) didn't showed MDR represented by figure 4.

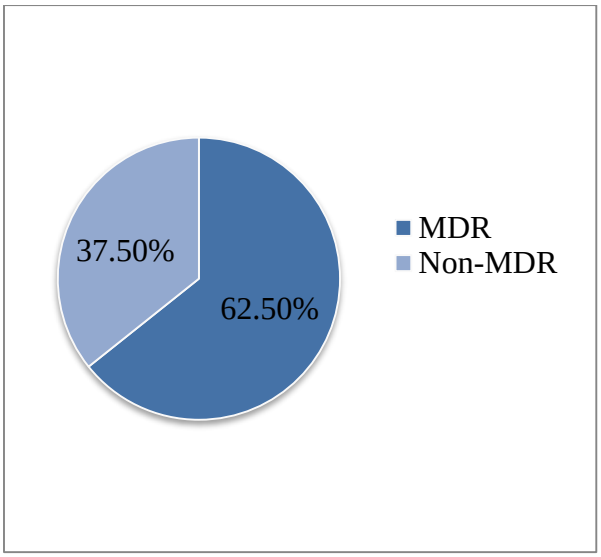


Figure 4: Multiple Drug Resistance pattern in MRSA strains

Table 2: Antibiotic susceptibility pattern of MSSA and MRSA

Antibiotics used	Antibiotic Susceptibility pattern					
	MSSA (n=33)			MRSA(n=8)		
	Sensitive (%)	Intermediate (%)	Resistant (%)	Sensitive (%)	Intermediate (%)	Resistant (%)
Ciprofloxacin	17(51.5)	3(9.1)	13(39.4)	0	0	8(100)
Clindamycin	33(100)	0	0	5(62.5)	3(37.5)	0
Co-trimoxazole	24(72.7)	3(9.1)	6(18.2)	4(50)	0	4(50)
Erythromycin	18(54.6)	9(27.3)	6(18.2)	3(37.5)	0	5(62.5)
Tetracycline	23(69.7)	10(30.3)	0	2(25)	6(75)	0
Linezolid	33(100)	0	0	8(100)	0	0

DISCUSSION

For understanding new and emerging resistance trends and in the management of both hospital and Community-acquired infections, surveillance on the antimicrobial susceptibility patterns of *S. aureus* is of chief importance. CA-MRSA being one of the burning global issues, different approaches has been made for the detection of prevalence and colonization of MRSA worldwide however, in Nepal sufficient research has not been carried out. This study aimed to generate the data for the determination of CA-MRSA nasal carriage among the asymptomatic prisoners of Kathmandu valley.

In this study, total 320 nasal samples were collected from prisoners of four different prisons and among them highest sample 110 (34.4%) collected from Nakhu prison. The prisoners included 95% male and 5% female. A study in the prison of USA showed that women were at higher risk for MRSA carriage on admission, compared with men [relative risk {RR}, 2.46 (95% confidence interval)] (Aiello et al., 2006). But in our study, none of the sample collected from female prisoner showed growth positive which may be due to very low female sample (5%) as shown in table 1.

Screening of prisoners for current study revealed that the prevalence of MRSA among *S. aureus* isolates is 19.5 % (8/41) shown in figure 1. It was reported that in the San Francisco County Jail system MRSA increased from 29% in 1997 to 74% in 2002 and in Texas jails and prisons, the proportion of MRSA among prison patients with *S aureus* infections increased from 25% (864/3520) in 1998 to 66% (5684/8633) in 2002 (Aiello et al., 2006). But our study showed a lower prevalence rate of MRSA carriage among the prisoners in comparison US jails. This may be due to small sample size and also the asymptomatic prisoners selected under study had no apparent health disorders, history of recent visit to hospital environments, use of antimicrobials in past three months and sharing of clothes during the study period.

Figure 1 also showed the highest number of *S.aureus* and MRSA were isolated from Nakhu prison as 31.7% (13/41)

and 75% (6/8) respectively. This may be due to the fact that highest sample collected from this prison and also a huge number of prisoners were kept in a single room which may have resulted in the transmission of MRSA via direct or indirect contact.

About distribution of *S.aureus* and MRSA among different age group prisoners as shown in figure 2, it was found that *S.aureus* isolated highest in 11-20 years group and closely highest in 21-30 as well as 31-40 years groups that is ranging from 10 to 40 years of age. But in case of MRSA, the vulnerable age group range from 21 to 40 years. Such results was also obtained in the study where patients with CA-MRSA were usually younger than those with HA-MRSA (median age 30 vs. 70 years; P<0.01) and CA-MRSA infections are more likely to involve the skin and soft tissue (Bassetti, Nicco, & Mikulska, 2009).

Treatment options for meticillin-resistant *Staphylococcus aureus* available in the European Union in January 2009 were listed as Trimethoprim–sulfamethoxazole, Fluoroquinolones, Linezolid, Minocycline, Clindamycin, Quinupristin, Vancomycin, Daptomycin and Tigecycline. Although clindamycin is a good choice for many types of CA-MRSA infection, the possibility of inducible resistance to clindamycin must be recognised. Isolates initially reported as susceptible to clindamycin (but resistant to erythromycin) may develop clindamycin resistance within days of starting treatment. A newer agent with activity against Gram-positive organisms, linezolid, is the second most studied antibiotic for MRSA infections. Linezolid is approved for severe skin and soft tissue infection in adults and offers a good first choice for empiric therapy in communities with a high rate of inducible clindamycin resistance among CA-MRSA. Linezolid has a 100% bioavailable oral formulation and has been associated with a shorter length of stay and a shorter duration of intravenous treatment than vancomycin (Bassetti et al., 2009)

In present study, MSSA showed 100% sensitivity toward tetracycline, clindamycin and linezolid while MRSA strains showed 100% sensitivity towards linezolid only, followed by clindamycin (62.5%) and co-trimoxazole (50%) as presented by table 2. This showed clindamycin and linezolid may be the choice of drug for MSSA infections.

Though no complete resistance towards Clindamycin was shown by MRSA isolates, 5 isolates showed erythromycin (15µg) resistance as shown in figure 3. These 5 isolates when tested for D-test, 80%(4/5) showed Inducible clindamycin resistance pattern which means clindamycin can't be used for treatment. In various studies conducted in Nepal, positive D-test among MRSA isolates were found to be 17/140 (12.1%) (Sah, 2015) and 11/24 (45.8%) (Lama, 2018). Since clindamycin is a drug used for treating both methicillin-susceptible and resistant staphylococcal infections, development of clindamycin resistance is a serious concern and should be studied in further detail and should be regularly tested since, D-test implantations in the laboratory is a simple and easy method . Use of D-test in a laboratory routinely will enable us in guiding the clinicians for judicious use of clindamycin in skin and soft tissue infections. Routine use of the D-test in the diagnostics also adds the early detection of its phenotypic resistance pattern that can help the clinician to avoid treatment failure possibilities.

Drug resistance, often as a consequence of horizontal gene transfer is initially encountered in hospitals and healthcare institutions, where there is a maximum selective pressure for resistance. Resistant strains are contained within hospitals temporarily, but eventually through a series of modifications and adjustments, invariably find their way to the community to emerge as fully fit and virulent pathogens. CA-MRSA strains are usually considered to be more virulent than HA-MRSA, leading to an important problem in terms of morbidity and mortality if they reach the hospital population. The circulation of CA-MRSA strains in hospitals has been already described and several reports suggest that CA-MRSA may be replacing HA-MRSA strains, with potentially catastrophic consequences (Bassetti et al., 2009) The simultaneous global emergence of genetically divergent CA-MRSA strains rivals the earlier spread of intercontinental HA-MRSA lineages, and highlights the evolutionary versatility of MRSA as a pathogen no longer limited to healthcare environments (Chambers & Deleo, 2009). A recent study suggests that higher levels of methicillin resistance in HA-MRSA strains may suppress expression of virulence factors, thereby limiting their ability to compete with CA-MRSA strains in community settings (Rudkin et al., 2012). Moreover, recent mathematical models predict that CA-MRSA strains harboring smaller SCCmec elements will eventually displace traditional HA-MRSA strains in hospitals, with significant clinical and

public health implications (Mediavilla, Chen, Mathema, & Kreiswirth, 2012).

Research on multidrug-resistant organisms such as MRSA in prisons should focus on characterising the extent of the problem with prevalence surveillance. The use of molecular epidemiological and social networking techniques should be carried out to elucidate the transmission dynamics and nature of the problem. Such studies help to develop sustainable and cost-effective interventions in targeted group.

CONCLUSION

The prevalance of MRSA among prisoners of Kathmandu valley was observed. MRSA isolates were resistant to most of the antibiotics; however, all isolated strains were sensitive to linezolid and were resistant to ciprofloxacin. All MSSA showed full sensitivity towards clindamycin and linezolid. Majority of strains showed resistance towards commonly used antibiotics. This indicates that the prison can be the hotspot for MRSA outbreak in the future. So, regular monitoring must be done in order to avoid outbreaks and minimise the risks it pose for the community.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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