Research Article

Multidrug-resistant *Staphylococcus aureus* Colonization in Healthy Adults Is more Common in Bhutanese Refugees in Nepal than Those Resettled in Ohio

Jhalka Kadariya,1 Dipendra Thapaliya,1 Sabana Bhatta,1 Ram Lal Mahatara,2 Sandra Bempah,1 Nabin Dhakal,3 and Tara C. Smith1

1Kent State University, College of Public Health, Department of Biostatistics, Environmental Health Sciences and Epidemiology, 750 Hilltop Drive, Kent, OH 44242, USA
2AMD AH os p i t a l, Madhumala Road, Damak, Jhapa, Nepal
3International Organization for Migration (IOM), Bhrikuti Chwok, Damak, Jhapa, Nepal

Correspondence should be addressed to Tara C. Smith; tsmit176@kent.edu

Received 4 October 2018; Revised 4 February 2019; Accepted 9 May 2019; Published 1 July 2019

Academic Editor: Cheng-Hsien Lu

Copyright © 2019 Jhalka Kadariya et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Although studies have shown that human migration is one of the risk factors for the spread of drug-resistant organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA), surveillance studies examining MRSA among refugee populations in the US are lacking. This study aimed to assess the prevalence and molecular characteristics of *S. aureus* among Bhutanese refugees living in Nepal and resettled in Northeast Ohio (NEO). One hundred adult Bhutanese refugees from each geographic location were enrolled between August 2015 and January 2016. The participants were interviewed to collect demographic information and potential risk factors for carriage. Nasal and throat swabs were collected for bacterial isolation. All *S. aureus* isolates were characterized by spa typing and tested for the presence of Panton-Valentine leukocidin (PVL) and mecA genes; selected isolates were tested by multilocus sequence typing (MLST). The overall prevalence of *S. aureus* was 66.0% and 44.0% in NEO and Nepal, respectively. In Nepal, 5.8% (3/52) of isolates were MRSA and 1.1% (1/88) in NEO. Twenty-one isolates in NEO (23.9%) were multidrug-resistant *S. aureus* (MDRSA), while 23 (44.2%) in Nepal were MDRSA. In NEO, 41 spa types were detected from 88 *S. aureus* isolates. In Nepal, 32 spa types were detected from 52 *S. aureus* isolates. spa types t1818 and t345 were most common in NEO and Nepal, respectively. The overall prevalence of PVL-positive isolates among *S. aureus* in Nepal and NEO was 25.0% and 10.2%. ST5 was the most common sequence type in both locations. Bhutanese refugees living in Nepal and resettled in NEO had high prevalence of *S. aureus* and MDRSA. The findings suggest a potential need for CA-MRSA surveillance among the immigrant population in the US and among people living in Nepal, and a potential need to devise appropriate public health measures to mitigate the risk imposed by community-associated strains of *S. aureus* and MRSA.

1. Introduction

*Staphylococcus aureus* is a common opportunistic bacterium. This bacterium is an important pathogen due to combination of toxin-mediated virulence, invasiveness, and antibiotic resistance [1]. Although it may be part of the normal human microbiota, it can cause wide range of diseases from skin and soft-tissue infections (STIs) to severe invasive disease such as infective endocarditis, osteomyelitis, and toxic shock syndrome [2]. *S. aureus* is also a major cause of food-borne illness worldwide [3]. An estimated 30% and 1.5% of the US population is colonized with methicillin-susceptible *Staphylococcus aureus* (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA), respectively [4, 5]. *S. aureus* can be recovered from many locations in and on the body, including the nose, throat, axillae, and groin, but the most important site for colonization is thought to be the anterior nares (nostrils) [4]. While colonization with *S. aureus* itself does not harm the host, colonization is a risk factor for developing subsequent symptomatic infections [6].
S. aureus accounts for nearly 20% of bloodstream infections in the hospital setting [7]. The treatment of S. aureus infections is often challenging due to the emergence of multidrug-resistant strains [8, 9]. Although drug resistance has been seen as a major risk in healthcare settings, a similar increasing trend is observed in community-acquired infections [10].

Traditionally regarded as a nosocomial pathogen [11], MRSA infection outside of hospital has increased in incidence over the last decade [12] and has emerged as a major public health concern worldwide [13]. For example, community-acquired methicillin-resistant S. aureus (CA-MRSA) is the leading cause of identifiable skin and soft-tissue infections (SSTIs) observed in US emergency rooms [14]. An estimated 80,461 invasive MRSA infections occurred nationally in 2011. Of these, 16,560 were community-associated infections [15]. The economic impact of CA-MRSA is tremendous. A recent publication suggested these infections impose an annual burden of $478 million - $2.2 billion on third-party payers and $1.4 billion - $13.8 billion on society in the US [16]. Because of lengthy hospital stays, increased costs, and higher mortality, MRSA infections have imposed an increased disease burden in the last decade [12, 17].

The disease burden caused by S. aureus is poorly understood in developing countries [18]. Although there have been some studies conducted in the context of Nepal, very little is known about the molecular epidemiology and transmission dynamics of S. aureus in this country. Most of the studies conducted in Nepal have been focused on the hospital environment, examining only the prevalence and phenotypic characterization of S. aureus [19–24]. As such, the molecular epidemiology of S. aureus in community settings has not been investigated, and S. aureus colonization parameters among the general population or specific groups such as Bhutanese refugees is unknown.

In the US, little is known regarding the prevalence and epidemiology of MRSA in immigrant populations. Although some infections, including tuberculosis and syphilis, may be detected during the pre- or postarrival screening, MRSA colonization is not examined, as there has been no policy or protocol for such screening by the US health institutions.

In the past several years, the US has granted asylum to Bhutanese immigrants of Nepalese origin that were displaced from Bhutan due to political reasons. As a result, Bhutanese of Nepali origin were one of the largest groups of refugees resettled in the US, accounting for 19% of the total 322,565 refugees admitted into the US between 2008 and 2012 [25]. This study investigated the prevalence and molecular epidemiology of S. aureus carriage in adult Bhutanese refugees living in camps in eastern Nepal and resettled in Northeast Ohio (NEO), United States.

2. Materials and Methods

2.1. Study Population. A community-based cross-sectional study was conducted among Bhutanese refugees living in Nepal and NEO in between August 2015 and January 2016. The study sites were Beldangi refugee camp in Jhapa district of Nepal and Akron and Cleveland metropolitan area in NEO. A convenience sample of 100 Bhutanese refugees aged 18 years and older, upon their consent to participate in the study, was recruited in each site. In both study sites, participants were recruited in community settings. Most of the participants in Nepal were enrolled in their home. A small portion of participants were invited to come to Primary Health Center for interview and sampling for their convenience. However, a meeting room was used for sampling. In NEO, the participants were recruited by visiting Nepalese grocery stores, Nepali restaurants, and the households of the participants and via distribution of flyers in the community.

2.2. Survey Instrument and Study Variables. Data obtained from the questionnaire included sociodemographic information such as family size, education, income, and occupation; medical information such as presence or absence of asthma, heart disease, diabetes, autoimmune disease, and cancer; presence or absence of risk factors such as recent history of hospitalization, hospital visit, history of antibiotic treatment, time spent in jail, participation in team or contact sports, ownership of pets, and history of S. aureus or MRSA infection in the respondent or his/her family members; occupational exposure; and some miscellaneous exposures such as exposure to manure, pork, chicken, turkey meats, live animals, and types of soap used at home. An outcome variable “S. aureus and MRSA colonization positive” was assessed in association with exposure variables such as environmental and occupational exposures.

2.3. Data Collection. A set of pretested semistructured questionnaires was used to collect information from study participants. A face-to-face interview was conducted with each participant by the research team. Questionnaires, first developed in English language were translated into Nepali language, and the Nepali language questionnaire set was used to interview the participants in both study sites. The purpose of the study, its potential harm, and benefits were explained to each participant. Verbal and written consent was obtained from each participant to participate in the study. A copy of Nepali consent form was provided to each participant to take with them. The Institutional Review Board (IRB) of Kent State University and of the Nepal Health Research Council (NHRC) approved the study.

2.4. Sample Collection and Bacterial Culture. Biological specimens were collected from nares and throats using sterile swabs as described previously [26] and transported to the laboratory on blue ice packs. Samples were processed within 24 hours of collection. Samples were inoculated into 5ml (1X concentration) Baird Parker Broth with tellurite enrichment (Sigma products-Sigma-Aldrich, St. Louis, MO) in a 15 ml sterile plastic tubes by twisting the swab vigorously and incubated for 24 hours at 37°C. One microliter cultured broth was inoculated onto Baird Parker agar (BPA) with EY tellurite enrichment (BD) and was incubated for 48 hours at 37°C. Isolates were subcultured onto Columbia colistin-nalidixic agar with 5% sheep blood (Columbia CNA; Ramel) and tested via the catalase test, coagulase test, and using a S. aureus.
latex agglutination assay (Pastorex Staph-plus, Bio-Rad). All *S. aureus* isolates were stored at -80°C. *S. aureus* prevalence was calculated as a percent of all individuals tested who were positive at either carriage site (nose or throat); MRSA and MDRSA prevalence were calculated as percentage of all *S. aureus* isolates who demonstrated these phenotypes.

2.5. Antimicrobial Susceptibility Testing (AST). *S. aureus* isolates were tested for antibiotic susceptibility via Vitek-2 System version R06.01 (BioMérieux, Durham, NC) per manufacturer's instructions. Isolates were tested for susceptibility to benzylpenicillin, oxacillin, tetracycline, erythromycin, ciprofloxacin, moxifloxacin, minocycline, clindamycin, trimethoprim–sulfamethoxazole (TMS), quinupristin/dalfopristin, gentamicin, levofloxacin, linezolid, daptomycin, vancomycin, rifampin, minocycline, tigecycline, and nitrofurantoin per Clinical and Laboratory Standards Institute guidelines [27]. Bacterial isolates resistant to three or more antibacterial or resistant to oxacillin were designated as multidrug-resistant MDRSA [28].

2.6. Molecular Characterization. Genomic DNA was extracted using the Wizard Genomic DNA preparation kit (Promega, Madison, WI). The staphylococcus protein A (*spa*) gene was amplified as described [29, 30]. The detection of the *mecA* and PVL genes was determined by PCR [31, 32]. *spa* types were assigned using Ridom® StaphType software (version 2.2.1; Ridom GmbH, Würzburg, Germany). The Based on Repeat Pattern (BURP) algorithm was used to group *spa* types into genetic clusters based on their genetic proximity [33]. A selected number of isolates were subjected to multilocus sequence typing (MLST) as described previously [34]. The most common, unique, and/or isolates of interest were chosen for MLST (e.g., those which were present in high numbers, low numbers, or unique in some manner including a novel *spa* type). Sequence types (STs) were assigned using organism specific MLST database (https://pubmlst.org/saureus/). PHYLOViZ software v2.0 was used to analyze Global optimal eBURST of STs and to draw minimum spanning tree and relatedness of STs as described by Francisco et al. [35, 36]. Positive (USA 300) and negative controls (reaction mixture without DNA template) were used in *mecA*, PVL, and *spa* PCR.

2.7. Statistical Analysis. Frequency distribution and proportions were calculated for the categorical variables while mean (± standard deviation), median, and range were calculated for continuous variables. Chi-square test or Fisher's exact test and logistic regression were used to assess the association between outcome variables and the potential risk factors. The crude odds ratio (OR) was calculated with 95% confidence intervals. Statistical significance was assessed at α = 0.05 level. All statistical analyses were conducted using SAS statistical software (Version 9.3, SAS Institute Inc., Cary, NC).

### 3. Results

3.1. Demographic Information. A total of 400 samples (200 nasal and 200 throat) were collected from 200 participants (100 from each study site). The mean age of the participants was 30.7 (SD 10.9) years in NEO and 36.8 (SD 13.3) years in Nepal. In NEO, 58% and 42% of the participants were male and female, respectively, while the percentage of male and female participants in Nepal was 29% and 71%, respectively. In Nepal, the majority of the participants was from the Kirat religion (79%), married (86%), and had a formal education (60%). In NEO, the majority of the participants had formal education (86%) (Table 1).

#### Table 1: Socio-demographic characteristics from NEO and Nepal.

| Category               | NEO (n=100) | Nepal (n=100) | P-value |
|------------------------|-------------|---------------|---------|
| Age in years (mean, SD)| 30.7, 10.9  | 36.8, 13.3    | <0.05   |
| Gender                 |             |               |         |
| Female                 | 42          | 71            |         |
| Male                   | 58          | 29            |         |
| Religion               |             |               | <0.001  |
| Kirat                  | 45          | 79            |         |
| Others                 | 55          | 21            |         |
| Marital Status         |             |               | <0.001  |
| Married                | 45          | 86            |         |
| Others                 | 55          | 14            |         |
| Education              |             |               | <0.001  |
| No formal Ed           | 14          | 40            |         |
| Had formal Ed          | 86          | 60            |         |

ED, education.

In Nepal, 39.0% (39/100; 95% CI 29.4%–48.5%) and 13.0% (13/100; 95% CI 6.4%–19.6%) of the participants were colonized with *S. aureus* in their throat and nose, respectively, while the prevalence of *S. aureus* colonization in nose and throat in NEO was 25.0% (25/100; 95% CI 16.5%–33.5%) and 63.0% (63/100; 95% CI 53.5%–72.5%), respectively. The difference of *S. aureus* colonization in nose and throat between Nepal and NEO was significant (*p* = 0.04 for nose and 0.001 for throat). The prevalence of positive isolates from both locations (nose and throat) from the same participants was 16% (7/44; 95% CI 5.1% - 26.7%) in Nepal and 33% (22/66; 95% CI 22% - 44.7%) in NEO. This difference was significant (*p* = 0.04).
Table 2: Exposure variables in association with S. aureus colonization in NEO (n=100).

| Variables                              | SA+ (%) | SA– (%) | P-value |
|----------------------------------------|---------|---------|---------|
| **Gender**                             |         |         |         |
| Male                                   | 42 (72.4) | 17 (27.6) | 0.13    |
| Female                                 | 24 (57.1) | 18 (42.9) |         |
| **Daycare going children**             |         |         |         |
| Yes                                    | 10 (52.6) | 9 (47.4) | 0.18    |
| No                                     | 56 (69.1) | 25 (30.9) |         |
| **Skin infections (within 6 months)**  |         |         |         |
| Yes                                    | 12 (75.0) | 4 (25.0) | 0.56    |
| No                                     | 54 (64.3) | 30 (35.7) |         |
| **Hospital admission (within 6 months)**|         |         |         |
| Yes                                    | 26 (61.9) | 16 (38.1) | 0.52    |
| No                                     | 40 (69.0) | 18 (31.0) |         |
| **Hospital visits (within 6 months)**  |         |         |         |
| Yes                                    | 41 (60.3) | 27 (39.7) | 0.11    |
| No                                     | 25 (78.1) | 7 (21.9)  |         |
| **Volunteers in health Institution**   |         |         |         |
| Yes                                    | 3 (50.0)  | 3 (50.0)  | 0.40    |
| No                                     | 63 (67.0) | 31 (33.0) |         |
| **Pork handle (within 6 months)**      |         |         |         |
| ≥1 times per week                      | 40 (71.4) | 16 (28.6) | 0.21    |
| Do not handle                          | 26 (59.1) | 18 (40.9) |         |
| **Poultry handle (within 6 months)**   |         |         |         |
| ≥1 times per week                      | 52 (71.2) | 21 (28.8) | 0.09    |
| Do not handle                          | 14 (51.8) | 13 (48.2) |         |
| **Length of stay in the U.S.**         |         |         |         |
| Less than 5 years                      | 45 (70.3) | 19 (29.7) | 0.27    |
| 5 or more years                        | 21 (58.3) | 15 (41.6) |         |
| **Family size**                        |         |         |         |
| ≤5 members                             | 29 (64.4) | 16 (35.6) | 0.83    |
| >5 members                             | 37 (67.3) | 18 (32.7) |         |
| **Occupation**                         |         |         |         |
| Daily wages labor                      | 35 (74.5) | 12 (25.5) | 0.13    |
| Others                                 | 31 (58.5) | 22 (41.5) |         |
| **Antibiotics use (within 6 months)**  |         |         |         |
| Yes                                    | 7 (58.3)  | 5 (41.7)  | 0.52    |
| No                                     | 59 (67.1) | 29 (32.9) |         |
| **Skin infections (within 6 months)**  |         |         |         |
| Yes                                    | 12 (75.0) | 4 (25.0)  | 0.56    |
| No                                     | 54 (64.3) | 30 (35.7) |         |
| **Participated in Sports (within 6 months)** |         |         |         |
| Yes                                    | 26 (62.0) | 16 (38.0) | 0.52    |
| No                                     | 40 (69.0) | 18 (31.0) |         |

The overall prevalence of MRSA (based on the presence of the mecA gene) was 3.0% (3/100; 95% CI 0 – 6.3%) in Nepal and 1.0% (1/100; 95% CI 0 – 2.9%) in NEO. No significant difference was observed in MRSA prevalence between Nepal and NEO (p = 0.62, OR 0.32; 95% CI 0.03%–3.19%). Tables 2 and 3 show participants’ demographics as well as medical, occupational, and environmental risk factors previously associated with the presence or absence of S. aureus. In NEO, a higher prevalence of S. aureus was found among males, participants who handled meat (pork and poultry) in the last six months, and participants who had skin infections in the last six months. In Nepal, a higher prevalence of S. aureus was found among females, participants who had children attending day care, and found who were diagnosed with skin infections and autoimmune diseases (Tables 2 and 3).
| Variables (n=100)                     | SA+ (%)   | SA– (%)   | P-value |
|--------------------------------------|-----------|-----------|---------|
| Gender                               |           |           |         |
| Female                               | 34 (47.9) | 37 (52.1) | 0.27    |
| Male                                 | 10 (34.5) | 19 (65.5) |         |
| Religion                             |           |           |         |
| Kirat                                | 37 (46.9) | 42 (53.1) | 0.32    |
| Others                               | 7 (33.3)  | 14 (66.7) |         |
| Education                            |           |           |         |
| Formal Education                     | 27 (45)   | 33 (55)   | 0.83    |
| No formal Education                  | 17 (42.5) | 23 (57.5) |         |
| Marital status                       |           |           |         |
| Married                              | 36 (41.9) | 50 (58.1) | 0.38    |
| Others                               | 8 (57.1)  | 6 (42.9)  |         |
| Family size                          |           |           |         |
| ≤5 members                           | 26 (46.4) | 30 (53.6) | 0.68    |
| >5 members                           | 18 (41.0) | 26 (59.0) |         |
| Daycare                              |           |           |         |
| Yes                                  | 11 (47.8) | 12 (52.8) | 0.81    |
| No                                   | 33 (42.9) | 44 (57.1) |         |
| Nearest farm                         |           |           |         |
| < 50 meter                           | 24 (41.4) | 34 (58.6) | 0.54    |
| ≥50 meters                           | 20 (47.6) | 22 (52.4) |         |
| Hand wash frequency                  |           |           |         |
| After each work                      | 39 (46.4) | 45 (53.6) | 0.28    |
| Before meal                          | 5 (31.2)  | 11 (68.8) |         |
| Skin infections (within 6 months)    |           |           |         |
| Yes                                  | 16 (51.6) | 15 (48.4) | 0.38    |
| No                                   | 28 (40.6) | 41 (59.4) |         |
| Autoimmune disease (within 6 months) |         |           |         |
| Yes                                  | 9 (60.0)  | 6 (40.0)  | 0.17    |
| No                                   | 34 (41.0) | 49 (59.0) |         |
| Ear Infection(within 6 months)       |           |           |         |
| Yes                                  | 13 (39.0) | 20 (61.0) | 0.51    |
| No                                   | 31 (46.0) | 36 (54.0) |         |
| Antibiotics use (within 6 months)    |           |           |         |
| Yes                                  | 8 (32.0)  | 17 (68.0) | 0.16    |
| No                                   | 36 (48.0) | 39 (52.0) |         |
| Skin disease (within 6 months)       |           |           |         |
| Yes                                  | 16 (51.6) | 15 (48.4) | 0.30    |
| No                                   | 28 (40.6) | 41 (59.4) |         |
| Hospital admission(within 6 months)  |           |           |         |
| Yes                                  | 3 (30.0)  | 7 (70.0)  | 0.34    |
| No                                   | 41 (45.6) | 49 (54.4) |         |
| Hospital visits (within 6 months)    |           |           |         |
| Yes                                  | 4 (25.0)  | 12 (75.0) | 0.09    |
| No                                   | 40 (47.6) | 44 (52.4) |         |
| Volunteers in health Institution     |           |           |         |
| Yes                                  | 12 (42.9) | 16 (57.1) | 0.88    |
| No                                   | 32 (44.4) | 40 (55.6) |         |
| Pork handle (within 6 months)        |           |           |         |
| Yes                                  | 7 (31.8)  | 15 (68.2) | 0.39    |
| No                                   | 37 (47.4) | 41 (52.6) |         |
| Poultry handle (within 6 months)     |           |           |         |
| Yes                                  | 24 (40.0) | 36 (60.0) | 0.32    |
| No                                   | 20 (50.0) | 20 (50.0) |         |
3.3. Antibiotic Susceptibility Testing. All isolates were tested for antibiotic susceptibility. All isolates in Nepal and NEO were resistant to penicillin. Oxacillin resistance was observed for 1.1% (1/89) and 5.8% (3/52) of the isolates in NEO and Nepal, respectively. Resistance to other antibiotics was higher for Nepal than Ohio. Additionally, MDRSA rates were higher in Nepal than Ohio (44.2% and 23.6%, respectively) (Figure 1).

3.4. Molecular Characterization of *S. aureus*. spa typing was carried out on all positive isolates. In NEO, a total of 41 spa types were detected from 88 *S. aureus* isolates. Overall, t1818 was the most common spa type (12/88; 13.6%), followed by t002 (7/88; 8.0%), t104 (6/88; 6.8%), t008, t084, t085, t091 (4/88 each; 4.5%), and t521 (3/88; 3.4%). All other spa types (t005, t0559, t0104, t0106, t012, t127, t131, t16153, t1664, t1654, t1818, t1839, t190, t1911, t2379, t2663, t273, t345, t4371, t442, t491, t521, t6163, t688, t7264, t774, t803, t818, t878, t934, and t9432) were ≤ 2.2% of *S. aureus* isolates.

In Nepal, a total of 32 spa types were detected from 52 *S. aureus* isolates. Overall, t345 was the most common spa type (5/52; 9.6%), followed by t311, t219 (4/52 each; 7.7%), t442, t127 (3/52 each; 5.8%), t021, t084, t091, t159, t164, and t934 (2/52 each; 3.8%). All other spa types (t102, t008, t085, t149, t11917, t12219, t1427, t15347, t15578, t15579, t15580, t15581, t1839, t2663, t304, t3175, t376, t4188, t701, t7766, t878) were less than 2.0% of the isolates.

*spa* types were grouped based on their genetic proximity to *spa* types associated with specific cluster complexes. BURP grouping using default parameters ("exclude parameters that are shorter than 5 repeats" and "*spa* types are clustered if costs are less or equal than 4") resulted in 7 *spa* CCs and 11 singletons (t021, t127, t159, t164, t818, t878) in NEO and 6 *spa* CCs and 16 singletons (t005, t091, t104, t122, t117, t164, t190, t213, t616, t818, t878, t654, t2663, t7264, t10559, and t16153) in NEO (Supplemental Figures 1-3).

39 *S. aureus* isolates from NEO and 29 from Nepal were subjected to MLST. A total of 26 different STs were detected from NEO. The most common ST was ST5 (17.9%; 7/39), followed by ST8 (10.3%; 4/39), ST1, ST15, ST3206, and ST96 (5.1% each; 2/39). No other STs constituted more than one tested *S. aureus* isolates (Table 4). A total of 22 different STs were detected from Nepal. The most common ST was ST5 (10.3%; 3/29), followed by ST15, ST3206, ST6, ST672, and ST80 (6.9 % each; 2/29). No other STs constituted more than one tested *S. aureus* isolate (Table 5).

The overall prevalence of PVL genes among *S. aureus* isolates in Nepal and NEO was 25.0% (13/52; 95% CI 13.2%–36.7%) and 10.2% (9/88; 95% CI 3.8%–16.4%), respectively. A statistically significant higher prevalence of PVL-positive isolates was observed in Nepal compared to NEO (p=0.02). All PVL-positive *S. aureus* (t1839, t345, t1094, t2663, t008, t121, t1906, and t005) from NEO were MSSA. Two-thirds of the PVL-positive isolates from NEO (66.7%; 6/9) and 46.2% (6/13) from Nepal were MDRSA. Only one PVL-positive isolate from NEO (t345) was *mecA*-positive. Three *mecA*-positive isolates from NEObelonged to ST1 (t127), ST6 (t304), and ST3206 (t1345). One *mecA*-positive isolate from Nepal belonged to ST8 (t008). All *mecA*-positive isolates were also phenotypically resistant to oxacillin.

4. Discussion

In this cross-sectional study, we investigated the prevalence and molecular epidemiology of *S. aureus* among Bhutanese refugees living in Nepal (n=100) and in NEO (n=100). The overall prevalence of *S. aureus* was lower in Nepal (44.0%) compared to NEO (66.0%). The *S. aureus* and MRSA prevalence of this study in Nepal is similar to previous studies that documented between 15.7%–43.8% *S. aureus* carriage rates and 2.3%–7.5% MRSA nasal colonization among healthcare workers and patients in Nepal [37–42]. However, the nasal carriage of *S. aureus* (13.0%) and MRSA (1.0%) in Nepal is
Table 4: Molecular characteristics of selected *S. aureus* isolates from NEO (N=38).

| Isolates | Source | mecA | PVL | AST | spa | MLST |
|----------|--------|------|-----|-----|-----|------|
| 27NEOT   | Throat | –    | –   | P   | t002| ST5  |
| 89NEOT   | Throat | –    | +   | P, G, C, L, T | t005| ST672|
| 33NEON   | Nose   | +    | –   | P, O, E | t008| ST8  |
| 3NEOT    | Throat | –    | –   | P, C, L, TS | t084| ST2885|
| 11NEON   | Throat | –    | –   | P   | t085| ST845|
| 38NEOT   | Throat | –    | –   | P, TS | t091| ST789|
| 9NEOT    | Throat | –    | –   | P, T | t104| ST8  |
| 68NEOT   | Throat | –    | –   | P   | t10559| ST2733|
| 23NEOT   | Throat | –    | +   | P, TS | t1094| ST2112|
| 80NEOT   | Throat | –    | +   | P, C, L | t1906| ST2884|
| 63NEOT   | Throat | –    | –   | P, E, CL | t122| ST2102|
| 8NEON    | Nose   | –    | –   | P   | t127| ST573|
| 69NEON   | Nose   | –    | –   | P   | t131| ST1290|
| 72NEOT   | Throat | –    | –   | P   | t164| ST20 |
| 48NEOT   | Throat | –    | –   | P   | t1654| ST667|
| 30NEOT   | Throat | –    | –   | P, T | t1818| ST96 |
| 1NEOT    | Throat | –    | +   | P, C, L, TS | t1839| ST3206|
| 82NEOT   | Throat | –    | –   | P   | t190| ST8  |
| 70NEOT   | Throat | –    | –   | P, TS | t1931| ST1  |
| 84NEOT   | Throat | –    | –   | P   | t2119| ST2871|
| 88NEOT   | Throat | –    | –   | P   | t2379| ST5  |
| 39NEOT   | Throat | –    | +   | P, C, L, E, CL | t2663| ST2233|
| 18NEOT   | Throat | –    | –   | P   | t273| ST1  |
| 1NEON    | Nose   | –    | +   | P, C, L, TS | t345| ST3206|
| 59NEOT   | Throat | –    | –   | P   | t4371| ST5  |
| 4NEOT    | Throat | –    | –   | P, TS | t442| ST5  |
| 13NEOT   | Throat | –    | –   | P, C, L, TS | t491| ST199|
| 9NEON    | Nose   | –    | –   | P, C, L, TS | t521| ST96 |
| 96NEOT   | Throat | –    | –   | P   | t6127| ST8  |
| 50NEOT   | Throat | –    | –   | P   | t616| ST944|
| 12NEOT   | Throat | –    | –   | P   | t688| ST5  |
| 8NEOT    | Throat | –    | –   | P   | t7264| ST5  |
| 55NEOT   | Throat | –    | –   | P   | t774| ST15 |
| 79NEOT   | Throat | –    | –   | P   | t803| ST15 |
| 28NEOT   | Throat | –    | –   | P   | t818| ST5  |
| 29NEOT   | Throat | –    | –   | P   | t878| ST2849|
| 58NEOT   | Throat | –    | –   | P, G, E, CL | t934| ST80 |
| 16NEOT   | Throat | –    | –   | P, C, L | t9432| ST2128|

P, benzylpenicillin; C, ciprofloxacin; E, erythromycin; CL, clindamycin; G, gentamicin; T, tetracycline; TS, trimethoprim–sulfamethoxazole; L, levofloxacin; O, oxacillin; AST, antibiotic susceptibility testing. Inclusion of antibiotic name denotes resistance.

lower than in a prior study conducted in a community setting in Nepal [43]. A previous study examining the prevalence of MRSA in a school children in Pokhara City of Nepal via nasal swab documented 17.4% (32/184) overall MRSA prevalence [43]. Such differences in *S. aureus* and MRSA prevalence may be due to geographical, population, and methodological differences. For example, we defined MRSA based on the presence of mecA gene, our sampling took place in eastern Nepal, and participants had to be at least 18 years old to be eligible for our study. Work by Rijal et al. (2008) took place in western Nepal, enrolled only minors (younger than 15 years old), and used a phenotypic method (Kirby-Bauer) for MRSA designation. Although the prevalence of MRSA in NEO is consistent with a previous report that suggested 0.84% of the noninstitutionalized US population were colonized with MRSA, the prevalence of *S. aureus* is significantly higher in this study (31.6% versus 66.0%) compared to that report [5].

*S. aureus* prevalence in our study was not associated with demographic factors such as age, gender, marital status, occupation, education, and income in both locations, nor with reported previous antibiotic exposure. The odds of *S.
**Table 5: Molecular characteristics of selected *S. aureus* isolates from Nepal (N=29).**

| Isolates | Source | mecA | PVL | AST | Spa | ST |
|----------|--------|------|-----|-----|-----|----|
| 68T5     | Throat | –    | –   | P, TS | t002 | ST5 |
| 26T5     | Throat | –    | –   | P, C  | t1917 | ST72 |
| 71T5     | Throat | –    | –   | P    | t15579 | ST2885 |
| 74N      | Nose   | –    | –   | P, C, L, E, CL | t15581 | ST3206 |
| 19N      | Nose   | –    | –   | P    | t008 | ST8 |
| 101N     | Nose   | –    | +   | P, L, M, TS | t021 | ST1482 |
| 102T     | Throat | –    | –   | P, TS | t084 | ST15 |
| 76T      | Throat | –    | –   | P    | t085 | ST15 |
| 12T      | Throat | –    | –   | P    | t091 | ST2081 |
| 24N      | Nose   | –    | –   | P, C, L | t1149 | ST291 |
| 105T     | Throat | –    | +   | P, G, C, L, E | t12219 | ST672 |
| 83T      | Throat | +    | –   | P, O, C, E, CL, Mi, T, R | t127 | ST1 |
| 48T      | Throat | –    | –   | P, G, E, CL | t1427 | ST361 |
| 70T      | Throat | –    | –   | P    | t15578 | ST2990 |
| 1T       | Throat | –    | –   | P, E, CL, TS | t159 | ST800 |
| 85T      | Throat | –    | –   | P, G, TS | t164 | ST20 |
| 67T      | Throat | –    | +   | P, TS | t1839 | ST573 |
| 65T      | Throat | –    | –   | P, TS | t2119 | ST2871 |
| 106T     | Throat | –    | +   | P, G, C, L, E | t2663 | ST2233 |
| 15T      | Throat | +    | –   | P, O | t304 | ST6 |
| 32N      | Nose   | –    | +   | P, TS | t311 | ST5 |
| 42T      | Throat | –    | –   | P, G, T | t3175 | ST672 |
| 77N      | Nose   | +    | +   | P, O, CL, R | t345 | ST3206 |
| 97T      | Throat | –    | –   | P, E, CL | t376 | ST80 |
| 82T      | Throat | –    | –   | P, C  | t4188 | ST199 |
| 33T2     | Throat | –    | –   | P, TS | t442 | ST5 |
| 107T     | Throat | –    | –   | P    | t701 | ST6 |
| 56T      | Throat | –    | –   | P    | t878 | ST2849 |
| 109N     | Nose   | –    | –   | P, G, E, CL | t934 | ST80 |

*S. aureus* colonization among participants in NEO was 2.5 times higher than colonization in Nepal (OR = 2.58, 95% CI: 1.45%–4.58%, p < 0.001). However, variability in participants' demographic characteristics as well as length of residency in the US may have contributed to these differences. For example, the demographic composition of participants from Nepal was different from those in NEO in regard to gender, religion, marital status, and education. No significant difference was observed in *S. aureus* colonization among participants who lived less than 5 years in NEO.

The prevalence of *S. aureus* in the nose and throat was higher in NEO than in Nepal (p<0.05). Although the anterior nares are considered as the most common site of *S. aureus* colonization [6], the result of this study showed that the colonization of *S. aureus* was higher in throat compared to the nose in both locations of the study. This result is in consistent with a previous study done in Sweden that found throat as more common site of colonization [44].

Of the 18 drugs tested, resistance was observed to 9 and 12 different antibiotics in NEO and Nepal, respectively (Figure 1). Compared to NEO, a significantly higher proportion of isolates (23.6% versus 44.2%) were MDRSA in Nepal. The higher proportions of isolates were resistant to different classes of antibiotics in Nepal as compared to NEO. *S. aureus* were frequently resistant to drugs that are used commonly in Nepal. For instance, ciprofloxacin is one of the most commonly prescribed antibiotics in Nepal [45], and trimethoprim/sulfamethoxazole is one of the most preferred antimicrobial in Nepal due to its wide spectrum and low cost [46]. One of the studies done in a tertiary care hospital in western Nepal demonstrated that approximately 56% and 27% of the MRSA isolates were resistant to trimethoprim/sulfamethoxazole and ciprofloxacin, respectively [24]. In Nepal, 42% and 27% of the isolates were resistant to trimethoprim/sulfamethoxazole and ciprofloxacin, respectively. Antibiotics overuse is a common drug use problem in Nepal [47]. For example, a study conducted in eastern Nepal documented that 84% of the prescriptions contained antibiotics comprising 42.8% of the total number of drugs [48]. In NEO, 22.5% and 18.0% of the isolates were resistant to trimethoprim/sulfamethoxazole and ciprofloxacin, respectively.

The prevalence of PVL-positive isolates was significantly higher among refugees living in Nepal compared to NEO.
risk factors were associated with outcomes. Although most of the medical and occupational it does not allow for causalinference between exposures and findings is limited. Since the study design is cross-sectional, geographic locations. As such, the generalizability of these number of human samples from populations in narrow locations.spa type t345 (ST 3206) was the most common spa type in Nepal. spa type t345 was previously isolated from patients infected with S. aureus in Bangalore, India [51]. spa type t818 (ST96) was most common in NEO. spa type t818 has been previously isolated from clinical specimens in France [52], clinical samples from intensive care units of a tertiary hospital in Korea [53], and clinical samples from intensive care unit patients in the Netherlands [54]. spa type t345 was found in both locations. However, spa type t818 was found only in NEO. Only one isolate of spa types t002 (ST5) and t008 (ST8) each was found in Nepal. In NEO, 7 (8.0%; 7/88) and 4 (4.5%; 4/88) isolates were t002 and t008, respectively. spa type t002 (ST5/USA100) and t008 (ST8/USA300) are common hospital-associated and community-associated S. aureus stain, respectively. ST5 was the most common sequence type in both locations.

There are number of limitations to this study. This study employed a convenience sampling method to collect a limited number of human samples from populations in narrow geographic locations. As such, the generalizability of these findings is limited. Since the study design is cross-sectional, it does not allow for causal inference between exposures and outcomes. Although most of the medical and occupational risk factors were associated with S. aureus colonization, the association was not statistically significant. Hence, the study was not able to establish the demographic as well as medical and occupational exposures potentially implicated with S. aureus colonization. Since the study participants in Nepal and NEO are different and resettled refugees having varying duration of time regarding their residency in NEO, there might be other confounding factors affecting S. aureus colonization. The lack of longitudinal follow-up limits the understanding of the dynamics of persistent colonization of S. aureus and MRSA in the study population as well as the effects of environmental and occupational exposure over time.

The strengths of the study include the acquisition of samples from two locations that enrolled participants from the same ethnocultural heritage. The inclusion of survey questions in addition to human samples provided information concerning exposure variables. To our knowledge, this is the first study conducted to assess the prevalence and molecular characterization of S. aureus in immigrant population in the United States and in a community setting in Nepal. Follow-up studies could examine changes in colonization in a longitudinal manner as immigrants are resettled in Ohio or other areas in the US.

5. Conclusions

In conclusion, the findings of this study indicate that Bhutanese refugees living in Nepal and resettled in NEO had high prevalence of S. aureus and MDRSA carriage. S. aureus colonization in throat was significantly higher than nasal colonization in both locations. The prevalence of MSSA was higher among resettled Bhutanese refugee living in NEO as compared to those living in Nepal. Information about S. aureus epidemiology in developing countries is important, as almost 1 billion people cross international boundaries each year. Population mobility imposes threats to the distribution of antimicrobial drug-resistant organisms [10]. MRSA has been isolated from immigrant people in Europe [55, 56]. For example, a total of 56 persons who had recently arrived in Sweden between 2002 and 2003 as immigrants had cases of MRSA, giving an overall risk of 15.9 cases/100,000 immigrants [56]. In Spain, of the 19 patients infected with CA-MRSA, 15 were immigrants from South America [55]. The findings of this study emphasize the potential need for surveillance among immigrant population in the US and among people living in Nepal and a potential need to devise public health interventions to mitigate the risk imposed by S. aureus infections.

Data Availability

Data are available upon request to the authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This study was funded by start-up funds (TCS) from Kent State University, Kent, Ohio, USA.

Supplementary Materials

The materials provided were added to the manuscript to provide additional detail of molecular analyses of spa and multilocus sequence types found in samples from Northeast Ohio and Nepal. (Supplementary Materials)

References

[1] J. Qiu, H. Feng, J. Lu et al., “Eugenol reduces the expression of virulence-related exoproteins in Staphylococcus aureus,” Applied and Environmental Microbiology, vol. 76, no. 17, pp. 5846–5851, 2010.
[2] A. Corrado, P. Donato, S. MacCari et al., “Staphylococcus aureus-depicted septic arthritis in murine knee joints: Local immune response and beneficial effects of vaccination,” Scientific Reports, vol. 6, Article ID 38043, 2016.
[3] J.-A. Hennekinne, M.-L. De Buyser, and S. Dragacci, “Staphylococcus aureus and its food poisoning toxins: characterization and outbreak investigation,” FEMS Microbiology Reviews, vol. 36, no. 4, pp. 815–836, 2012.
[4] R. J. Gorwitz, D. Kruszon-Moran, S. K. McAllister et al., “Changes in the prevalence of nasal colonization with Staphylococcus aureus in the United States, 2001-2004,” The Journal of Infectious Diseases, vol. 197, no. 9, pp. 1226–1234, 2008.
[5] P. L. Graham, S. X. Lin, and E. L. Larson, “A U.S. Population-Based Survey of Staphylococcus aureus Colonization,” Annals of Internal Medicine, vol. 144, no. 5, pp. 318–325, 2006.
[6] H. F. L. Wertheim, D. C. Melles, M. C. Vos et al., “The role of nasal carriage in Staphylococcus aureus infections,” The Lancet Infectious Diseases, vol. 5, no. 12, pp. 751–762, 2005.

[7] H. Wisplinghoff, T. Bischoff, S. M. Tallent, H. Seifert, R. P. Wenzel, and M. B. Edmond, “Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study,” Clinical Infectious Diseases, vol. 39, no. 3, pp. 309–317, 2004.

[8] F. D. Lowy, “Staphylococcus aureus infections,” The New England Journal of Medicine, vol. 339, no. 8, pp. 520–532, 1998.

[9] C. R. Jackson, J. A. Davis, and J. B. Barrett, “Prevalence and characterization of methicillin-resistant staphylococcus aureus isolates from retail meat and humans in georgia,” Journal of Clinical Microbiology, vol. 51, no. 4, pp. 1199–1207, 2013.

[10] D. W. MacPherson, B. D. Gushulak, W. B. Baine et al., “Population mobility, globalization, and antimicrobial drug resistance,” Emerging Infectious Diseases, vol. 15, no. 1727, 2009.

[11] B. Diederik and J. Kluytmans, “The emergence of infections with community-associated methicillin resistant Staphylococcus aureus,” Journal of Infection, vol. 52, no. 3, pp. 157–168, 2006.

[12] M. Z. David and R. S. Daum, “Community-associated methicillin-resistant Staphylococcus aureus: epidemiology and clinical consequences of an emerging epidemic,” Clinical Microbiology Reviews, vol. 23, no. 3, pp. 616–687, 2010.

[13] S. Pu, F. Han, and B. Ge, “Isolation and characterization of methicillin-resistant staphylococcus aureus strains from Louisiana retail meats,” Applied and Environmental Microbiology, vol. 75, no. 1, pp. 265–267, 2008.

[14] C. A. Arias and B. E. Murray, “Antibiotic-resistant bugs in the 21st century—a clinical super-challenge,” The New England Journal of Medicine, vol. 360, no. 5, pp. 439–443, 2009.

[15] R. Dantes, Y. Mu, R. Bellflower et al., “National burden of invasive methicillin-resistant Staphylococcus aureus infections, United States, 2011,” JAMA Internal Medicine, vol. 173, no. 21, pp. 1970–1979, 2013.

[16] B. Y. Lee, A. Singh, M. Z. David et al., “The economic burden of community-associated methicillin-resistant Staphylococcus aureus (CA-MRSA),” Clinical Microbiology and Infection, vol. 19, no. 6, pp. 528–536, 2013.

[17] R. M. Klevens, M. A. Morrison, J. Nadle et al., “Invasive methicillin-resistant Staphylococcus aureus infections in the United States,” Journal of the American Medical Association, vol. 298, no. 15, pp. 1763–1771, 2007.

[18] E. K. Nickerson, T. E. West, N. P. Day, and S. J. Peacock, “Staphylococcus aureus disease and drug resistance in resource-limited countries in south and east Asia,” The Lancet Infectious Diseases, vol. 9, no. 2, pp. 130–135, 2009.

[19] N. Kumari, T. Mohapatra, and Y. Sigh, “Prevalence of methicillin-resistant staphylococcus aureus (MRSA) in a tertiary-care hospital in eastern nepal,” Journal of Nepal Medical Association, vol. 47, no. 170, 2013.

[20] B. Shakya, S. Shrestha, and T. Mitra, “Nasal carriage rate of methicillin resistant Staphylococcus aureus among at National Medical College Teaching Hospital, Birgunj, Nepal,” Nepal Medical College Journal: NMJC, vol. 12, no. 1, pp. 26–29, 2010.

[21] B. Shrestha, B. M. Pokhrel, and T. M. Mohapatra, “Antibiotic susceptibility pattern of nosocomial isolates of Staphylococcus aureus in a tertiary care hospital, Nepal,” Journal of Nepal Medical Association, vol. 48, no. 175, pp. 234–238, 2009.

[22] B. Shrestha, B. M. Pokhrel, and T. M. Mohapatra, “Phenotypic characterization of nosocomial isolates of Staphylococcus aureus with reference to MRSA,” The Journal of Infection in Developing Countries, vol. 3, no. 7, pp. 554–560, 2009.

[23] S. Subedi and K. N. Brahmadathan, “Antimicrobial susceptibility patterns of clinical isolates of Staphylococcus aureus in Nepal,” Clinical Microbiology and Infection, vol. 11, no. 3, pp. 235–237, 2005.

[24] H. K. Tiwari, A. K. Das, D. Sapkota, K. Sivarajan, and V. K. Pahwa, “Methicillin resistant Staphylococcus aureus: prevalence and antibiogram in a tertiary care hospital in western Nepal,” The Journal of Infection in Developing Countries, vol. 3, no. 9, pp. 681–684, 2009.

[25] M. Bhata, L. Assad, and S. Shakya, “Socio-Demographic and dietary factors associated with excess body weight and abdominal obesity among resettled bhutanese refugee women in northeast ohio, united states,” International Journal of Environmental Research and Public Health, vol. 11, no. 7, pp. 6639–6652, 2014.

[26] T. C. Smith, W. A. Gebreyes, M. J. Aley et al., “Methicillin-resistant staphylococcus aureus in pigs and farm workers on conventional and antibiotic-free swine farms in the USA,” PLoS ONE, vol. 8, no. 5, Article ID e63704, 2013.

[27] CLSI, Performance Standards for Antimicrobial Susceptibility Testing; Twenty - Second Informational Supplement, Clinical and Laboratory Standards Institute, Wayne, PA, USA, 2012.

[28] A.-P. Magiorakos, A. Srinivasan, R. B. Carey et al., “Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance,” Clinical Microbiology and Infection, vol. 18, no. 3, pp. 268–281, 2012.

[29] B. Shopsin, M. Gomez, S. O. Montgomery et al., “Evaluation of protein A gene polymorphic region DNA sequencing for typing of Staphylococcus aureus strains,” Journal of Clinical Microbiology, vol. 37, no. 11, pp. 3556–3563, 1999.

[30] L. Koreen, S. V. Ramaswamy, E. A. Graviss, S. Naidich, J. M. Musser, and B. N. Kreiswirth, “spa typing method for discriminating among Staphylococcus aureus isolates: implications for use of a single marker to detect genetic micro- and macrovariation,” Journal of Clinical Microbiology, vol. 42, no. 2, pp. 792–799, 2004.

[31] G. Lina, Y. Piémont, F. Godail-Gamot et al., “Involvement of Panton-Valentine leukocidin—producing Staphylococcus aureus in primary skin infections and pneumonia,” Clinical Infectious Diseases, vol. 29, no. 5, pp. 1128–1132, 1999.

[32] G. Bosgelmez-İmaz, S. Ulusoy, B. Arıdo˘gan, and F. Coğkun-Ari, “Evaluation of different methods to detect oxacillin resistance in Staphylococcus aureus and their clinical laboratory utility,” European Journal of Clinical Microbiology & Infectious Diseases, vol. 25, no. 6, pp. 410–412, 2006.

[33] A. Mellmann, T. Weniger, C. Berssenbrügge et al., “Based Upon Repeat Pattern (BURP): an algorithm to characterize the long-term evolution of Staphylococcus aureus populations based on spa polymorphisms,” BMC Microbiology, vol. 7, no. article 98, 2007.

[34] M. C. Enright, N. P. J. Day, C. E. Davies, S. J. Peacock, and B. G. Spratt, “Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of Staphylococcus aureus,” Journal of Clinical Microbiology, vol. 38, no. 3, pp. 1008–1015, 2000.

[35] A. P. Francisco, M. Bugalho, M. Ramírez, and J. A. Carriço, “Global optimal eBURST analysis of multilocus typing data using a graphic matroid approach,” BMC Bioinformatics, vol. 10, article no. 152, 2009.
[36] A. P. Francisco, C. Vaz, P. T. Monteiro, J. Melo-Cristino, M. Ramirez, and J. A. Carriço, "PHYLOViZ: phylogenetic inference and data visualization for sequence-based typing methods," BMC Bioinformatics, vol. 13, no. 1, article 87, 2012.

[37] B. Shrestha, B. M. Pokhrel, and T. M. Mohapatra, "Staphylococcus aureus nasal carriage among health care workers in a Nepal Hospital," The Brazilian Journal of Infectious Diseases, vol. 13, no. 5, pp. 322–322, 2009.

[38] R. Khanal, P. Sah, P. Lamicchane, A. Lamsal, S. Upadhyaya, and V. K. Pahwa, "Nasal carriage of methicillin resistant Staphylococcus aureus among health care workers at a tertiary care hospital in Western Nepal," Antimicrobial Resistance and Infection Control, vol. 4, no. 1, article 39, 2015.

[39] P. R. Joshi, M. Acharya, R. Aryal et al., "Emergence of staphylococcal cassette chromosome mec type I with high-level mupirocin resistance among methicillin-resistant Staphylococcus aureus," Asian Pacific Journal of Tropical Biomedicine, vol. 7, no. 3, pp. 193–197, 2017.

[40] S. Khatri, N. D. Pant, R. Bhandari et al., "Nasal carriage rate of methicillin resistant staphylococcus aureus among health care workers at a tertiary care hospital in kathmandu, nepal," Journal of Nepal Health Research Council, vol. 15, no. 1, pp. 26–30, 2017.

[41] J. Pant and S. Rai, "Occurrence of Staphylococcus aureus in hospital environment and staffs in teaching hospital in Kathmandu, Nepal," Journal of Nuclear Analytical Methods in the Life Sciences, vol. 8, pp. 72–73, 2007.

[42] P. Sah, K. Rigal, B. Shakya, B. Tiwari, and P. Ghimire, "Nasal carriage rate of Staphylococcus aureus in hospital personnel of National medical college and teaching hospital and their susceptibility pattern," Journal of Applied Health Sciences, vol. 3, pp. 21–23, 2013.

[43] K. R. Rigal, N. Pahari, B. K. Shrestha et al., "Prevalence of methicillin resistant staphylococcus aureus in school children of pokhara," Nepal Medical College journal : NMCJ, vol. 10, no. 3, pp. 192–195, 2008.

[44] P. Nilsson and T. Ripa, "Staphylococcus aureus throat colonization is more frequent than colonization in the anterior nares," Journal of Clinical Microbiology, vol. 44, no. 9, pp. 3334–3339, 2006.

[45] M. P. Joshi, T. Sugimoto, and B. Santos, "Geriatric prescribing in the medical wards of a teaching hospital in Nepal," Pharmacoepidemiology and Drug Safety, vol. 6, no. 6, pp. 417–421, 1997.

[46] B. Dwari, S. Bajracharya, S. Gupta et al., "Fixed drug eruption due to co-trimoxazole: a case report," Journal of Institute of Medicine, vol. 28, 2007.

[47] S. Prasad, A. Dubey, M. Rana, P. Mishra, and pp. Subish, "Drug utilization with special reference to antimicrobials in a subhealth post in western nepal," Journal of Nepal Health Research Council, vol. 3, 2005.

[48] S. R. Harmeeet, M. Nagarani, and R. Moushumi, "A study on the drug prescribing pattern and use of antimicrobial agents at a tertiary care teaching hospital in eastern Nepal," Indian Journal of Pharmacology, vol. 30, no. 3, pp. 175–180, 1998.

[49] D. R. Bhatta, L. M. Cavaco, G. Nath et al., "Association of Panton Valentine Leukocidin (PVL) genes with methicillin resistant Staphylococcus aureus (MRSA) in Western Nepal: A matter of concern for community infections (a hospital based prospective study)," BMC Infectious Diseases, vol. 16, no. 1, p. 199, 2016.

[50] R. H. Pokhrel, M. S. Aung, B. Thapa et al., "Detection of ST772 Panton-Valentine leukocidin-positive methicillin-resistant Staphylococcus aureus (Bengal Bay clone) and ST22 S. aureus isolates with a genetic variant of elastin binding protein in Nepal," New Microbes and New Infections, vol. 11, pp. 20–27, 2016.

[51] C. Bouchiat, N. El-Zenenni, B. Chakrakodi, S. Nagaraj, G. Arakere, and J. Etienne, "Epidemiology of Staphylococcus aureus in Bangalore, India: emergence of the ST217 clone and high rate of resistance to erythromycin and ciprofloxacin in the community," New Microbes and New Infections, vol. 7, pp. 15–20, 2015.

[52] O. Dauwalder, G. Lina, G. Durand et al., "Epidemiology of invasive methicillin-resistant staphylococcus aureus clones collected in france in 2006 and 2007," Journal of Clinical Microbiology, vol. 46, no. 10, pp. 3454–3458, 2008.

[53] T. Kim, J. Yi, K. H. Hong, J.-S. Park, and E.-C. Kim, "Distribution of virulence genes in spa types of methicillin-resistant Staphylococcus aureus isolated from patients in intensive care units," Korean Journal of Laboratory Medicine, vol. 31, no. 1, pp. 30–36, 2011.

[54] M. I. Rijnders, R. H. Deurenberg, M. L. Boumans, J. A. Hoogkamp-Korstanje, P. S. Beisser, and E. E. Stobberingh, "Population structure of staphylococcus aureus strains isolated from intensive care unit patients in the netherlands over an 11-year period (1996 to 2006)," Journal of Clinical Microbiology, vol. 47, no. 12, pp. 4090–4095, 2009.

[55] A. Manzur, A. M. Dominguez, M. Pujol et al., "Community-acquired methicillin-resistant Staphylococcus aureus infections: An emerging threat in Spain," Clinical Microbiology and Infection, vol. 14, no. 4, pp. 377–380, 2008.

[56] M. Stenhem, A. Ørtqvist, H. Ringberg et al., "Imported methicillin-resistant Staphylococcus aureus, sweden," Emerging Infectious Diseases, vol. 16, no. 2, pp. 189–196, 2010.