Original Research Article

Prevalence of methicillin-resistant *Staphylococcus aureus* on paper currency notes

Abstract

The main objective was to determine the prevalence of Methicillin Resistant *Staphylococcus aureus* (MRSA) in paper currency. The paper currencies in circulation in Pokhara Metropolitan City were inspected. Bills of various denominations (Rs 5, 10, 20, 50, 100, 500 and 1000) were collected from five different locations; namely Food and Vegetable Shop, Bus conductor, Hospital Pharmacy, Butcher Shop and Grocery Shop. Collected sample were cultured and incubated for 24 hours at 37^{o}C in Brain Heart Infusion (BHI) Broth. The inoculums were further cultured on Mannitol Salt Agar (MSA) and Blood Agar (BA) media to obtain colonies, which were examined and evaluated for various parameters like gram staining and biochemical tests for identification. Then, antibiotic susceptibility test of the isolates was performed using standard procedures. A total of 35 sample of paper currency were processed, all of which showed positive growth. Out of 86 total isolates, 21 (24.42%) were *Staphylococcus aureus* followed by *Coagulase Negative Staphylococci 19*(22.09%), *Diptheroids* 14(16.3%), *Bacillus* spp 13(15.11%), *Micrococci* 9(10.46%), *Streptococcus pneumonia* 4(4.65%), *Viridans Streptococcus* 4(4.65%) and *Streptococcus pyogens* 2(2.32%). The total prevalence of MRSA in this study was 7 (33.33%). Paper currency contaminated with MRSA pose a high threat to those handling the bills as well as the community. Thus this study suggests proper hygiene measures be adopted after handling of paper currency to minimize the risk of contamination and emergence of diseases.

Key words: Contamination, Methicillin Resistant *Staphylococcus aureus*, Paper Currency

INTRODUCTION

Paper currency notes are extensively used globally to exchange the purchase of goods and services circulating within the largest population around its surrounding. Circulating and expose of these notes often contaminate from the environment or from the people's hand and unhygienic practice [1] including sneezing and coughing on palm, using mucus for note counting, improper handwashing after using a toilet which ultimately makes currency as an environmental vehicle for the transmission of microorganisms from one person to another [2][3]. Infection with pathogens through contact with fomites and vehicles is widespread and more serious in the era of the COVID-19 Pandemic. Dirty and moist paper money provide substrate for microflora which could be transmitted through butchers house, conductors and vendors are often overlooked as enteric disease reservoir [4][3] and Ministry of Health 2007). [5] resulting in various infectious diseases [6] [7] [8]. In a developing country like Nepal, people often use paper money than digital payment that may result in the rapid spread of contamination every day leading to serious public health disasters in near future [7].

A previous study showed the presence of a high microbial load on paper currency and coins [3]. However, there is no evidence that the presence of potential microorganisms on paper currency or coins leads to infections. Therefore, this study aims to investigate the prevalence of Methicillin Resistance *Staphylococcus aureus* (MRSA) on paper notes in circulation in selected sites and sources of Pokhara.

Materials and methods

A descriptive cross-sectional study was conducted taking altogether 35 samples of paper notes (Rupees 5, 10, 20, 50, 100, 500, and 1000) excluding damaged, really dirty, or physically ripped/torn and polymer bills collected from different sources in Pokhara and analyzed at Microbiology laboratory of Janapriya Multiple Campus, Pokhara, Nepal during January 2019 to May 2019. The samples were collected randomly from different users from different sources like a butcher shop, grocery shop, vegetable/fruit shop, bus conductors, and hospital pharmacy nearby Prithivi chowk and Janapriya Multiple Campus, Pokhara. During the collection, users were requested to keep those samples in sterile polythene bags. Then, the

collected specimens were transported to the research laboratory as soon as possible and processed accordingly.

As reported previously, each sample was placed in 10 mL of BHI broth and subsequently incubated at 37 °C for 24 hours after shaking for 5-10 minutes [3]. After that, the pre-enriched samples were sub-cultured on Mannitol salt agar and Blood agar and incubated aerobically at 37 °C for 24 hrs. Further isolation was done by sub-culturing on selective media and nutrient agar to obtain a pure culture.

Bacterial isolates were identified phenotypically based on colony morphology, gram reaction, spore staining, and biochemical tests including catalase, oxidase, urease, and coagulase test. The identified bacterial isolates were taken for the antibiotic susceptibility test by the disc diffusion method. The inoculum was prepared by suspending the organisms into 2 mL of sterile saline (0.9% w/v NaCl) and the turbidity of this inoculum was adjusted to 0.5 McFarland standards. The inoculum was cultured at 37 °C on Mueller Hinton agar (MHA) media with a sterile cotton swab. Bacterial isolates were tested against antibiotics such as Vancomycin (30µg), Penicillin-G (10µg), Oxacillin (10µg), Cefoxitin (30µg), Chloramphenicol (30µg), Erythromycin (15µg), Ampicillin (10µg) and Co-Trimoxazole (25µg). The zones of inhibition (mm) were measured at 18-24 hours of incubation. The antibiotic susceptibility was interpreted based on CLSI guidelines [9].

Screening and Confirmation of MRSA

Further antimicrobial susceptibility test was carried out for *S. aureus* isolates to test their methicillin susceptibility, using 10 microgram oxacillin and 30 microgram cefoxitin disc placed on Muller-Hinton agar. The zones of inhibition were measured and interpreted according to the Clinical and Laboratory Standards Institute. Cefoxitin is considered more accurate than oxacillin (CLSI 2018). *S. aureus*, which showed a zone of inhibition of ≤ 10 mm with oxacillin (10µg) and zone of inhibition ≤ 21mm with cefoxitin (30µg) is considered as MRSA on MHA after overnight incubation at 35 ℃, were considered as MRSA [9].

Result

Among the 35 samples subjected to laboratory investigation, all samples were found to be heavily contaminated by microbial flora. Discrete colonies were isolated and identified as gram-positive bacteria through gram staining. Among the total of 86 gram-positive isolates,

27 (31.4%) were found to be gram-positive rods and 59 (68.60%) were gram-positive cocci as shown in figure 1.

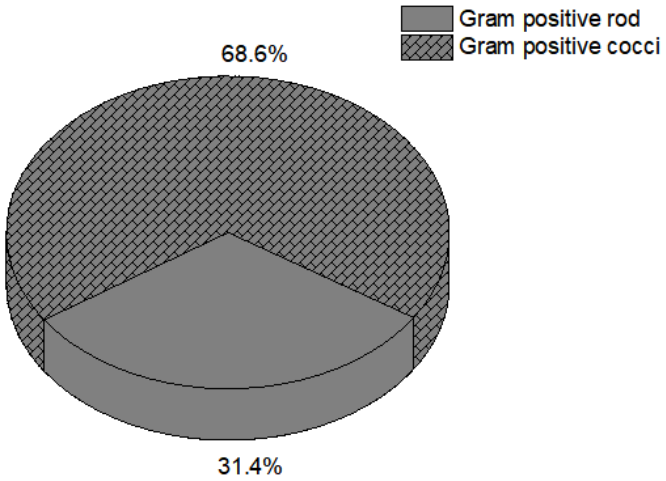

Figure 1: Percentage of Gram-positive rod and Gram-positive cocci bacteria among the total gram-positive isolates

Distribution of different organisms among gram-positive isolates

Out of the total 86 gram-positive isolates, 21 were *Staphylococcus aureus*, 19 were coagulase-negative staphylococcus (CONS), 14 were Diptheroids, 13 were *Bacillus* spp, 9 were *micrococcus*, 4 were *Streptococcus pneumoniae*, 4 were *Viridans streptococcus* and 2 were *Streptococcus pyogenes.*

Table 1: Distribution of different organisms from isolated gram positive colonies.

S.N	Organisms	% (n= No. of isolates)
1	*Staphylococcus aureus*	24.42 (n= 21)
2	Coagulase Negative Staphylococcus (CONS)	22.09 (n= 19)
3	Diptheroids	16.30 (n= 14)
4	*Bacillus* spp	15.11 (n= 13)
5	*Micrococci*	10.46 (n= 9)
6	*Streptococcus pneumoniae*	4.65 (n= 4)
7	*Viridans streptococcus*	4.65 (n= 4)
8	*Streptococcus pyogenes*	2.32 (n= 2)
	Total	**86 (100%)**

Prevalence of Gram positive bacteria on different denominations from various sources

A total of 15 (17.45%) isolates were obtained from Rs 5 currency notes followed by 14 (16.28%) in Rs 10 bills, 13 (15.12%) in Rs 50 bills, 12 (16.28%) in Rs 20 bills, 12 (16.28%) in Rs 100 bills, 12 (16.28%) in Rs 500 bills and least 8 (9.30%) in Rs 1000 bills. Denomination of Rs 5 showed the highest gram-positive load while Rs 1000 showed the least.

Similarly, the highest numbers of gram-positive isolates were found in currency notes collected from the butcher shop and grocery shop while the least from the hospital pharmacy. Among the total number of gram-positive isolates, 19 (22.09%) were isolated from the butcher shop, 19 (22.09%) from the grocery shop, 18 (20.93%) from bus conductor, 16 (18.61%) from food and vegetable shop and 14 (16.28%) from hospital pharmacy respectively.

Table 2: Distribution pattern of gram-positive bacteria isolated in paper currency notes of different denominations

Name of organisms	Denomination and No of isolates						
	Rs 5	Rs 10	Rs 20	Rs 50	Rs 100	Rs 500	Rs 1000
Staphylococcus aureus	5	3	4	4	3	2	-
CONS	4	2	1	2	2	5	3
Diptheroids	2	2	3	1	3	1	2
Bacillus spp	-	2	2	3	1	3	2
Micrococci	1	3	1	2	1	-	1
Streptococcus pneumoniae	2	1	1	-	-	-	-
Viridans streptococcus	1	-	-	1	1	1	-
Streptococcus pyogenes	-	1	-	-	1	-	-
Total	**17.5 (N= 15)**	**16.3 (N=14)**	**13.9 (N=12)**	**15.1 (N=13)**	**13.9 (N=12)**	**13.9 (N=12)**	**9.3 (N=8)**

Table 3: Distribution pattern of gram positive bacteria isolated in paper currency notes collected from different sites

Name of organisms	Sites and No of isolates				
	Food and Vegetable Shop	Bus Conductor	Hospital Pharmacy	Butcher Shop	Grocery Shop
Staphylococcus aureus	3	4	4	5	5
CONS	5	3	4	3	4
Diptheroids	3	4	2	3	2
Bacillus spp	3	3	1	3	3
Micrococci	1	2	1	2	3
Streptococcus pneumoniae	-	1	1	1	1
Viridans streptococcus	1	-	-	2	1
Streptococcus pyogenes	-	1	1	-	-
Total	**16 (18.61%)**	**18 (20.93%)**	**14 (16.28%)**	**19 (22.09%)**	**19 (22.09%)**

Antibiotic susceptibility test of isolated *Staphylococcus aureus*

Antibiotic susceptibility pattern was determined using the Kirby-Bauer disc diffusion method. An Antibiogram of *S. aureus* revealed a higher degree of susceptibility towards the tested antibiotics. Interestingly, all *S. aureus* isolates were sensitive to Vancomycin (100%), Chloramphenicol (100%), and Co-trimoxazole (100%) while resistant to Penicillin-G and Ampicillin (100%). Meanwhile, 90.48% were sensitive to Erythromycin and 66.67% to Cefoxitin and Oxacillin as shown in table 4.

Table 4: Antibiotic Susceptibility Pattern of *Staphylococcus aureus*

Antibiotic used	Susceptibility pattern	
	Sensitive (%)	**Resistant (%)**
Vancomycin (VA_{30})	21 (100)	0
Erythromycin (E_{15})	19 (90.48)	2 (9.52)
Cefoxitin (CX_{30})	14 (66.67)	7 (33.33)
Penicillin-G ($P_{10\ units}$)	0 (0)	21 (100)
Ampicillin (AMP_{10})	0 (0)	21 (100)
Co-Trimoxazole (COT_{25})	21 (100)	0
Chloramphenicol (C_{30})	21 (100)	0
Oxacillin (OX_{10})	14 (66.67)	7 (33.33)

Distribution of MSSA and MRSA among isolated *S. aureus*

Out of a total of 21 *Staphylococcus aureus*, 14 (66.67%) were found to be methicillin-sensitive *Staphylococcus aureus* (MSSA) and 7 (33.33%) were methicillin-resistant *Staphylococcus aureus* (MRSA) as shown in figure 2.

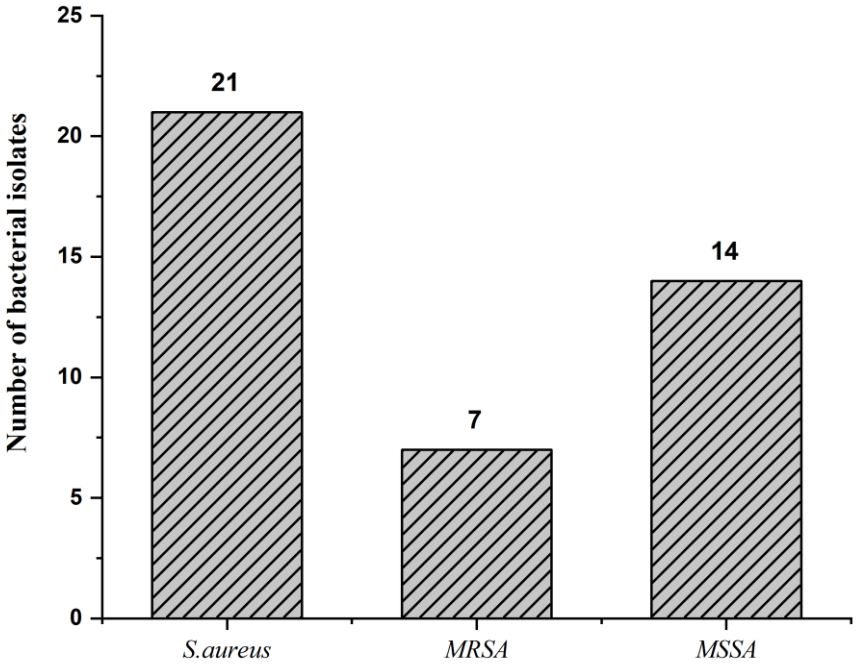

Figure 2: Distribution of MSSA and MRSA among isolated *S. aureus*.

Distribution of MRSA according to sources and denominations.

Out of 21 *S. aureus* isolates, the highest number was isolated from the butcher shop and grocery shop 5 (23.81%) followed by pharmacy and bus conductor 4 (19.1%). Meanwhile, 3 (14.29%) were isolated from food and vegetable shop. Among the 7 methicillin-resistant *S. aureus*, 2 (9.52%) isolates were found to be isolated from each food and vegetable shop, butcher shop, and grocery shop whereas MRSA was not detected from pharmacy samples as shown in table 5.

Similarly, among 7 MRSA isolates, 2 (9.25%) were detected from Rs 5, Rs 50, and Rs 100, 1 (4.76%) from Rs 10 while MRSA was not detected from Rs 20 and Rs 1000 as shown in table 6.

Table 5: Distribution of MRSA according to different sample sites.

S.N	Sampling site	*Staphylococcus aureus*	MRSA
1.	Food and Vegetable shop	3 (14.29%)	2 (9.52%)
2.	Bus Conductor	4 (19.05%)	1 (4.76%)
3.	Pharmacy	4 (19.05%)	0 (0%)
4.	Butcher shop	5 (23.81%)	2 (9.52%)

5.	Grocery Shop	5 (23.81%)	2 (9.52%)
	Total	**21 (100%)**	**7 (33.33%)**

Table 6: Distribution of MRSA according to different denominations

S.N	Denominations	*S. aureus*	MRSA
1.	Rs 5	5 (23.81%)	2 (9.25%)
2.	Rs 10	3 (14.29%)	1 (4.76%)
3.	Rs 20	4 (19.05%)	-
4.	Rs 50	4 (19.05%)	2 (9.25%)
5.	Rs 100	3 (14.29%)	2 (9.25%)
6.	Rs 500	2 (9.52%)	-
7.	Rs 1000	-	-
	Total	**21 (100%)**	**7 (33.33%)**

Discussion

In this study, often all Nepali paper currency had contaminants. This finding was similar to studies conducted in Saudi Arabia [7]. The result was evident that the paper currency notes act as a significant carrier of human pathogens and transfer harmful pathogens through them.

This study demonstrated the high level of bacterial contamination on paper notes with the higher number of gram-positive bacteria. Similar findings were also recorded on Indian rupees [8], Bangladesh Teka [10], Ghanaian [11], Nepal [12], Pakistan [13], Uganda[14], and Saudi Riyal [7]. This study demonstrates the highest number of bacterial isolates as well as *S. aureus* on samples collected from the butcher shop and Grocery shop 19 (22.09%), which is also supported by the study conducted in India [2], [15], and Bangladesh [10]. The variation in the number of positive samples from different sources may be connected with differences in the hygiene of the currency owners and sanitary conditions in the environment. The high level of bacterial contamination of currency notes could be attributed to the fact that they are being used frequently by a very high number of people [14].

The prevalence of MRSA on Butcher and vegetable shop 2 (9.52%) was consistent with the study conducted by the Khanal group [16]. Meanwhile, detection of MRSA in Rs 5, 10, and

100 but not in Rs 20, Rs 500, and Rs 1000 may be due to more circulation and frequent use for the trade of these denominations than the latter one among the people.

The findings of this research work provide an important clue of dissemination of MRSA in the environment through the circulating paper money posing a public health threat. Therefore, further study should be done to validate the results of the present study and need to take action to reduce the spread of pathogenic microflora through paper currency.

Conclusion

The presence of a high load of microbial contamination and Methicillin Resistance *Staphylococcus aureus* in paper currency suggests that banknotes play an important role in the causation and spreading of potentially harmful diseases. This also clearly indicates that pathogenic microorganisms are circulating among people through paper currency in day-to-day life. This is an important finding as the spread of pathogenic bacteria could be minimized through possible preventive measures such as maintaining good hygienic behavior among money handlers.

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