Research Article

Prevalence and factors associated with human colonisation and vancomycin minimum inhibitory concentration of *Staphylococcus aureus* in Colombo, Sri Lanka.

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Abstract

Introduction: *Staphylococcus aureus* is known to cause a wide variety of diseases of varying severity in humans. The objectives of this study were to determine the prevalence of *S. aureus* and methicillin resistant *S. aureus* (MRSA) colonisation, assess factors associated with MRSA colonisation, identify antibiotic susceptibility patterns of MRSA isolates, determine distribution of vancomycin minimum inhibitory concentrations (MIC) of *S. aureus* and factors associated with high vancomycin MIC values (≥2 µg/ml).

Methods: A descriptive cross sectional study was conducted among 341 participants aged 18 to 84 years selected randomly from the Colombo district from 11th December 2018 to 10th April 2019. Samples were collected from the nose, axilla, groin of participants and identification and sensitivity testing of *S. aureus* were performed based on standard operation procedures given in the Laboratory manual in microbiology published by the Sri Lanka College of Microbiologists (2011).

Results: Prevalence of *S. aureus* and MRSA colonisation in the sample population was 34.6% and 13.78% respectively. All MRSA isolates were susceptible to linezolid. MRSA susceptibility to gentamicin (86%), co-trimoxazole (88 %), and chloramphenicol (80%) was high. Erythromycin had the lowest susceptibility (20%). Inducible clindamycin resistance was demonstrated in 31% of isolates. Meat consumption and consumption of antibiotics within the previous six months showed a significant association with MRSA colonisation. Vancomycin MIC of *S. aureus* ranged from ≤ 0.5 to 2 µg/ml.

Conclusion: As prevalence of MRSA colonisation is 13.78%, more attention should be paid when managing patients with suspected *S. aureus* infections presenting from the community. Further research on associated factors and environmental triggers are warranted with a larger sample size and different study design to formulate national regulations and guidelines.

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Introduction

Staphylococcus aureus is responsible for a wide spectrum of clinical conditions ranging from simple skin and soft tissue infections such as folliculitis to more severe life threatening conditions such as pneumonia, osteomyelitis, endocarditis and sepsis.[1] It is estimated that 20-30% of the healthy population are long-term carriers of *S. aureus*.[2] Nasal carriage of *S. aureus* has been associated with the development of infection.[3] The common sites of methicillin resistant *S. aureus* (MRSA) colonisation in the human body are the anterior nares, throat, perianal region, groin and axillae.[4]

Anti-microbial resistance (AMR) is a global health issue and currently Sri Lanka faces a challenge in combating AMR as a major health problem.[5] Empirical antimicrobial therapy of patients presenting from the community with severe infections would be aided by knowing the prevalence of MRSA and its antibiotic sensitivity pattern in the general population. There is growing evidence that the presence of antimicrobial compounds, antimicrobial resistant genes, other selection pressures (e.g. metals and biocides) and bacteria in the natural environment contribute to the increase in AMR. Limited data on factors contributing to AMR limits further action towards it in Sri Lanka.[6]

Vancomycin is the mainstay of treatment for MRSA infections as well as a recognised treatment option for methicillin sensitive *S. aureus* (MSSA) infections in patients allergic to β-lactam antibiotics. Development of reduced susceptibility of vancomycin can cause treatment failure in patients.

There is an increase in the proportion of MRSA isolates with high vancomycin MIC within the susceptible range, a phenomenon known as vancomycin 'MIC creep'.[7] This may contribute to treatment failure when patients are treated with the usual drug dose. Widespread use of vancomycin to treat infections caused by Gram positive cocci including MRSA has led to the development of vancomycin resistance.[8]

The current study was performed to determine prevalence, factors associated with colonisation, distribution of vancomycin MIC of *S. aureus* and other possible factors associated with high vancomycin MIC values (≥2 µg/ml) in a sample population in the Colombo district.

Method

This was a community based descriptive cross-sectional study. The sample for the study was chosen from the 2017 voters' name list published by the Election Commission, Sri Lanka. Adults over 18 years of age in the Colombo district were selected as study participants. Participants with known pathological conditions leading to immunosuppression and those who needed proxy consent were excluded.

The sample size and sampling technique: Calculation of the population sample size was done using the Lwanga and Lemeshow equation.[9]

$N = Z^2 P (1- P)/d^2$
P = 33% Anticipated population proportion for *S. aureus* among population living in Colombo district.[8]
Z= Confidence level 95%
d= Absolute precision required on either side of proportion
N=1.962 x 0.33x0.67 / 0.0523
N=339.7
Ultimate sample size – 340

Modified multistage sampling technique was applied to select the study participants from all 13 divisional secretariat units in the Colombo district. Five Grama Niladari divisions were randomly selected from each divisional secretariat unit (Figure 1) and the number of study participants from each unit was determined considering the total number of the population in each unit. Selection of study participants was done from the 2017 voters' list based on computer generated random numbers.

S.aureus isolates from 57 study participants were selected randomly from each divisional secretariat area for vancomycin MIC determination considering the total number of the population in each area. Data collection was done from 11th December 2018 to 10th April 2019.

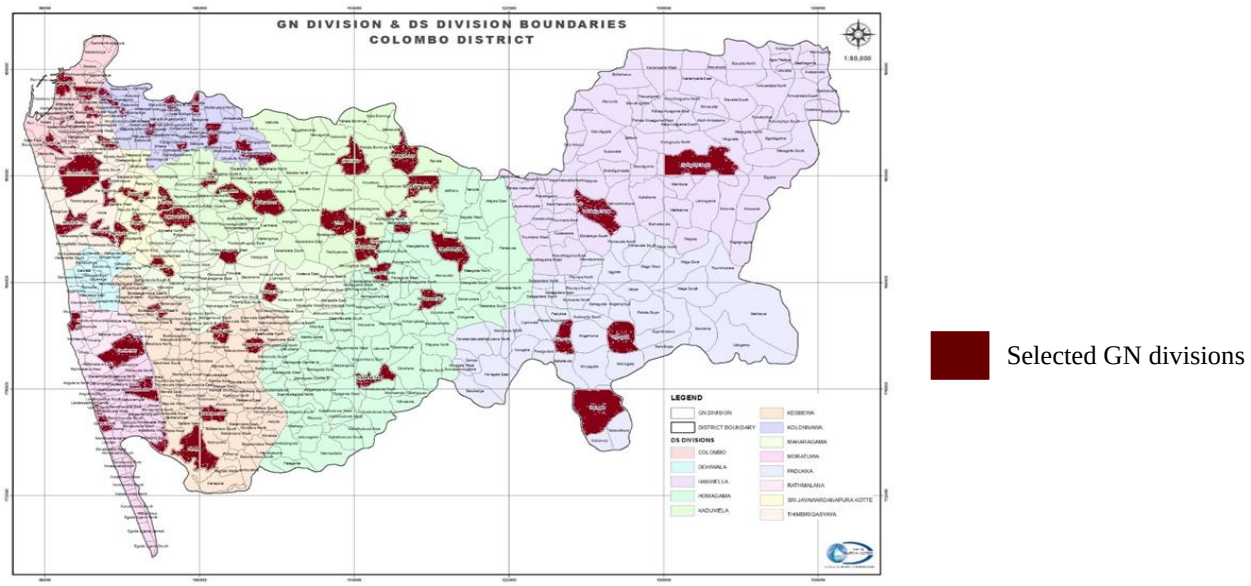

Figure 1: Map of randomly selected Grama Niladari divisions

For detection of *S. aureus*, three samples were collected from the anterior nares, axillae and groin of selected subjects using commercially prepared cotton wool swabs after obtaining informed written consent. Specimens were transported to the microbiology laboratory within two hours of collection. Specimens were processed according to the standard operating procedures in the

Laboratory manual in Microbiology (Sri Lanka College of Microbiologists, 2011).[10] The samples were incubated at 35 ^{o}C in 7% sodium chloride nutrient broth and after overnight incubation subcultured onto mannitol salt agar and re-incubated at 35-37 ^{o}C overnight. Colony morphology, Gram stain, catalase test, slide/tube coagulase and DNase tests were performed for identification of *S. aureus*. Resistance to cefoxitin (30µg) was used to detect MRSA. Antibiotic susceptibility testing of MRSA isolates was done using the disk diffusion method on Mueller Hinton Agar. Vancomycin MIC was determined using the Vitek 2 (bioMerieux) automated antibiotic sensitivity testing system with the Vitek 2 AST-P628 panel. The Clinical Laboratory Standard Institute (CLSI) M–100 - 28th edition guidelines were used to interpret antimicrobial susceptibility. Data analysis was facilitated by SPSS version 25.0.

Results

The number of study participants included for analysis was 341 with a total response rate of 92% of whom 51% were males

Table 1: Distribution of age of the participants

Age category	N	%
< 25 Years	81	23.75
26-35 Years	67	19.6
36-45 Years	54	15.8
46-55 Years	43	12.7
56-65 Years	53	15.5
66-75 Years	35	10.3
>76 Years	8	2.3
Total	**341**	**100.0**

Table 2: Occupational distribution of study participants

Occupational Category	N	%
Unemployed + students	95	27.8
Labourers	79	23.1
Non-executives	83	24.3
Executives	24	7.1
Professionals	9	2.6
Retired	51	14.9
Total	**341**	**100.0**

Table 3: Distribution of *Staphylococcus aureus* colonisation among study participants

***S. aureus* Type**	**Number**	**%**	**95%CI**
S. aureus	119	34.9	0.30-0.40
MRSA	47	13.7	0.11-0.19
MSSA	76	22.2	0.18-0.27
MRSA & MSSA	4	1.2	0.01-0.03
No *S. aureus*	222	65.1	0.60-0.70
Total	**341**	**100.0**	

Table 4: Association of gender and age categories with colonisation of MRSA

Exposure	**MRSA colonisation**		**OR**	**95%CI**
	Yes	No		
Age				
>60Years	7	57	0.728	0.31-1.70
< 60 years	40	237		
Gender				
Male	24	150	1.02	0.54-1.85
Female	23	144		
Total	47	294		

Table 5: Factors associated with MRSA colonisation

Exposure	MRSA colonisation		OR	95%CI
	Yes	No		
Agrochemical				
Yes	6	26	1.262	0.45-3.47
No	41	269		
Meat				
Yes	41	205	2.93	1.202-7.16
No	6	88		
Fish				
Yes	44	263	2.62	0.62-11.57
No	3	32		
Prawn				
Yes	39	243	1.19	0.51-2.81
No	8	52		
Open Water				
Yes	13	81	0.93	0.46-1.89
No	34	214		
Inward Treatment				
Yes	7	39	1.17	0.49-2.82
No	40	256		
Antibiotics (past 6 months)				
Yes	20	73	2.24	1.18-4.23
No	27	221		
Household treated in hospital				
Yes	8	49	0.91	0.38-2.13
No	39	246		
Household HCW				
Yes	4	23	0.82	0.83-2.86
No	43	272		

When factors associated with positive MRSA colonisation were considered, statistically significant association was noted with consumption of meat and antibiotic use within the past six months.

Exposure to agrochemicals, consumption of fish and prawns, in-ward treatment within the past six months demonstrated a contributory association which was not statistically significant.

Use of open water sources, presence of a health care worker (HCW) at home and presence of a household member who had in-ward treatment within the past six months were not shown to be associated with MRSA colonisation.

Table 6: Distribution of antibiotic sensitivity patterns

Antibiotic	Sensitive N(%)	Intermediate N(%)	Resistant N(%)
Gentamicin	43(86)	-	7(14)
Co-trimoxazole	44(88)	4(8)	2(4)
Erythromycin	10(20)	-	40(80)
Clindamycin	24(48)	1(2)	25(50)
Tetracycline	37(74)	1(2)	12(24)
Linezolid	50(100)	-	-
Ciprofloxacin	32(64)	1(2)	17(34)
Chloramphenicol	40(80)	4(8)	6(12)

Table 7:
Distribution of vancomycin MIC

Vancomycin MIC	N	%
≤ 0.5 µg/ml	30	52.63
1 µg/ml	22	38.60
2 µg/ml	**05**	**8.77**
Total	**57**	**100.00**

Table 8: Significance of associated factors for high vancomycin MIC values (≥2 µg/ml) of *Staphylococcus aureus*

Exposure	Vancomycin MIC ≥2 µg/ml		OR	95%CI
	Yes	No		
Agrochemical				
Yes	1	5	0.426	0.039- 4.586
No	4	47		
Meat				
Yes	4	48	4.286	0.605-30.377
No	1	7		
Fish				
Yes	4	48	3.000	0.268-33.639
No	1	4		
Prawn				
Yes	4	43	1.194	0.119- 11.986
No	1	9		
Open water				
Yes	1	19	2.303	0.240-22.133
No	4	33		
In-ward Treatment				
Yes	1	9	0.837	0.083-8.401
No	4	43		
Antibiotics (past 6 months)				
Yes	2	25	1.389	0.214-9.012
No	3	27		
Household treated in hospital				
Yes	2	10	0.357	0.052-2.430
No	3	42		
Household HCW				
Yes	1	7	0.622	0.060-6.405
No	4	45		

Discussion

The 2017 Sri Lankan census showed the male: female ratio in the Colombo district as 51.6:48.4. The majority of the population (75.2%) were between the ages of 20 and 80.[12] The study sample can therefore be considered as a representative sample.

The prevalence of *S. aureus* was 34.5% (95% confidence interval as 30%-40%) and MRSA prevalence was 13.7% in the present study. In 2009, Van Belkum et al. demonstrated a prevalence of *S. aureus* as 30% within a community.[13] According to Bennet et al. (2015), this value could range from 30%-50%.[1] Abeygoonewardena et al. (2015), studied the prevalence of *S. aureus* in a selected urban community in Sri Lanka and demonstrated a prevalence of 28%.[14] This latter study was generated from a restrictively localised area and the time period of the study was not specifically mentioned. This may therefore not be a representative sample of the Colombo district. In addition, they only considered samples from nasal sites.

A systematic review of MRSA prevalence from 152 studies in the Asia-pacific region was reported by Wong et al. (2018) as ranging from 0% to 23%.[15] MRSA prevalence in India was the highest, ranging from 16-23%, while the lowest prevalence was from Vietnam (7.9%) and Taiwan (3.5%). The MRSA prevalence in the current study at 13.7% falls in the middle of this range.

MRSA prevalence in Sri Lanka has been previously reported as 5.7% (Abeygoonawardena et al. 2015)[14], and 7.4% (Corea et al. 2003).[16] These two studies sampled only nasal sites. In addition, both studies were done with study participants from restricted locations. The former study[14] was done from residents in Ratmalana and the latter from patients admitted to National Hospital of Sri Lanka.[16] The data from these two studies therefore cannot be compared with the present study which was done in a representative sample of the Colombo district.

Although there was no significant association of gender or age with MRSA colonisation in the current study, previous studies have demonstrated such associations[3,16] which need further study. The current study showed a significant risk association between meat consumption and MRSA detection (OR-2.93:95%CI = 1.202-7.16). The addition of antibiotics is common in poultry supplements. Enrofloxacin, amoxicillin and sulphadiazine are the most frequently used antibiotics in animal husbandry in Sri Lanka.[18] Several studies have discussed the association between AMR and fish consumption, exposure to river, sea and swimming pool waters and pig farming with the development of drug resistance in *S. aureus*.[19,20] Further studies would be useful in determining whether these factors contribute to MRSA colonisation in humans.

Although hospitalisation within the previous six months and the presence of a healthcare worker as a household member have been associated with MRSA colonisation,[17,21] the current study could not demonstrate a statistically significant association with these factors.

From the 57 isolates evaluated for vancomycin MIC, only five isolates had vancomycin MIC of ≥2 µg/ml. Increase in mortality, prolonged hospital stays, and treatment failure have been associated with high vancomycin MICs[2,25] although the actual MICs considered as high varied from >1µg/ml to 2µg/ml. As we were unable to obtain MIC values between 1-2 µg/ml by the Vitek 2 system, we defined the high MIC value as ≥ 2 µg/ml in our study.

In our study, we could not demonstrate statistical significance between high vancomycin MIC of *S. aureus* and factors such as recent hospital admission, exposure to antibiotics, open water, agrochemicals, and consumption of meat, fish and prawns and presence of a household contact with exposure to a health care environment. Some studies have detected a significant association between recent prior exposure to vancomycin and high vancomycin MIC of *S. aureus*.[24,26] In our study, from the 57 isolates evaluated for vancomycin MIC, 47.4% had exposure to antibiotics and 17.5% had in-ward treatment. However, none had vancomycin exposure during the previous six months. That may be a reason for not detecting an association between antibiotic exposure and high MIC in our study

A case-control study to determine the relationship between MRSA bacteraemia with vancomycin MIC value of 2 µg/ml in patients who undergo haemodialysis showed that a history of surgery within the past six months and ICU admission were significant risk factors in this group.[27] Several

other authors have also assessed risk factors in their studies in relation to high vancomycin MIC of *S. aureus*.[24,26] Many of those factors were related to a hospital setting and none of the authors addressed environmental exposures as in our study. It is important to evaluate environmental exposures as *S. aureus* can develop antimicrobial resistance due to these environmental triggers.[8] This needs to be further investigated in future studies with larger sample size and different study designs.

Conclusions

Prevalence of *S. aureus* colonisation among a sample population living in Colombo was 34.6% while prevalence of MRSA was 13.78 %. Meat consumption and consumption of antibiotics within the last 6 months demonstrated a significant association with MRSA colonisation.

Vancomycin MIC of *S. aureus* isolated from colonising flora of population in the sampled Grama Niladari divisions in the Colombo district ranged from ≤ 0.5 to 2 µg/ml. None of the isolates were found to be vancomycin-intermediate sensitive or vancomycin-resistant in this study. There was no significant association between environmental triggers and high vancomycin MIC values (≥ 2 µg/ml) of *S. aureus*.

Recommendations: Based on this study, as MRSA colonisation is considerable in the community, more attention should be paid when managing suspected *S. aureus* infections presenting from the community. Further research on associated factors and environmental triggers for the development of antimicrobial resistance in *S aureus* are warranted with a larger sample size and a different study design (case-control) to formulate national regulations and guidelines.

Declaration

Conflicts of Interest: There are no conflicts of interest
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Statement on author contributions: All authors have contributed.
CNW and WMSPJB:data collection, analysis and results interpretation.
CNW and WMSPJB: Manuscript writing.
SKJ: supervision of research and manuscript writing.

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