Research article

Characteristics of community acquired and hospital acquired methicillin resistant *Staphylococcus aureus* isolates in the National Hospital of Sri Lanka

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Abstract

Introduction and Objectives: Highly virulent community acquired methicillin resistant *Staphylococcus aureus* (MRSA) strains emerged recently causing infections in healthy young adults without predisposing factors. This descriptive cross-sectional study was conducted to compare socio-demography of patients and microbiology and molecular characteristics of Community acquired (CA) and Hospital acquired (HA) methicillin resistant *S. aureus* strains isolated at the National Hospital of Sri Lanka.

Methods and Results: Antimicrobial susceptibility test and Panton Valentine Leukocidine (PVL) gene detection was carried out on 100 MRSA isolates. CDC epidemiological criteria were used for differentiation of CA and HA MRSA. Of those 100 isolates, 21(21%) were CA-MRSA and 79(79%) were HA-MRSA. Patients did not show any significant difference in acquiring CA MRSA and HA MRSA in relation to their age, sex and gender except ethnicity. The majority of these isolates were from pus samples. CA-MRSA isolates were significantly more sensitive to ciprofloxacin, fusidic acid, tetracycline, cotrimoxazole, and gentamicin compared with HA-MRSA isolates ($p<0.001$). Inducible, constitutive clindamycin resistance ($p<0.001$) and multidrug resistant phenotypes were significantly higher ($p<0.001$) among patients with HA-MRSA infection. All isolates were susceptible to glycopeptides, rifampicin and linezolid. Mupirocin resistance was seen in 6% and all isolates came from patients who harboured HA-MRSA strains ($p<0.338$). The PVL gene ($P<0.001$) was present in 20 (95.2%) of CA-MRSA isolates.

Conclusion: This study highlights the importance of accurate differentiation of CA and HA MRSA using epidemiological, microbiological and molecular characteristics. Further, awareness of the existence of these types will optimise individual treatment strategies.

Key words: CA-MRSA, HA-MRSA, Antimicrobial resistance, Sri Lanka

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Introduction

Methicillin resistant *Staphylococcus aureus* (MRSA) is one of the major nosocomial pathogens in Sri Lanka that causes mild to life threatening infections. Prevalence of MRSA in Sri Lanka varies among the hospital settings from 47% to 62% while most are resistant to many antimicrobials tested.[1,2,3] In the late 1990s, a phenotypically and genotypically distinct highly virulent MRSA clone emerged as community-acquired/associated MRSA (CA-MRSA) causing skin and soft tissue infection, and severe haemorrhagic pneumonia in children and young adults without any predisposing conditions.[4] It usually carries smaller staphylococcal cassette chromosome mec (SCCmec) elements e.g. IV, V that do not contain other resistance genes and many clones spread independently worldwide. They produce Panton-Valentine Leukocidin toxin (PVL) which is responsible for both skin infection and severe haemorrhagic necrotizing pneumonia through tissue necrosis and abscess formation.[5] Many studies have shown significant association of PVL gene with CA-MRSA isolates compared with HA-MRSA isolates despite its controversial significance.[5,6] However, the prevalence of CA-MRSA varies markedly worldwide. Song *et al*. showed 25.5% prevalence of CA-MRSA in Asian countries with Sri Lanka demonstrating a higher prevalence of 38.8% in a multicentre surveillance study.[7] Local studies to assess the burden and characteristics of CA-MRSA and HA-MRSA infections in the country are lacking.

The purpose of this study was to compare the presence of PVL gene and antimicrobial susceptibility of CA and HA MRSA strains and to compare the socio-demographic features in patients with CA-MRSA and HA-MRSA infections in the National Hospital of Sri Lanka (NHSL).

Method

A descriptive cross-sectional study was conducted from November 2013 to March 2014 for statistically calculated 100 consecutive, non-repetitive MRSA isolates collected from the microbiology laboratory, National Hospital of Sri Lanka (NHSL).

HA-MRSA infection was defined as isolation of MRSA in a patient 48 hours after admission, with a history of hospitalization, surgery, dialysis, or residence in a long-term health care facility within the last one year prior to the culture date or who had an indwelling intravenous line, catheter or any other percutaneous medical device present at the time of isolation. Isolates with none of the above were classified as CA-MRSA.[8] An interviewer administered pre-piloted questionnaire was filled after written informed consent. Bed head tickets, clinic records and the guardian's histories were used in addition.

MRSA isolates were identified using standard protocols[9,10] in the microbiology laboratory, Medical Research Institute (MRI). Methicillin resistance was screened with cefoxitin 30µg disk and confirmed by PBP2a latex agglutination test (OXOID; DR0900) according to the manufacturer's protocol.[11] Antibiotic susceptibility was determined according to Clinical Laboratory Standard Institute (CLSI-guidelines 2013), for penicillin, rifampicin, cotrimoxazole, ciprofloxacin, gentamicin, tetracycline, linezolid and fusidic acid.[4] Inducible clindamycin resistance was identified by 'D-zone' tests with erythromycin (15µg) and clindamycin (2µg) disks.

Inducible and constitutive Macrolide-lincosamide-streptogramin B (MLSB) phenotypes were assessed by CLSI guidelines 2013.[11] Double-Disk diffusion testing method as described by Swenson *et al*, 2010 was used to detect high-level and low-level susceptibility with mupirocin 200µg and 5µg disk.[12] Glycopeptide susceptibility of MRSA was tested with vancomycin and teicoplanin (EzyMIC, Himedia) strips according to manufacturer's guidelines. Interpretations of susceptibility were done using CLSI 2013 guidelines.[11] A multi-drug resistant (MDR) isolate was defined as non-susceptibility to more than 3 antimicrobial classes and a pan drug resistant (PDR) isolate was defined as non-susceptibility to all antimicrobial agents.[12]

Conventional PCR was done to detect the PVL gene as described by Lina *et al*. (2009) using lukS PV(5-ATCATTAGGTAAAATGTCTGGACATGATCC A-3) and lukFPV(5-GCATCAA STGTATTGGATAGCAAAAGC-3) as primers.[13] *S. aureus* ATCC 25923 was used as the positive control[14] while *S. aureus* ATCC 25913 was used as the negative control.[15] DNA was extracted with the Wizard® Genomic DNA Purification Kit.[16] After amplification for 30 cycles (30s denaturation at 95 °C, 60s annealing at 55 °C and one minute extension at 72 °C), the PCR products were resolved by electrophoresis through 2% agarose gel. This was followed by ethidium bromide staining and analysis to visualise bands at 433bp. PCR was optimized to identify the best annealing temperature (55 °C) and primer concentration (0.3µM). Analytical sensitivity of the procedure was done for ten-fold serial dilutions of *S. aureus* (the positive control). Lower limit of detection was $3X10^4$ CFU/mL. Sensitivity was increased up to $3X10^3$ CFU/mL by increasing the number of cycles to 40. All hundred samples were subjected to the optimised PCR procedure with positive and negative strains included in each run.

Descriptive analysis was employed in investigating the distributions of variables between the HA and CA groups using SPSS16. Categorical variables between the two groups were compared by means of the chi-square test or Fisher's exact test. Range was used to assess the statistical dispersion of the data set. Statistical significance was assumed if P value was <0.05.

Results

One hundred MRSA isolates were tested in the study. The mean and median ages of the CA-MRSA group were 45 years and 57 years and that for HA-MRSA were 46.67 years and 56years respectively. The basic demographics of MRSA positive patients are shown in Table 1.

Table 1: Socio-demography of patients with CA-MRSA and HA-MRSA infections

	CA MRSA count	HA MRSA count	Odds ratio	95% Confidence Interval		P value
				Lower	Upper	
Female	**21**	**7**	0.724	0.257	2.040	0.541
Male	**58**	**14**				
Sinhalese	**14**	**70**	.257	.082	.806	0.020
*Non-Sinhalese	**7**	**9**				
< 45years	**9**	**34**	.993	.375	2.624	0.988
>45years	**12**	**45**				

*Non-Sinhalese: Tamil, Muslim and other ethnic groups

In this study, the prevalence of HA-MRSA was 79% (70.9%- 87.1%) and CA-MRSA was 21% (12.9% - 29.1%). The majority (92) were clinical samples and 8 were screening samples. Skin and soft tissue infections were the most common infection among all subjects. Blood and respiratory specimens had only HA-MRSA infections (Table 2).

Table 2: Type of samples

	CA-MRSA		HA-MRSA	
	No	**%**	**No**	**%**
Screening	3	37.5	5	62.5
Clinical	18	19.6	74	80.4
Blood stream infection	-		5	6.8
Respiratory tract infection	-		3	4.1
Skin/soft tissue infection/pus	16	88.9	61	82.4
Sterile fluid	1	5.6	5	6.8
Urinary tract infection	1	5.6	-	

Antibiotic susceptibility pattern is shown in Table 3. Resistance rates were significantly higher for fusidic acid, cotrimoxazole, tetracycline, ciprofloxacin, gentamicin, and clindamycin among isolates of HA-MRSA which showed the MDR phenotype.

All isolates were susceptible to rifampicin and linezolid but resistant to penicillin. The distribution of glycopeptide MIC values among the two groups differed. There were no glycopeptide intermediate or resistant *S. aureus* isolates. Prevalence of mupirocin resistance was 6%. All resistant isolates were in the HA-MRSA group. Four isolates showed high-level resistance and two isolates showed low-level resistance.

The proportion of PVL gene among HA-MRSA isolates was 3.8% whereas proportion of PVL among CA-MRSA isolates was 95.2 % ($p< 0.001$).

Table 3: Interpretation of antimicrobial susceptibility testing of the isolates

Antibiotic	Susceptibility result	HA MRSA *n*	CA MRSA *n*	P value
Fusidic acid 30µg	Resistant	**35**	**0**	<0.001
Cotrimoxazole	Resistant	**34**	**1**	<0.001
Tetracycline 30µg	Resistant	**56**	**1**	<0.001
Ciprofloxacin 5µg	Resistant	**61**	**1**	<0.001
Gentamicin 10µg	Resistant	46	3	<0.001
Inducible and constitutive clindamycin resistance (Erythromycin15µg+Clindamycin 2 µg)	Resistant	62	9	<0.001
Rifampicin 5µg	Resistant	**0**	**0**	NA
Linezolid 30µg	Resistant	**0**	**0**	NA
MDR (Multi drug resistance)	MDR	**57**	**0**	<0.001
XDR / PDR	XDR/PDR	**0**	**0**	NA
Vancomycin MIC	Range	0.5-2µg/ml	0.5-2µg/ml	NA
	MIC 50	1 µg/ml	1 µg/ml	
	MIC90	1.5 µg/ml	1.5 µg/ml	
Teicoplanin MIC	Range	0.25-3 µg/ml	0.25-1µg/ml	NA
	MIC 50	0.5 µg/ml	0.5 µg/ml	NA
	MIC90	1 µg/ml	0.5 µg/ml	

MDR -Multi drug resistant phenotype, PDR -Pan-drug resistance, XDR - Extreme drug resistance, MIC-Minimum inhibitory concentration, NA-Not applicable.

Discussion

The prevalence of CA-MRSA varies markedly worldwide. The NHSL is the largest tertiary care hospital in the country and caters to patients from all parts of the country. This study demonstrates that high proportions (21%) of isolates are CA-MRSA at NHSL. CA-MRSA categorization was done according to the patient's history which is based entirely on epidemiological information. However, the boundaries between HA-MRSA and CA-MRSA are becoming blurred due to the movements of patients and infections between hospitals and the community.[1]

The majority of the study population had skin and soft tissue infections. HA-MRSA was common in invasive samples such as blood and lower respiratory samples showing that HA-MRSA is prone to cause more invasive disease. None of the demographic factors such as age and gender (except ethnicity) were significant associates among the two groups which may be due to the small sample size. However, evidence suggests that CA-MRSA causes infection in healthy, predominantly young hosts who have no predisposing co-morbidities and in certain groups (ethnic groups, MSM, sport teams).[17,18,19] Clindamycin is used to treat serious infections caused by MRSA strains to suppress toxin production.[4] Constitutive and inducible clindamycin resistance was significant among HA-MRSA isolates in our study confirming global evidence.[2,3,15,17] CA-MRSA isolates in NHSL were significantly more susceptible to other antibiotics such as fusidic acid, cotrimoxazole, tetracycline, ciprofloxacin and gentamicin. The MDR phenotype was significantly higher in the HA-MRSA isolates than the CA-MRSA isolates. This highlights the importance of enforcing rational use of antimicrobials in the hospital setting.

The dissemination of MRSA has led to a tremendous increase in the use of glycopeptides worldwide. All our isolates were within the susceptible range of glycopeptide MIC values, similar to other local studies.[2] MIC50 shows how good an antimicrobial works intrinsically against a species while MIC90 reflects different resistance mechanisms. The vancomycin MIC's of the isolates suggest a drift towards antibiotic resistance. Teicoplanin MIC50, MIC90 and the range among CA-MRSA were less than that of HA-MRSA, reflecting the infrequent use of teicoplanin in the community setting. However, these data should be confirmed by large inter centre studies. There were five isolates which had vancomycin MIC of 2 μg/ml and seven isolates had teicoplanin MIC value ≥ 1.5μg/ml. We did not assess the clinical outcomes, hVISA strains (MIC 0.5–2 μg/ml) and vancomycin creep in our study. There are reports of poor clinical outcome and increased mortality in *S. aureus* infection with MIC's at the upper end of the susceptible range.[20, 21]

All isolates in our study showed susceptibility to linezolid and rifampicin, similar to other local studies.[2,3] Mupirocin is a topical agent which is used to treat skin infections and to eliminate nasal carriage of *S. aureus*. Usually high-level resistance is identified as an 'independent predictor' of decolonization failure while low-level resistant strains can recolonize very commonly.[22] In our study, prevalence of mupirocin-resistance was 6% which highlights the importance of ensuring restrictive use of mupirocin to prevent widespread resistance. Although double disk diffusion method has good sensitivity and specificity compared to broth dilution MIC, false negatives may occur rarely when there is a frame shift mutation in the mupA gene or silent mupA gene on the chromosome.[5]

Our study demonstrated significant presence of PVL gene among CA-MRSA ($p < 0.001$). Similar findings have been found globally.[6,17,21]

Conclusion

This study highlights the importance of collective use of clinical, microbiological and molecular tests for accurate differentiation of CA and HA infections. Antibiotic policy should be developed separately for the two groups of MRSA infections to optimise patient management.

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Informed written consent was taken from the patients before taking data for the questionnaire.

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