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Observations on community associated methicillin resistant *Staphylococcus aureus* carriage

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ABSTRACT

Background & Objectives: Community associated methicillin resistant *Staphylococcus aureus* (CA-MRSA) infections are becoming increasingly important. Present study was conducted to know CA-MRSA prevalence in apparently healthy individuals, colonization sites and antibiotic susceptibility pattern.

Methods: It was a prospective, hospital based study in which 200 healthy individuals (accompanying the patients attending outdoor services at a tertiary care center) with no history of recent hospitalization/surgery, and antibiotic intake were randomly enrolled as study subjects. A total of 600 samples one each from nose, throat and axilla, were collected. 100 admitted patients were enrolled as controls to look for acquisition of hospital acquired MRSA (HA-MRSA).

Results: A total of 204 *S. aureus* isolates were recovered from 116 subjects in the study group; maximum yield was from throat, followed by anterior nares. Of these, 41.2% (84/204) were MRSA as detected by oxacillin MIC by agar dilution method. Over all CA-MRSA colonization at one or more body sites was found in 23.5% (47/200) of study subjects. The antibiotic susceptibility testing showed 25% of CAMRSA to be resistant to clindamycin.

Interpretation and conclusions: A considerably high proportion of the population (23.5%) in study group was colonized with CA-MRSA and throat was the commonest site for both *S. aureus* and MRSA carriage.

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1. Introduction

Staphylococcus aureus (SA) is one of the most common bacterial infections encountered in day-to-day practice by clinicians. In spite of best of the efforts to treat and control it, newer drug resistant SA variants are posing a challenge. Over the past 50 years, Methicillin Resistant *S. aureus* (MRSA) has been a major threat to human health either as hospital acquired (HA-MRSA) or recently as community associated (CA-MRSA) pathogen,^{1,2} owing to its ability to colonize at various body sites and being multi-drug resistant as well. CA-MRSA refers to MRSA infection with onset in the community in an individual lacking established MRSA risk factors, such as recent hospitalization, surgery, residence in a long-term care facility, receipt of dialysis or presence of invasive medical devices.³ CA-MRSA has emerged very rapidly since it first appeared in late 1990s, affecting virtually every geographical region, be it rural or urban, causing not only relatively minor skin and soft tissue infection but also quite severe pneumonitis, necrotizing fasciitis and osteomyelitis.⁴ Clinical, epidemiological, molecular and antibiotic resistance profiles show that CA-MRSA strains are different from those of hospital acquired MRSA (HA-MRSA).⁵ CA-MRSA remains susceptible to several non-beta lactam groups of antimicrobial agents unlike HA-MRSA.⁶ Moreover epidemiologically, CA-MRSA resembles more with methicillin sensitive SA (MSSA) rather than HA-MRSA, with crowding, repeated physical contact, poor hygiene and skin trauma being the main conditions leading to colonization and infection.⁴ Relatively little is known regarding colonization pattern of CA-MRSA while nasal carriage of HA-MRSA is strongly related to its infection.⁷ Worldwide studies are going on to reveal colonization sites and their association with infection. Literature search did not give much data on colonization pattern of CA-MRSA and antibiotics susceptibility pattern from North India too. Therefore this study was planned with the aim of observing the prevalence of colonization by CA-MRSA at various body sites and their antimicrobial susceptibility pattern in this part of the world. Locally generated data can be used for policy decision regarding CA-MRSA screening and guidelines for empirical treatment and decolonization.

2. Methods

It was a prospective study, carried out between October 2006 and November 2007 in a tertiary hospital associated with King George's Medical University, Lucknow. The Institute's Ethics Committee approved the study. A total of 200 apparently healthy individuals, accompanying the patients attending outdoors for the first time, were randomly enrolled after obtaining written informed consent. Inclusion criteria included no history of hospitalization/surgery/antibiotic intake within last one-year. Three swabs were taken from each individual – nasal, axillary and throat as per method described in standard texts. To compare this study group with hospital acquired MRSA (HA-MRSA); 100 randomly selected indoor patients from a surgical ward were enrolled as controls. One nasal swab each was collected from these patients at the time of admission to look for MRSA carriage and those found

positive were excluded from further study. From remaining patients another nasal swab was taken 72 h after admission to look for acquisition of HA-MRSA (Fig. 1).

All swabs were immediately brought to the laboratory and inoculated on mannitol salt agar. Plates were incubated at 37 °C for overnight in ambient air incubator. Colonies were identified by colony morphology, Gram staining and biochemicals if needed. Staphylococci thus isolated were subjected to slide and tube coagulase tests to pick out *S. aureus*.⁸ Methicillin resistance was determined by oxacillin MIC using agar dilution method. Reference grade oxacillin powder was obtained from Hi Media Laboratories, India. Mueller Hinton agar (Microexpress, Tulip) with 2% NaCl was used; plates were prepared containing doubling dilutions of oxacillin ranging from 0.125 mg/l to 64 mg/l. The final inoculum used was 10⁴–10⁵ cfu/spot. They were incubated at 30 °C for full 24 h. *S. aureus* strain ATCC 29213 was used as control with each batch of tests. MIC for control strain was within one, two-fold dilution step of 0.25 mg/l of oxacillin. MIC was read as the point of complete inhibition of growth. Trailing end points or reduced numbers of colonies other than a single colony in the spot were not taken as complete inhibition. Oxacillin MIC of ≤2 mg/l was taken as susceptible, that of ≥4 mg/l as resistant.⁹ *S. aureus* showing oxacillin resistance were subjected to *mecA* gene detection by conventional PCR method. DNA preparation was done from a single colony of oxacillin resistant strain of *S. aureus* after overnight growth at 37 °C in Brain Heart Infusion Broth.¹⁰ PCR reaction was set to detect *mecA* gene from the prepared DNA using Forward primer 5'-AAAATCGATGGTAAAGGTTGGC-3' and Reverse primer 5'-AGTTCTGCAGTACCGGATTTTGC-3'.¹¹ Reaction conditions and master mix was as per Siripornmongkolchai T et al¹⁰

Antibiotic susceptibility pattern of MRSA strains thus obtained was checked using Kirby Bauer method; antibiotic disks used were vancomycin 5 µg (Hi Media Laboratories, Mumbai, India); ciprofloxacin 5 µg; clindamycin 2 µg; erythromycin 15 µg and gentamicin 10 µg (Span Diagnostics Ltd., Surat, India). Before use each batch of disks was tested with standard strains of *S. aureus* ATCC 29213. Zone diameters were interpreted as per CLSI recommendations.⁹

2.1. Statistics

Stata 9.2 statistical software was used for calculations. The proportions are expressed in terms of % and 95% CI of the proportions are provided. Chi square statistics were used to compare the significance between the proportions and Fisher's exact *p* values are reported.

3. Results

Amongst cases, 58.0% (116/200) of individuals were colonized by *S. aureus*; of which 57% (67/116) were colonized at more than one body site; colonization at one or more body sites by CA-MRSA was 23.5% (47/200). Throat was the single commonest site for *S. aureus* (107/200; 53.5%) as well as MRSA carriage (44/200; 22%) followed by anterior nares (71/200; 35.5% *S. aureus* and 27/200; 13.5% MRSA) (Fig. 1). Of 116 subjects colonized with *S. aureus*, throat swab picked up 107 (proportion 92.2%, 95%CI 86–96) as

Fig. 1 – Study design and final outcome. SA = *Staphylococcus aureus*, CA-MRSA = Community associated methicillin resistant *Staphylococcus aureus*, HA-MRSA = Hospital acquired methicillin resistant *Staphylococcus aureus*.

against 71 and 26 from anterior nares and axilla (proportion 61.2%, 95%CI 52–70; 22.4%, 95%CI 15–31). None of the individuals were carrying *S. aureus* in axilla alone (Table 1). Axilla had maximum percentage (13/26, 50%) of CA-MRSA among SA isolates and Nose harbored the least percentage (27/71, 38.02%) (Fig. 1). Amongst control group, 26 of the 100 patients enrolled carried MRSA in their nose at the time of admission hence they were excluded from the study. Of remaining, 37.8% (28/74) patients yielded MRSA when swabbed 72 h after admission. All of the *S. aureus* strains showing oxacillin resistance by phenotypic method were also having *mecA* gene detected by PCR. The antibiotic sensitivity profile of these HA-MRSA revealed 100%

resistance to ciprofloxacin and 85.6% resistant to clindamycin as compared to 22.6% and 25% resistant to ciprofloxacin and clindamycin in CA-MRSA isolates respectively, this difference was statistically significant at $p < 0.0001$ for ciprofloxacin, clindamycin and gentamicin and at $p = 0.0007$ for erythromycin (Fig. 2).

4. Discussion

Prevention of CA-MRSA infections and controlling their spread in community and spread to hospital is a critical

Table 1 – Colonization by *S. aureus* & CA-MRSA at various body sites amongst cases (n = 116).

	<i>S. aureus</i>	CA-MRSA
Single site colonization only		
Throat only	41	19
Nose only	8	3
Axilla only	0	0
Colonization at multiple body sites		
Throat & Nose	41	12
Throat & Axilla	4	1
Nose & Axilla	1	0
Throat, Nose & Axilla	21	12
Total	116	47

Fig. 2 – Resistance to antibiotics CA-MRSA vs. HA-MRSA. All values are in %; Cipro = Ciprofloxacin; Clinda = Clindamycin; Erythr = Erythromycin; Genta = Gentamicin; Vanco = Vancomycin.

issue. Significant observation in this study was the large number (116/200, 58%) of apparently healthy individuals from community harboring *S. aureus* strains while 47/200 i.e. 23.5% were colonized with MRSA, presumably CA-MRSA as per the study inclusion criteria. A previous study estimates prevalence of CA-MRSA to be around 18.1% of total SA positives in east Delhi area¹² and 3.89% in children of 5–15 years age group in a study from Chandigarh.¹³ We are reporting higher rate of CA-MRSA carriage in comparison to other studies from India, the reasons of which could be that the population we chose to screen was an adult population attending the hospital. Besides, previous studies have not taken a throat swab and hence some of the *S. aureus* carriage may have been missed by them. However genotypic study to confirm CAMRSA status is required and this is a limitation of our study. Sites of colonization are important as a potential source of subsequent infection to the host and as a reservoir for organism. Previous studies have shown anterior nares to be the most consistent area from where *S. aureus* can be isolated.¹⁴ In present study however *S. aureus* was isolated most frequently from throat. Similar findings have been reported in a study carried out in Sweden where they found 40% of the patients and 54% of the staff positive for *S. aureus* in the throat, compared to 31% and 36%, respectively, in the anterior nares.¹⁵ Our results demonstrate that *S. aureus* was infrequently carried in anterior nares alone; if anterior nares were colonized, with few exceptions, it was present in throat also (Table 1). Throat seems to be a better choice if only one site is to be sampled. This could have important prophylactic implications since decolonization of only nasal carriers has been tried with different patients groups to limit MRSA spread. The source of bacteria in many of these cases could be other carriage sites on the patients.

In the control group acquisition of HA-MRSA was 37.8%; some isolates may have been missed as only nasal swab was taken for this group. On comparing the sensitivity pattern we found HA-MRSA to be significantly more resistant to all groups of antibiotics (Fig. 2). Earlier, sensitivity to clindamycin was taken as a surrogate marker of CA-MRSA detection¹⁶; in present study however 25% of CA-MRSA was found resistant to clindamycin. This trend needs to be closely followed up to determine appropriate empirical treatment policy for CA-MRSA infections.

Conflicts of interest

All authors have none to declare.

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