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Observations on community associated methicillin resistant *Staphylococcus aureus* carriage

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ARTICLE INFO

Article history:
Received 18 February 2013
Accepted 21 April 2013
Available online 30 April 2013

Keywords:
Antibiotic susceptibility
Community associated MRSA
Staphylococcal carriage sites

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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http://dx.doi.org/10.1016/j.cegh.2013.04.001

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 10^4-10^5 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 $\leq$2 mg/l was taken as susceptible, that of $\geq$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 Siripornmongcolchai T et al[10]

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