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Finalized Article

Nasal colonization of Methicillin resistant Staphylococcus aureusamong patients during hospital admission--emergence of community-associated MRSA strains.

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Abstract

Patients colonized with Methicillin resistant Staphylococcus aureus (MRSA) in hospital are considered as one of the risk factors for infection with MRSA. Worldwide spread of MRSA in both hospital setting and community poses public health threat. This study was undertaken to determine the frequency of MRSA colonization among patients at time of hospital admission. Five hundred adult patients were screened within 24 hrs of admission in different wards in Dhaka Medical College Hospital by taking nasal swabs from anterior nares and were analyzed. All isolated Staphylococcusaureus were screened to detect methicillin resistance by modified Kirby-Bauer disc diffusion method using oxacillin and cefoxitin disc and then all MRSA isolates were subjected for MIC testing against oxacillin by agar dilution method and PCR for mecAgene detection. Out of 500 patients Staph aureus nasal colonization was observed among 112 (22.4%) patients and among those 7.6% was MRSA.MRSA colonization rate was 23.29% among patients who had history of prior hospitalization and was 4.92% among community residents who had no previous hospitalization history in last 12 month. A significant number of patients (7.6%) were colonized with MRSA at the time of admission. Screening for MRSA carriers among this population is necessary for hospital acquired infection control.

Key words: MRSA, Staphylococcus aureus, PCR

Introduction:

Colonization with *Staphylococcus aureus has* been identified as an important risk factor for the development of Staph *aureus* infections in both community and hospitalsettings[1-3]. Anterior nares are the most consistent site of Staph aureus colonization[4]. *Staph aureus* first developed resistance to penicillin in the 1940s and then to methicillin in early 1960s. Methicillin resistant *Staph aureus* (MRSA) is resistant to methicillin and other β-lactamase-resistant penicillins (oxacillin, nafcillin) and cephalosporins[5]. Number of MRSA infections has doubled in the last 10 years, and number of deaths in the United States owing to complications of this infection is higher than the number of deaths from AIDS[6]. MRSA infection is largely confined to hospitals and long term care facilities, typically linked to persons with health care associated risk factors such as hospitalization or nursing home care, chronic dialysis, antibiotic treatment, or exposure to invasive devices or procedures, called healthcare-associated MRSA (HA-MRSA) infection[7]. The frequency of community-associated MRSA (CA-MRSA) [8]is increasing. CA-MRSA is an emerging pathogen diagnosed from an outpatient or within 48hrs of hospitalization if the patient lacks healthcare-associated MRSA risk factors[7]. These infections have been associated with carriage of Staphylococcus cassette chromosome (SCC) *mec* type IV complex andgenes encoding Panton-Valentine leukocidin toxin[9,10]. Different studies[11,12] reported that PVL genes were differentially distributed among CA-MRSA strains and PVL is not only the key virulence determinant of CA-MRSA.

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Other virulence factors are associated with CA-MRSA, such as phenol-soluble modulins (PSMs) and α-hemolysin[13,14]. In Bangladesh, the rate of MRSA infection ranges from 32% to 63% in hospitals[15]. The frequency of MRSA is alarming here due to indiscriminate and incomplete uses of antibiotics[16,17]. Recognition and isolation of persons either colonized or infected with MRSA is recommended for minimizing the spread of MRSA within hospitals. In Bangladesh, there is no adequate information on MRSA nasal colonization that is the important risk factor for both HA-MRSA and CA-MRSA infection. The present study was designed to determine the MRSA nasal colonization in patients at the time of admission to the hospital and to evaluate CA-MRSA carriage.

Methods:

This cross sectional study was carried out in the Department of Microbiology in Dhaka Medical College during the period of January 2010 to December 2011.

Five hundred adult patients were screened within 24 hours of their admission to Dhaka Medical College Hospital by taking nasal swab from both anterior nares and were analyzed. Data related to age, sex, history of prior hospitalization(within past 12 month) or directly from home and their medical history, such as-diabetes mellitus, chronic obstructive pulmonary disease, cerebrovascular disease, chronic kidney disease were collected from hospital records or directly from patients using predesigned data collection form.

Case definition:

Community acquired MRSA:

MRSA strains isolated from the patients having the following criteria were considered as community acquired MRSA (CA-MRSA).

- Samples collected within 24 hours after admission to the hospital.
- No history of hospitalization in the past year (within 12 month).
- No indwelling catheters or medical devices that pass through skin into the body.

Healthcare associated MRSA:

MRSA strains isolated from the patients having history of hospitalization within last 12 months were considered as healthcare associated MRSA (HA-MRSA).

Collection of nasal swab: A single sterile cotton swab was moistened with sterile normal saline and was then inserted into each nostril and nasal septum and immediately processed for culture. Nasal swab samples were plated on blood agar media and incubated at 37^{o}c. Isolates were identified as *Staph aureus* by colony morphology, Gram staining and biochemical tests (catalase, coagulase and mannitol fermentation test)[18].

Detection of MRSA: *Staph aureus* isolates were screened for methicillin resistance by disc diffusion method using oxacillin (1µg) and cefoxitin (30µg) disc and by determination of minimum inhibitory concentration (MIC) of oxacillin by agar dilution method as per recommendation of CLSI method[19] and by detection of mec-A gene by PCR.

Polymerase chain reaction (PCR): PCR for detection of mec-A and PVLgenes was performed using specific primers. DNA was extracted from bacterial pellets by simple boiling method[20].

DNA amplification: Isolated DNA was amplified by using specific primers for *mec-A*[21,22]. The following oligonucleotide primers were used:

Primer	Oligonucleotide sequence (5'-3')	Amplicon Size
mec-A	Forward- AAAATCGATGGTAAAGGTTGGC Reverse- AGTTCTGCAGTACCGGATTTTGC	533 bp

PCR was performed in a final reaction volume of 25µl, containing 12.5 µl Master mix, 1.5µl of each primer, 2µl of extracted DNA and 6.5µl nuclease free water (Promega Corporation, USA).

Visualization and Interpretation of results: After staining with ethidium bromide (0.5µg/ml) and destaining, gel was observed under UV Transilluminator (Gel Doc, Major science, Taiwan) and DNA bands were identified according to their molecular size by comparing with 100 bp DNA ladder. Samples showing the presence of specific DNA band corresponding to 533 bp were considered positive for presence of mec-A gene.

Antimicrobial susceptibility test: All MRSA isolates were tested for susceptibility against ceftriaxone (30µg), ciprofloxacin (5µg), doxycycline (30µg), erythromycin (15µg), gentamycin (10µg), rifampicin (5?g), vancomycin (30µg), fusidic acid (10µg) and linezolid (30µg) by disc diffusion method as recommended by CLSI[19]. The discs from each batch were standardized by testing against reference stain of *Staphaureus* ATCC-25923.

RESULTS:

After screening 500 nasal swabs, 255 (51%) were culture positive for *Staphylococcus*. Out of 255 *Staphylococcus*, 112 (22.4%) were *Staph aureus* and 143 (28.6%) were coagulase negative *Staphylococcus* (TableI).

Out of 112 *Staph aureus*, 38 (33.93%) strains were detected as MRSA and 74 (66.07%) strains were detected as MSSA by different phenotypic method and by detection of *mec-A* gene by PCR (TableII).

Among 73 patients having previous history of hospitalization, 23 (31.50%) *Staph aureus* were isolated, of them 17 (23.29%) were MRSA. Of the 427 patients who had no history of previous hospitalization, 89 (20.84%) were *Staph aureus*, of them 21 (4.92%) were MRSA (Table III).

Both health-care related and community-associated-MRSA strains were resistant to anti-*staphylococcal* β-lactam antibiotics (oxacillin, cefoxitin, ceftriaxone). Both CA-MRSA and HA-MRSA strains were highly resistant to ciprofloxacin (90.47% and 94.11% respectively). HA-MRSA colonization strains showed resistance to erythromycin (88.23%), gentamycin (82.35%) and doxycycline (70.59%). Rate of resistance to both vancomycin and rifampicin were 17.65% and fusidic acid was 23.53% among health-care related MRSA isolates. Most (76.19%) of CA-MRSA strains were resistant to erythromycin. Most of CA-MRSA isolates were susceptible to rifampicin (95.24%), fusidic acid (90.48%) and doxycycline (47.62%).All CA-MRSA strains were susceptible to vancomycin and linezolid (TableIV).

Table I : Isolation rate of Staphylococcus from nasal swab sample (n=500)

Staphylococci	No. (%)
Staphylococcus aureus	112 (22.40)
Coagulase -ve Staphylococcus	143 (28.60)
Total	**255 (51.00)**

Table II :Shows isolation rateof MRSA and MSSA among Staphylococcus aureus (n=112).

Staphylococcus aureus	No. (%)
MRSA	38 (33.93)
MSSA	74 (66.07)
Total	**112 (100)**

Table III : Isolation of Staph aureus and MRSA among previously hospitalized patients and patients from community (n=500).

Study population	*Staph aureus* No. (%)	*MRSA* No. (%)
Previously hospitalized patients (n=73)	23 (31.50)	17 (23.29)
Patients from community (n=427)	89 (20.84)	21 (4.92)
Total (n= 500)	**112 (22.40)**	**38 (7.60)**

Table IV: Antimicrobial susceptibility pattern of healthcare associated-MRSA and community associated-MRSA strains.

Antimicrobial agents	HA (n=17) Resistant No. (%)	Sensitive No. (%)	CA (n=21) Resistant No. (%)	Sensitive No. (%)
Ceftriaxone	16 (94.12)	01 (5.88)	19 (90.47)	02 (9.53)
Ciprofloxacin	16 (94.12)	01 (5.88)	19 (90.47)	02 (9.53)
Erythromycin	15 (88.24)	02 (9.53)	16 (94.12)	05 (23.81)
Gentamycin	14 (82.35)	03 (17.65)	15 (88.24)	06 (28.57)
Doxycycline	12 (70.59)	05 (23.81)	11 (50.38)	10(47.62)
Vancomycin	03 (17.65)	14 (82.35)	00 (0.00)	21 (100)
Rifampicin	03 (17.65)	14 (82.35)	01 (5.88)	20 (95.24)
Fusidic acid	04 (23.53)	13 (76.47)	02 (9.53)	19 (90.47)
Linezolid	01 (5.88)	16 (94.12)	00 (0.00)	21 (100)

Discussion:

Methicillin-resistant *Staph aureus* (MRSA) is not only confined to healthcare facilities or healthcare associated, but also a significant number of persons carry this organism without having any history of hospitalization or any risk factor, called community-associated MRSA(CA-MRSA)[23]. MRSA is a serious threat to hospitalized patients globally and now represents a challenge for public health, as community-acquired infections appear to beon the increase in various regions and countries[24, 25].Nasal colonization is important risk factor for both hospital and community acquired MRSA infection[1]. It is necessary to take steps to prevent the spread of MRSA infection in hospital and community.

This study revealed that 38 (7.6%) patients were colonized with MRSA at the time of hospital admission which was similar to the study of from the USA[26] and Santos *et al (2010)*[27] from Brazil where colonization rate was 7.3% and 6.1% respectively. The prevalence of MRSA at admission was 3.4% and 1.1% in patients from the USA and from Saudi Arabia[28,29] respectively which is lower than this study. Very low prevalence (0.03%) of MRSA nasal carriage at the time of hospitalization was observed in Netherland[30]. Such lower isolation rate in the studies of different countries was probably due to the fact that in those countries MRSA control program is well established and irrational antibiotic prescribing is restricted. This higher rate of MRSA colonization in this study is probably due to lack of MRSA control program, poor knowledge about personal hygiene among general population and overcrowding environment.

This study also evaluated that MRSA colonization rate was 23.29% among patients who had previous history of hospitalization in contrast to patients who had no previous hospitalization history (4.92%). Chatterjee *et al (2009)*[31] from India, Santos *et a (2010)*l[27] from Brazil reported similar

results who also found significant relationship between MRSA colonization and hospitalization. The reason of higher rate of MRSA colonization in patients with history of previous hospitalization may be explained by the fact that, health care system including hospital personnel (patients and health care workers) act as important reservoir of MRSA acquisition that may be transmitted to other patients.

In this study, 427 patients were admitted directly from community who had no history of hospitalization in last 12 months. In this community group of patients isolation rate of MRSA (CA-MRSA) was 4.92%. This result was similar to the study of Chatterjee *et al.(2009)*[31] in India where CA-MRSA carriage rate was 3.16%. A study by Hidron *et al(2005)*[26] from the USA reported that 2.2% of patients colonized with MRSA were admitted directly from community, which was lower than this study. Lower rate of MRSA colonization in the community people may be explained by the fact that they are less exposed to the source of MRSA as there is less chance of contact with health-care system and less exposure of antibiotics.

Out of 38 MRSA-colonization strains, 21 (55.26%) and 17 (44.74%) were community and health-care associated MRSA strains, respectively. In this study both CA-MRSA and HA-MRSA strains were resistant to anti-staphylococcal β-lactam antibiotics (oxacillin, cefoxitin, ceftriaxone). Both CA-MRSA and HA-MRSA strains were highly resistant to ciprofloxacin (90.47% and 94.12% respectively). CA- MRSA strains were resistant to erythromycin (76.17%) and gentamycin (76.49%) which was in agreement with study of Neelaet *a(2008)l*[32] from Malaysia and Kim *et al(2004)*[33].All CA-MRSA strains were susceptible to vancomycin and linezolid. Most of CA-MRSA isolates were susceptible to rifampicin (95.24%), fusidic acid (90.48%) and doxycycline (47.62%). On the other hand, health-care related MRSA colonization strains showed resistance to erythromycin (88.24%), gentamycin (82.35%) and doxycycline (70.59%). Rate of resistance to rifampicin and fusidic acid were 17.65% and 23.53% respectively among health-care related MRSA isolates, which were higher than resistance rate of 4.76% and 9.52% among community associated MRSA isolates.

Antimicrobial susceptibility by disc diffusion method showed that 3 (17.65%) isolates were resistant to vancomycin. CA-MRSA isolates tend to become susceptible to non-β-lactam antibiotics than HA-MRSA[34]. Chura *et al(2011)*[35] reported that there was significant diversity in MRSA clones arising in the community worldwide, because geographical differences in typical antimicrobial resistance profiles. A study in Bangladesh reported that widespread and suboptimal use of antimicrobial agents was an important factor for high prevalence of resistant strains[17].

Conclusion:

This study demonstrated that a significant number of MRSA (7.6%) carrier patients are seeking admission everyday in Dhaka Medical College Hospital. MRSA colonization rate was 4.92% among community residents who had no hospitalization history. Carrier patients can transmit MRSA to other inpatients in hospital by skin-to-skin contact or by contact with contaminated items. So, early detection of MRSA carrier, contact isolation and decolonization may prevent MRSA transmission in hospital and community. MRSA colonization rate was higher among patients who had history of previous (within 12 month) hospitalization. So, maintaining clean environment of hospital and hygiene practices among hospital personnel and patient attendant during handling the patients may prevent MRSA transmission from hospital to hospital or to community.

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Conflict of interest:

We do not have any potential conflicts of interest.

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