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MRSA INFECTION IN PUS AND WOUND SWAB SAMPLES IN HOSPILIZED PATIENTS IN BANGLADESH

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ABSTRA

This study was carried out to assay the methicillin resistance of *Staphylococcus aureus* isolated from pus and wound swab samples of patients of local Hospital, Dhaka, Bangladesh. In this study, a total of 211 *S. aureus* isolates were isolated from 1,063 specimens of pus and wound swab samples. Methicillin resistant *S. aureus* (MRSA), were screened by testing resistance to oxacillin in Muller Hinton agar plate following the standard bacteriological technique. The overall prevalence of MRSA was 29.38% where MRSA infection was significantly higher among males (53.22%) than females (46.77%) and the isolation rate was higher in wound (7.11%) than pus sample (5.4%). All clinical isolates of this study have shown 100% *S. aureus* susceptible to vancomycin and 92.30% were susceptible to fusidic acid. The present study indicates that vancomycin and fusidic acid resistance is also getting spread day by day, both in community as well as hospital settings.

KEYWORDS: *S. aureus,* methicillin, resistance.

INTRODUCTION

MRSA stands for methicillin-resistant *Staphylococcus aureus* (*S. aureus*) bacteria. This organism is known for causing skin infections in addition to many other types of infections. There are other designations in the scientific literature for these bacteria according to where the bacteria are acquired by patients, such as community-acquired MRSA (also termed CA-MRSA or CMRSA), hospital-acquired or health-care-acquired MRSA (also termed HA-MRSA or HMRSA), or epidemic MRSA (EMRSA). Statistical data suggest that as many as 19,000 people per year have died from MRSA in the U.S.; data supplied by the CDC in 2010 suggest this number has declined by about 28% from 2005 to 2008, in part, because of prevention practices at hospitals and home care. Although *S. aureus* has been causing infections (Staph infections) probably as long as the human race has existed, MRSA was first noted in 1961, about two years after the antibiotic methicillin was initially used to treat *S. aureus* and other infectious bacteria. The resistance to methicillin was due to a penicillin-binding protein coded for by a mobile genetic element termed the methicillin-resistant gene (mecA). In recent years, the gene has continued to evolve so that many MRSA strains are currently resistant to several different antibiotics such as penicillin, oxacillin, and amoxicillin (Amoxil, Dispermox, Trimox). HA-MRSA is often also resistant to tetracycline (Sumycin), erythromycin (E-Mycin, Eryc, Ery-Tab, PCE, Pediazole, Ilosone) and clindamycin (Cleocin). *S. aureus* is sometimes termed a "superbug" because of its ability to be resistant to several antibiotics.[1-3] In addition, these organisms have been termed "flesh-eating bacteria" because of their occasional rapid spread and destruction of human skin. The present study was undertaken in order to isolate, identify and prevalence of MRSA in pus and wound swab samples and also analyze drug sensitivity and resistance pattern of MRSA. Attempt was also taken to compare oxacillin and cefoxitin disc for identification of MRSA and MSSA.

MATERIALS AND METHODS

The study was designed as cross sectional study for the period of six months (1st March to 31st August, 2013) and pus and wound swab samples were taken from patients attending outpatient department (OPD or community source) as well as patients admitted into wards, cabins, ICU and neonatal intensive care unit (NICU) of local Hospital, Dhaka, Bangladesh. Blood Agar, Mac-conkey agar and Muller-Hinton agar media were used in present study and Hydrogen peroxide and Human plasma tests were done. Cephalexin (CL), Ripampicin (RA), Gentamicin (CN), Ciproflaxacin (CIP), Vancomicin (VAN), Cotrimoxale (SXT), Netilmicin (NET), Amoxycillin (AMC), Erythromycin (ERY) and Amikacin (AK) were taken to assay the antibiotic sensitivity pattern of the isolates.

Bacterial isolation and biochemical identification Collected samples were immediately transported to the microbiology laboratory and inoculated onto MacConkey agar and Blood agar plates (Hi-Media Laboratories, Mumbai). These plates were incubated at 37^0C for 24-48 h. Plates were observed for growth and a Gram smear was performed from different types of colonies. On blood agar with yellow colored, round and elevated colonies with β-haemolysis were seen; those were taken to be *S. aureus* (Fig 1). For pure culture samples of these Gram positive cocci, catalase and coagulase tests were also performed.[4]

Antibiotic sensitivity and detection of MRSA All the confirmed *S. aureus* strains were subsequently tested for methicillin resistance based on Kirby-Bauer disk diffusion method[5] using oxacillin discs (1µg) obtained from Hi-Media Laboratories Pvt. Ltd, Mumbai (India). The isolates were considered methicillin resistant if the zone of inhibition was 10 mm or less. *S. aureus* isolates were tested for methicillin resistance by modified Kirby-Bauer disk diffusion technique according to NCCLS.[22] Antibiotics CL, RA, CN, CIP, VAN, SXT, NET, AMC, ERY and AK were used to determine the drug resistant and sensitivity pattern of the selected MRSA. This study sampled a large number of recent *S. aureus* isolates to compare the performance of the cefoxitin disk test at the new breakpoints to that of the 1-µg oxacillin disk test (Fig 1).

RESULTS AND DISCUSSION

Comparative status of Pus and Wound samples Out of 1063 clinical samples 824 of them were pus and 239 were from wound swab (Fig 2) indicating higher percentage of MRSA in pus sample.

Prevalence of MRSA & MSSA and Rate of MRSA in Pus and Wound swab samples A total of 211 *S. aureus* were isolated, among which 29.38% were confirmed as MRSA, 70.61% as MSSA, and 63.87% of them were other organisms (Table 1). From the confirmed 29.38% MRSA result showed that most of the MRSA were 72.58% from wound swab samples and 27.41% were from pus sample (Fig 3).

MRSA according to sex and IPD-OPD The study showed that most of female patients were MRSA positive (46.77%) and the percentage of MRSA positive male patients were 53.22% (Table 2). In case of the percentage of MRSA in in-patient (IPD) and out-patient (OPD), it was found 5.18% and 6.72%, respectively (Table 3).

Sensitivity pattern of isolated MRSA All of the isolates were sensitive to vancomycin, (92.30%). The sensitivity against other antibiotics were fusidic acid (34.65%), co-trimoxazole (36%), Rifampicin (35%), Gentamicin (23%), Ciproflaxacin (11.53%), Netilmicin (9.60%), Cephalexin (11.53%) respectively (Fig 4). 16 MSSA and 34 MRSA isolates were tested against oxacillin and cefoxitin disc in order to compare the sensitivity pattern. All of the MRSA isolates were also resistant to cefoxitin whereas all of the MSSA sample were sensitive to cefoxitin disc (Table 5).

Analysis of multidrug resistance pattern of MRSA It was observed among the detected isolates, when many antibiotics (e.g 10) were used at the same time in one isolate, the resistance pattern were decreasing (Table 4).

The present study showed that MRSA is a problem as a nosocomial pathogen in our hospitals. Studies around the world have shown that the prevalence of MRSA is increasing. In the present study Out of total 1063 clinical samples 211 were *S. aureus* isolates, 62 were found to be MRSA, which is 29.38% of the total number of *S. aureus* isolates. In studies carried out in similar settings by Tiwari *et al* [6] have reported the percentage of MRSA out of the total *S. aureus* isolates to be as high as 54.9%, 29.23% 31.1%, and 69.1%, respectively. Rajaduraipandi *et al*[7] have reported that as high as 35.7% of MRSA strains were obtained from throat swabs and 33.6% of strains were obtained from pus among clinical isolates. Anupurba *et al*[8] have also reported that maximum number of MRSA were from pus and wound swab samples.

Sex wise significantly high MRSA isolation from male cases compared to that from females (53.22%) were found in this study might be due to lack of knowledge, comparatively over use of antibiotics without prescriptions and its incomplete course among males might have led to high MRSA isolation from them without prescriptions and its incomplete course among males might have led to high MRSA isolation from them. Iqbal *et al*[9] has mentioned that urine collected from the OPD of the hospital has found 40.0% MRSA isolates. Kawsar *et al* [10] has reported a three months long study has found 50 cases of *Staphylococcus* infection of which 84.0% cases are *Staph. aureus*. Interestingly out of 42 cases of *Staph. aureus* 85.7% are found as beta lactamase producers and only 4.8% cases are MRSA. In the present study showed that from the OPD of the hospital has found 6.72% MRSA isolates. It was observed in present study that all the isolates (MRSA) were uniformly susceptible to Vancomycin which is in total compliance with Vidhani *et al*[11], Tyagi *el al* [12], Rajaduraipandi *et al*[7] which have reported that all of the *S. aureus* isolates in their studies were sensitive to vancomycin. The sensitivity of MRSA to amoxiclav was found to be very low in our study–approximately 11.53% and consistent with other studies.[6 -7, 11, 13] We found a high level of resistance to ciprofloxacin, although not as high as reported by other studies 75.75%[7], 84%[13] and 90%.[14] Over the past twelve years there have been dramatic changes in the susceptibility of *S. aureus* in both hospitals and community. The older β-lactams, penicillin and ampicillin are ineffective against more than 80 % of isolated strains and resistance to many of the non-β-

lactam agents such as the tetracyclines, gentamicin, chloramphenicol, erythromycin and clindamycin has gradually increased and reached alarming levels by the 1990s in many parts of the world.[15-17] In recent years[18], usage of cefoxitin instead of oxacillin are recommended, when using the disk diffusion method to determine resistance against methicillin for *S. aureus*. Cefoxitin results are easier to interpret and are thus more sensitive for the detection of *mecA*-mediated resistance than oxacillin results .The recommended resistance and susceptibility breakpoints for the 30-µg cefoxitin disk test used to detect *mecA*-mediated resistance in *S. aureus* were changed by CLSI[18] from ≤19 mm and ≥20 mm to ≤21 mm and ≥22 mm, respectively. Several mechanisms for the methicillin resistance seen in *S. aureus* have been elucidated. The most important is the production of a unique penicillin-binding protein (PBP) that has a low affinity for β-lactam antibiotics and whose effects are determined by several structural genes (*mec, mec RI, mec I*).[19-20] Other known mechanisms of methicillin resistance are the production of the usual PBPs, but with modified affinities for the β-lactam drugs and the hyper production of penicillinase enzyme.[4,20] MRSA and MSSA strains can easily spread from infected patients to medical staffs, who often become transient carriers[21] because MRSA, are usually also resistant to other non-β-lactam antibiotics, infections with them are life-threatening in immunocompromised patients, often difficult to manage, and problematic to eradicate. MRSA have created a huge clinical burden in the hospital settings as well as in the community. Clinicians and the health care workers of this country must be aware of the wide and unique spectrum of disease caused by MRSA. Increased vigilance should be employed in the diagnosis and management of suspected and confirmed *Staphylococcal* infections. The phenomenon of CA-MRSA has swept worldwide within a couple of decades. The crux of the matter is whether this is an immutable evolutionary process following on the steps of penicillin resistance, or if it can potentially be reversed or contained. Current evidence favors the former theory, although there may be a window period for collaborative human efforts to halt the process. To draw a parallel from the development of penicillin resistance in *S. aureus*, it may take years to decades before methicillin resistance becomes as prevalent as penicillin resistance worldwide While there is a clear need locally to keep track of the CAMRSA situation and to formulate guidelines for empirical therapy and for minimizing spread of CA-MRSA should the situation worsen, it is equally important to focus on antibiotic stewardship. In conclusion, the present study reports the detection of prevalence of methicillin and multi-drug resistance among clinical *S. aureus* isolates. Percentage of resistant isolates was remarkably lower than that reported in previous studies. This study therefore provides additional evidence that the rate of emergence of MRSA is in a continuous increase over years. Unfortunately, one can speculate that in the near future all *S. aureus* isolates would become drug resistant. This necessitates the need for combined international efforts to control the spread of antibiotic resistance among bacterial pathogens. Further methods like biotyping, serotyping, phagetyping, Polymerase Chain Reaction (PCR) and Restriction Endonuclease Analysis of Plasmid (REAP) and PCR for the detection of *mec A* gene should be considered whenever possible. Strict rules and regulations regarding the prudent use of antibiotics must be developed in each hospital and also in the national level. Awareness programs on the misuse and overuse of antibiotics should be launched. The primary importance is to decrease the prevalence of MRSA by measures such as rapid and reliable identification of the organisms along with their susceptibility patterns to other antibiotics, isolation and treatment of patients and carriers, and strict adherence to proper hand washing practices by healthcare providers.

Figure-1: Flow-diagram of MRSA isolation

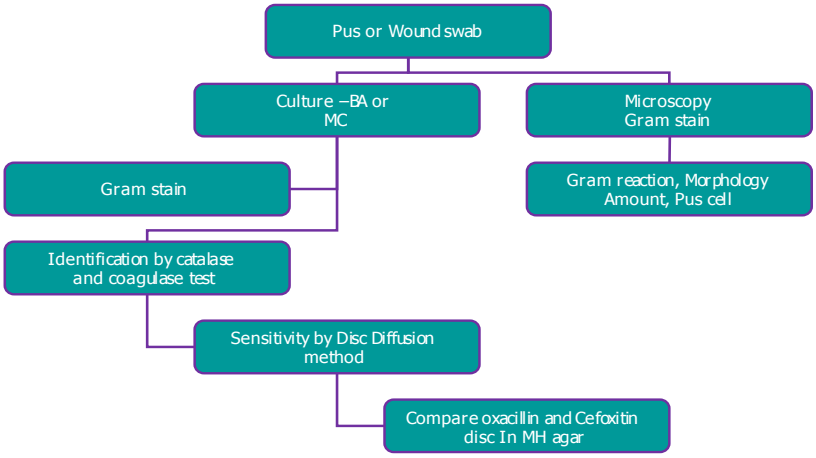

Fig. 1: Isolation of MRSA.

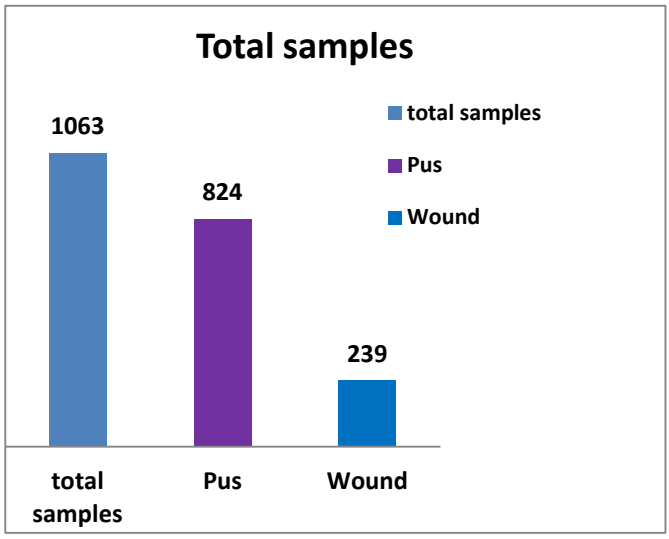

Fig 2: Comparative status of Pus and Wound samples.

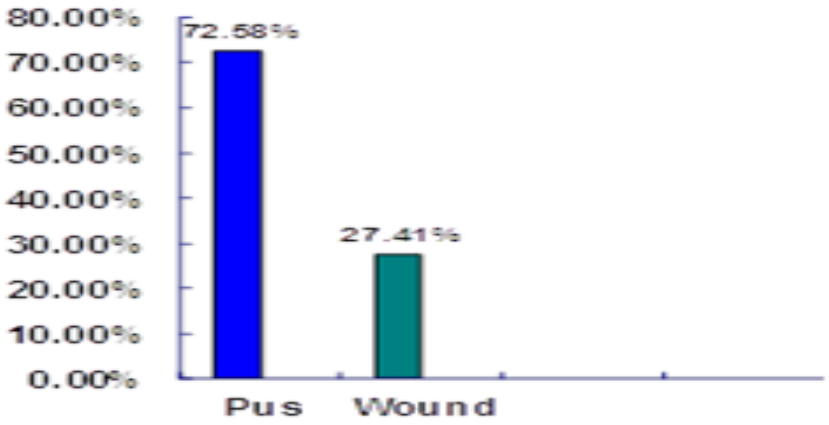

Fig 3: Isolation rate of MRSA in Pus and Wound swab samples.

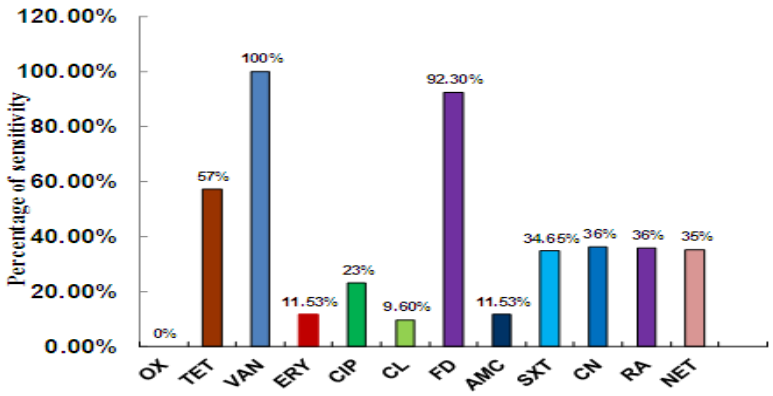

Fig 4: Sensitivity pattern of isolated MRSA.

Table 1: Prevalence of MRSA and MSSA.

Number of organism isolated	Total No.	Percentage
MSSA	149	70.61%
MRSA	62	29.38%
Other Organism	679	63.87% (N=1063)

Table 2: Isolation rate of MRSA according to sex.

Gender	Total number	No. of MRSA positive(%)
female	382	29 (46.77%)
male	681	33 (53.22%)

Table 3: MRSA in outdoor and indoor patient.

Location	Total No.	Percentage (%)
Indoor	617	32(51.61%)
Outdoor	446	30(48.38%)

Table 4: Multidrug resistance pattern of MRSA.

Number of drug resistant	Total No.	Percentage (%)
1 drug resistant	62	100%
2 drag resistant	62	100%
3 drag resistant	62	100%
4 drag resistant	47	76.92%
5 drag resistant	40	65.38%
6 drag resistant	38	61.53%
7 drag resistant	33	53.84%
8 drag resistant	14	22.58%
9 drag resistant	9	14.51%
10 drag resistant	8	12.90%

Table 5: Comparison of Oxacillin and Cefoxitin.

Name of antibiotic	Sensitive(MSSA)	Resistant (MRSA)
Oxacillin	16	62
Cefoxitin	16	62

REFRENCES

1. Barber M. Methicillin-resistant Staphylococci. J Clin Pathol., 1961; 14(4): 385-393.
2. Batchelor FR, Doyle FP, Nayler JK, Rolinson GN. Synthesis of penicillin: 6-aminopenicillanic acid in penicillin fermentations. Nature., 1959; 183(4656): 257-8.
3. Jevons MP. Celbenin Ⅱ-resistant staphylococci. BMJ, 1961; 1: 124–25.
4. Dubey D, Padhy RN. Surveillance of multidrug resistance of two Gram- positive pathogenic bacteria in a teaching hospital and in vitro efficacy of 30 ethnomedicinal plants used by an aborigine of India. Asian Pac J Trop Dis., 2012; 2(4): 273-281.
5. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol., 1966; 45(4): 493-6.
6. Tiwari HK, Sapkota D, Sen MR. High prevalence of multidrug-resistant MRSA in a tertiary care hospital of northern India. *Infect Drug Resist.*, 2008; 1: 57-61.
7. Rajaduraipandi K, Mani KR, Panneerselvam K, Mani M, Bhaskar M, Manikandan P. Prevalence and antibiotic susceptibility pattern of methicillin resistant *Staphylococcus aureus*: a multicentre study. *Indian J Med Microbiol.*, 2006; 24: 34-8.
8. Anupurba S, Sen MR, Nath G, Sharma BM, Gulati AK and Mohpatra TM. Prevalence of methicillin resistant *Staphylococcus aureus* in tertiary referral hospital in eastern Uttar Pradesh. Indian J. Med. Microbiol., 2003; 21: 49-51.
9. Iqbal J, Rahman M, Kabir MS. Ciprofloxacin resistance among community-derived methicillin-resistant *Staphylococcus aureus* (MRSA) strains. Southeast Asian J Trop Med Public Health., 1999; 30(4): 779-80.
10. Kawsar NM, Mondal MEA, Khan NK, Jubaida N, Chowdhury JP, Khan L (2008). Antimicrobial susceptibility patterns of beta-lactamase producing *Staphylococcus aureus*. J Armed Forces Med Coll Bangladesh., 2008; 4(2): 14-7.
11. Vidhani S, Mehndiratta PL, Mathur MD. Study of methicillin resistant *S. aureus* [MRSA] isolates from

high risk patients. *Indian J Med Microbiol.,* 2001; 19: 13-6.

12. Tyagi A, Kapil A, Singh P. Incidence of Methicillin Resistant Stahylococcus aureus [MRSA] in Pus Samples at a Tertiary Care Hospital, AIIMS, New Delhi. *JIACM.,* 2008; 9: 33-5.
13. Mallick SK, Basak S. MRSA -too many hurdles to overcome: a study from Central India. *Tropical Doctor.,* 2010; 40: 108–10.
14. Pulimood TB, Lalitha MK, Jesudason MV, Pandian R, Selwyn J, John TJ. The spectrum of antimicrobial resistance among methicillin resistant *Staphylococcus aureus* [MRSA] in a tertiary care centre in India. *Indian J Med Res.,* 1996; 103: 212-5.
15. Bell JM, Turnidge JD, SENTRY APAC Participants: High prevalence of oxacillin-resistant Staphylococcus aureus isolated from hospital patients in Asia-Pacific and South Africa: Results from SENTRY Antimicrobial Surveillance Program, 1998 –1999. Antimicrob Agents Chemother, 2002; 46: 879-881.
16. Hackbarth CJ, Chambers HF. Methicillin-resistant Staphylococci, Genetics and mechanisms of resistance. *Antimicrob Agents Chemother.,* 1989; 33: 995-999.
17. Tomasz A, Drugeon HB, de Lancester HM. New mechanism for methicillin-resistant *Staphylococcus aureus*: Clinical isolates that lack the PBP–2a gene and contain normal penicillinbinding protein with modified penicillin-binding capacity. *Antimicrob Agents Chemother.,* 1989; 33: 1869-1874.
18. CLSI. Performance standards for antimicrobial susceptibility testing; 17th informational supplement. 2007; CLSI M100-S17. CLSI, Wayne, PA.
19. Fruit AC, Wielders CLC, Verhoef J, Schmitz FL: Epidemiology and susceptibility of 3051 *Staphylococcus aureus* isolated from 25 university hospitals participating in the European SENTRY Study. *J Clin Microbiol.* 2001; 39: 3727-2732.
20. Cookson B, Peters B, Webster M, Phillips I, Rahman M, Noble W: Staff carriage of epidemic methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol.,* 1989; 27: 1471-1476.
21. Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scott's diagnostics in microbiology. Maryland Heights: Elsevier/Mosby; 2002; 1-783.
22. National Committee for Clinical Laboratory Standards (NCCLS) Methods for dilution antimicrobial susceptibility tests for bacteria that grows aerobically. Approved standard, 2000; M7-A5 National Committee for Clinical Laboratory Standards, Wayne, PA.