Prevalence of *mec*A: Genotyping screening of community acquired-MRSA isolates in Karachi, Pakistan

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Abstract: Among resistant nosocomial and community pathogens, MRSA has become the most serious pathogen, causing life threatening infections worldwide. In *S.aureus*, quick and exact recognition of methicillin (cefoxitin) resistance has become essential. The benchmark for MRSA identification among *S.aureus* is the detection of the *mecA* gene that causes the expression of protein (PBP2a) culpable for classic β-lactam resistance. However, the utter reliance on amplification of *mec*A gene as a hallmark in confirmation of methicillin (cefoxitin) resistant *S. aureus* is the matter of distrust by some investigators. The current investigation designed to analyse the prevalence of *mec*A gene among phenotypically positive MRSA isolates using molecular method and to correlate its prevalence to conventional techniques. Furthermore, antimicrobial sensitivity of *mec*A positive staphylococci was determined by Kirby Baeuer method. For this purpose, 201 clinical staphylococcal specimens were recovered from various diagnostic laboratories in Karachi City, Pakistan. Phenotypic existence of methicillin resistance in *S. aureus* was observed to be 51.7%. In contrast, when organisms were subjected for amplification of *mec*A gene by PCR, *mec*A positive isolates were 36/104 (35%) MRSA isolates. Current work raise question towards the usefulness of molecular identification of *mec*A gene in confirmation of methicillin resistance without correlating with conventional methods. Therefore, it is essential to consider the other possible resistance mechanisms for β-lactams that may interact with *mec*A gene in the development of methicillin resistance mechanism in Staphylococcus.

Keywords: MRSA, *mec* A, CA-MRSA, PCR, antibiotic susceptibility.

INTRODUCTION

Staphylococcus aureus is one of the notorious microbes within the community and hospitals. The proportion of mortality from invasive Staphylococcal illnesses was high in pre-antibiotic age, however, the addition of penicillin in clinical care had a crucial impact on management of infectious diseases (Grubb, 1998). Methicillin was commercially introduced in late1950s to combat the problem associated with preponderance of penicillinase-producing staphylococcal strains resistant to natural Penicillin (Enright *et al*., 2002). Presently, MRSA strains accepted as serious clinical issue globally to most classes of antimicrobials (Speller *et al*., 1997).

Emergence of penicillin's non-susceptible isolates among Staphylococcal strains initially in hospitals and currently in community setting is ambiguous because the choice of empirical treatment must include antimicrobials having spectrum of activity against resistant isolates (Enright *et al*., 2002). Clinical management of diseases caused by MRSA is challenging as these strains showed resistance to all β-lactam agents. Community related isolates tends to be resistant to only few antibactacterials, in contrast to Hospital related MRSA strains which are multiple antibiotic resistant and often remains susceptible to agents other than beta lactams, including sulfonamides, tetracyclines and clindamycin (John and Schreiber, 2006).

Staphylococcus aureus strains spread in the community setting are generally susceptible to methicillin, in contrast, MRSA observed more frequently in the hospital settings. However, the trend of community-acquired illnesses caused by MRSA strains has been rising worldwide in last 10 years. In light of this, the present work was planned to assess the increasing reports of CA-MRSA and investigate the threat of recently emerging community pathogen and its consequences from Pakistan perspective. The emergence of MRSA in community is a current phenomenon, boosting paramount concern since it would cause difficulties in treatment of infections in the outpatient setting.

The resistance in *S. aureus* is due to the acquisition of an inducible PBP2a (Penicillin binding protein) encoded by resistance gene, *mec*A, that is located on mobile genetic element, the Staphylococcal cassette chromosome mec (SCCmec). Different classes of SCCmec were generally identified by PCR method. In addition, low affinity PBP2a, substitutes the other constitutive PBPs, enables

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the bacterium to sustain the high concentrations of β-Lactam antimicrobial agents (Chambers, 2001). Resistance to β-lactams is associated mainly due to alterations in the *mec*A gene, however, the genetic elements other than *mec*A may also be investigated for the description of possible antimicrobial resistance (Matsuhashi *et al*., 1986).

Recently, introduction of decisive molecular methods has a vital job in the identification of *mec*A, e.g. PCR method and DNA hybridization (Mehrotra *et al*., 2000). Amplification of *mec*A by molecular techniques is considered as a benchmark to detect methicillin resistant strains in the community since this gene is extensively conserved among staphylococcus (Al-Abbas, 2012). The current investigation aimed to analyse the usefulness and reliability of *mec*A gene amplification in the diagnosis of methicillin resistant staphylococcal strains.

MATERIAL AND METHODS

Clinical specimen

In all 201 clinical samples of Staphylococci were recovered from various laboratories in community settings at Karachi, Pakistan. Clinical samples of *S. aureus* recovered from tissue aspirates, wound, urine, ear swabs and nasal secretions from Jan 2017 to June 2017. *S. aureus* isolates were identified by conventional laboratory methods which included colonial morphology, microscopic morphology, catalase, mannitol salt agar fermentation, coagulase and DNase.

Antimicrobial susceptibility testing

Antibiotic sensitivity was performed for all 201 *S. aureus* specimens using the following chemotherapeutic agents: oxacillin, cefoxitin, tetracycline, erythromycin, vancomycin, nalidixic acid, clindamycin, rifampin, minocycline and cephradine by Kirby-Bauer technique according to CLSI regulations.

Amplification of mecA gene

Single bacterial colony of an overnight subculture was inoculated in hundred micro litre nuclease free water. The bacterial suspension was subjected to heat for 10-20□min at 80°C for lysis of cells. For amplification, bacterial lysate served as template DNA. Polymerase reaction mixture contain 2× PCR Master mix (Fermentas Life Sciences, USA) (*Taq* DNA polymerase (recombinant) 0.05□U/µl, Magnesium Chloride 4mM, dNTPs mix 0.4□mM each) and 0.5µM of each forward (5-GTAGAAATGACTGAACGTCCGATAA-3) and reverse (5-CCAATTCCACATTGTTTCGGTCTAA-3) primers explained by Geha (Geha *et al*., 1994). These generate a PCR amplicon of 310 base pairs. Bacterial lysate (5µl) was incorporated as a source of DNA to PCR Master Mix. Final volume of PCR mixture was 25µl. The initial denaturation was achieved by amplification reaction for 3min at 95°C. For denaturation, cycles were performed for 1min at 94°C, annealing of primers were carried out at 55°C for 1min. extension and final extension were done at 72°C for 2min. and 72°C for 5min respectively. Total 34 cycles were carried out for amplification reaction. Amplified product was observed on 1% agarose gel along with 1500bp ladder (Fermentas US).

RESULTS

Distribution of MRSA Source among clinical samples

Methicillin resistant Staphylococci were recovered from various sites of infections, wound and ear were the main the main sites of infections i.e. 24.5% and 28.5% followed by urine and nasal cavity i.e. 23.5% respectively. (fig. 1).

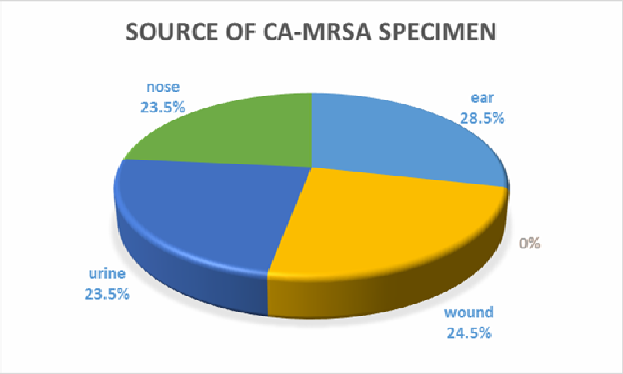


Fig. 1: Site of CA-MRSA infections

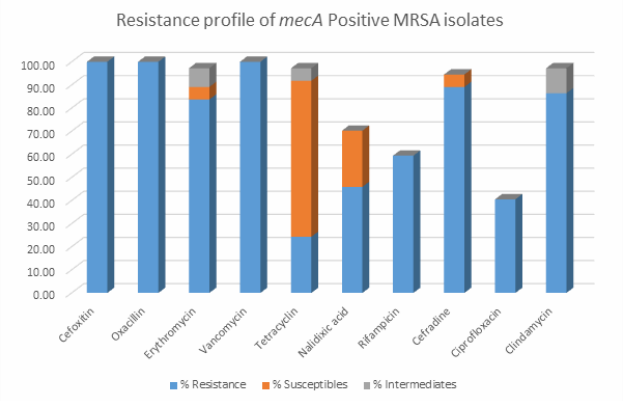


Fig. 2: *mec* A positive isolates resistance pattern

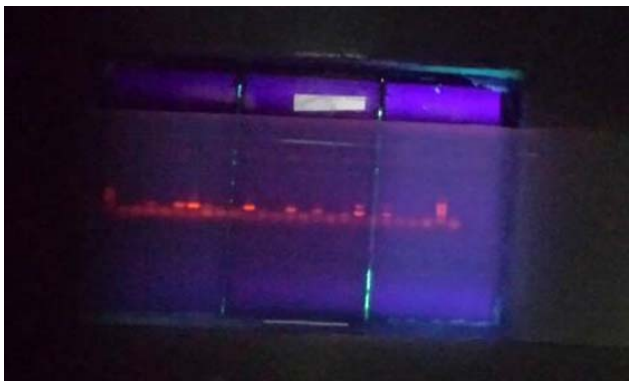
Drug susceptibility pattern

Of 201 *S. aureus* isolated from community samples, 104 (51.7%) isolates were diagnosed as MRSA and 97 (48.3%) were susceptible to methicillin/cefoxitin (MSSA). Moreover, all these specimens were tested for their susceptibility against 10 antibiotics; the results are presented in fig. 2.

Amplification of mecA Gene

The *mec*A gene was analysed in all phenotypically positive MRSA; 36/104 (36.5%) whereas remaining MRSA were failed 68/104 (64%) to show the band of

310□base pair particular for the presence of *mec*A gene. (fig. 3).



1% Agarose gel electrophoresis showing mecA gene of PCR primer pair (L1: DNA ladder- 1500 bp; L2, L3, L4, L5, L8, L9, L10, L12, L13, L15, L17, L18: mecA negative, L6, L7, L11, Ll4, L16, L19: mecA positive, L19: fem B positive)

Fig. 3: Amplification of *mecA* and *fem B* gene by PCR

DISCUSSION

Developing a consistent test for the speedy and decisive determination of MRSA strains is still highly necessitated. Such achievement will play a key role in preventing the dissemination of problematic microbe in community. The current work aimed to illustrate the present picture of Staphylococcal resistance in the community and the resistance of pathogen to antibiotics that are generally prescribed to treat this organism in Karachi, Pakistan. One of the main observation in this research is the high preponderance (*n*=104, 51%) of MRSA, in addition, they had also demonstrated multiple antibiotic resistance among *S.aureus*. The increased resistance in clinical isolates was observed earlier by various researcher: in USA (61.8%) (Jarvis *et al*., 2012), in Egypt (54%) (Hafez *et al*., 2009), in Taiwan (61%) (Huang *et al*., 2000), in Saudi Arabia (51%) (Alghaithy *et al*., 2000). In addition to cefoxitin and oxacillin, MRSA had showed resistance to erythromycin, cephradine, nalidixic ancid and teteracyclin. In diagnosis of MRSA, presence of *mecA* gene has been understood a prime evidence. This statement was endorsed by different authors worldwide: USA (Murakami *et al*., 1991), in Saudi Arabia (Al-Ruaily and Khalil, 2011), in India (Mehndiratta *et al*., 2009). However, the present study reported low burden of the *mec*A within MRSA (34.6%), this finding may unbolt the door to investigate other alternative mechanism which may involve in developing methicillin resistance. On the other hand, the absence of resistant gene among *S. aureus* was also reported around the globe (Aziz *et al*., 2014, Bignardi *et al*., 1996). Also an earlier report from Nigeria revealed the complete absence of *mec*A besides 5 major SCCmec types in phenotypically identified MRSA isolates, suggesting a possibility of β-lactamase hyper production as an origin of this phenomenon (Olayinka *et al*., 2009). Furthermore, Ba et al identified modifications in amino acids of PBPs cascade 1, 2 and 3 that might be the basis of resistance phenomenon (Ba *et al*., 2013). These modifications were observed to include 3 amino acid substitutions which were similar and were present in PBPs cascade 1, 2 and 3. In addition, the identical amino acid was found to have 2 other distinct substitutions in protein (PBP1). The amino acid substitution either identical or distinct were found in clinical isolates from distinct multilocus types (Ba *et al*., 2013). These observations showed an important clue towards the phenomenon aside from the existence of *mec*A gene responsible for methicillin (β-lactam) resistance in MRSA strains as well as absolute reliance on molecular technique are not adequate for characterization of Methicillin resistant strains.

CONCLUSION

Presently, *mec*A negative isolates of MRSA are not commonly observed, thereby, it is crucial to identify the origin of resistance in *S. aureus*. It is particularly important for diagnostic labs that depend only on amplification of *mecA* as well as recognized as gold standard for diagnosis of strains of MRSA. Lastly, it is clearly understood that in *S.aureus* there are multiple different resistance mechanisms for beta-lactam agents.

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