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Pheno and genotypic screening of *mec A* among carrier Methicillin Resistant *Staphylococcus aureus* (MRSA)

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Abstract

Staphylococcus aureus is an opportunistic pathogen in human beings as well as in animals and is responsible for a broad spectrum of diseases ranging from skin infections to other severe diseases. The methicillin resistant *S. aureus* (MRSA) strains are highly pathogenic and are proved to carry a number of virulence factors that intern enable them causing difficult to treat/ manage diseases. Clinically, MRSA acquires significance owing to their transmissibility in clinical set-ups and manifestation frequently as nosocomial infections worldwide. The clinical importance of carrier *S. aureus* and MRSA observed on par with their clinical counter parts by causing community acquired infections. In this study, carrier *S. aureus* (*n*=272) isolated from normal subjects were comparatively evaluated for their degree of susceptibility/ resistance to oxacillin and cefoxitin by the conventional discs diffusion methods as well as by oxacillin agar screening method. Further, among the preliminarily confirmed MRSA strains (*n*=52), a total of 21 MRSA were screened for *mec A* gene by PCR. It was identified that10 (47.61%) isolates were positive for *mec A* gene and were further discussed.

Key words: *S. aureus, carrier, mrsa, mec A, PCR and antibiotic discs.*

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INTRODUCTION

S. aureus is a facultative anaerobic and opportunistic Gram- positive pathogen in human beings and animals (Hague *et al.,*2011; Abdalla*et al.,* 2012). Humans are the natural reservoirs of *S. aureus*, and asymptomatic staphylococcal colonization is far more common than infection. *S. aureus* is carried by approximately 30% of the healthy population as its colonization is common in nasopharynx, perineum, or skin, shortly after birth and may recur anytime thereafter (Olowe *et al.,* 2007). The human system is considered as a major ecological niche for *S. aureus* and hence the species is well adapted to colonize the human skin. Though day to day exposure to the bacterium does take place, some persons are observed to be the carriers over longer periods of time (Sollid *et al.,* 2014). Currently, infection due to methicillin resistant *S. aureus* (MRSA) has been a global challenge and is a major nosocomial pathogen causing significant morbidity and mortality. The infected or colonized patients as the major reservoirs of MRSA transmit the pathogen among health care workers in institutions and the transient bacterial hand carriage among such persons is the predominant mode of patient to patient transmission (Deepa *et al.,* 2010).

To augment, *S. aureus* has the capability to acquire resistance against all classes of antibiotics and methicillin resistance, an important resistance trait. The development of resistance to most of the new antibacterial drugs is a reflection of its ability to adapt and survive in extreme conditions (Mulligan *et al.,* 1993). As the heterogeneous / homogenous nature of resistant gene expression among the isolates has been the root of difficulty in MRSA detection / prevalence determination, the *mec A* gene test by PCR or protein expressed by *mec A* gene (PBP2) could redress the screening of *S. aureus* and identifying MRSA that would strongly assist in an effective surveillance system so as to describe epidemiological trends and infection control strategies in each area.

In this context, the aim of the study was to evaluate the disc(oxacillin and cefoxitin) diffusion and oxacillin agar screening tests as to detect MRSA and to compare the outcomes with *themec A* gene detection sensitivities of PCR. This would minimize any chance of missing MRSA and improve the determination of MRSA prevalence, and these aspects are evaluated and discussed in this article.

MATERIALS AND METHODS

A total of 272 carrier *S. aureus* strains isolated from volunteering learners from the institution were subjected to antimicrobial susceptibility test against a

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panel of 14 antibiotics [Penicillin (10 ìg), Oxacillin (1ìg), Cefoxitin (30 ìg), Vancomycin (10 ìg), Gentamycin (10 ìg), Tobramycin (10 ìg), Tetracyclin (30 ìg), Ciprifloxacin (5 ìg), Levofloxacin (5 ìg), Ofloxacin (5 ìg), Moxifloxacin (5 ìg), Norfloxaxin (10 ìg), Gatifloxacin (5 ìg) and Co- Trimoxazole (25 ìg)] and the clear zones were measured so as to determine their nature of their susceptibility (susceptible, intermediately resistant and resistant) to each test antibiotic.

Methicillin/cefoxitin resistance screening

The test cultures (0.5 McFarland suspensions) were spread with a sterile cotton wool swab on Mueller–Hinton agar supplemented with 2% NaCl. Subsequently, Oxacillin (1 ìg) and Cefoxitin (30 ìg) disks were applied with sterile forceps, and the overnight incubated plates were checked for any inhibition zone diameter (ZD) and were assessed using the ZD interpretative standard (CLSI, 2011).

Oxacillin agar screening method

Similarly, the test isolates (*n*= 272) were evaluated on oxacillin agar (4% NaCl with 6 ìg/ml oxacillin in Muller Hinton agar plates) by inoculating 0.5 Mc Farland suspensions. After 24 hours of incubation, any growth of the test carrier *S. aureus* was considered as Oxacillin resistant (Brown *et al.,* 2005).

PCR amplification of the *mec A* gene

For PCR based detection, the test carrier *S. aureus*strains were subjected to DNA extraction as recommended by Madadgar *et al*. (2008). The *mecA* gene among the carrier isolates (21 of 52 *S. aureus*) was confirmed by PCR amplification using the primers *mec*A1 (5'-GTAGAAATGACT-GAACGTCCGATAA-3′) and *mec*A2 (5'-CCAATTCCACATTGTTTCGGTC-TAA-3') (Geha *et al.*, 1994). A 25 ìl reaction master mix containing PCR buffer (1X), dNTP mix (0.25mM of each), Taq DNA polymerase (0.25 U), $MgCl_2$ (1.5mM) and Primer mix 4 ìl and 3 ìl of DNA was used. The initial denaturation at 94°C for 4 min was followed by 30 cycles of amplification (denaturation at 94°C for 45 sec, annealing at 50°C for 45 sec, and extension at 72°C for 60 sec) ending with a final extension at 72°C for 2 min and the amplicons analysed by 1.5% agarose gel electrophoresis with ethidium bromide dye under UV transilluminator and were documented.

RESULT AND DISCUSSION

Of the 272 isolates, 52 were noted to be MRSA to Oxacillin and Cefoxitin discs diffusion and oxacillin agar test. While18 (34.61%) and 14 (26.92%) isolates were respectively confirmed by using oxacillin, and cefoxitin disc diffusion methods, as much as 42 (80.76%) carrier strains were determined by using oxacillin agar screening (Figure 1).

Figure 1. Methods of MRSA determination and total number of isolates

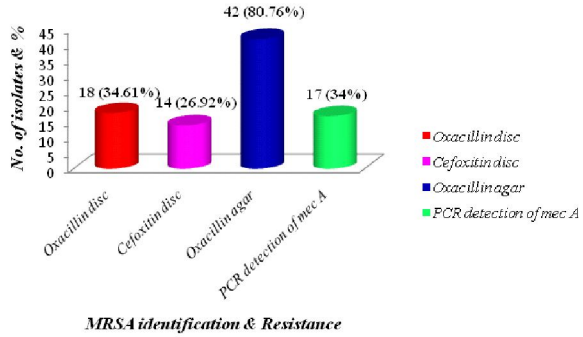


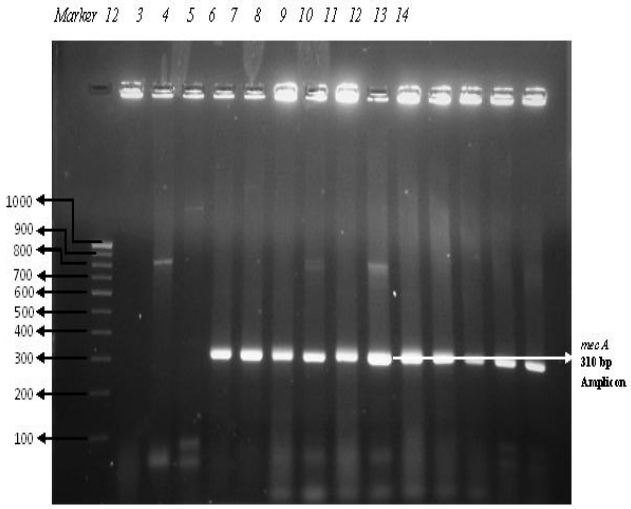
Table 1. Methods of MRSA determination and their distribution among the volunteers

Isolate No.	Results of various detection methods of methicillin resistant Staphylococcus aureus (MRSA)			
	Oxacillin	Cefoxitin	Oxacillin	mec A
1	S	R	G	+
2	R	S	NG	-
3	R	S	G	+
4	R	R	NG	-
5	R	R	G	-
6	R	S	NG	-
7	R	S	G	-
8	R	S	G	+
9	R	S	G	-
10	R	S	NG	-
11	R	S	G	+
12	R	R	G	-
13	S	R	G	-
14	S	R	G	-
15	R	R	G	+
16	S	S	G	-
17	S	S	G	+
18	S	R	NG	+
19	S	S	G	+
20	S	S	G	+
21	S	S	G	+
Total	**12 (57.14%)**	**8(38.09%)**	**16 (76.19%)**	**10 (47.61%)**

R- Resistant, S- Sensitive, G- Growth, NG- No Growth, +- Positive and - - Negative

In this study, the antibiotic resistance was confirmed by either one of the three methods or by all the screening methods. Notably, one of the twenty one test isolates was categorised to be resistant type by all the studied methods. Precisely, of the 21 isolates, 12 (57.14%), 8(38.09%) and 16 (76.19%) were identified to be resistant by oxacillin, cefoxitin disc and oxacillin agar screening tests respectively. Similarly, among the 21 carrier isolates, 10 (47.61%) were positive for *mec A* (310 bpamplicon; Figure 2) genotype and of which 90% of the (9 of 10 *mec A* positive genotypes) carrier isolates was re-identified to be resistant phenotypes by the oxacillin screening agar test. However, only 40% and 30 % oxacillin and cefoxitin discs tests turned isolates were respectively positive for *mec A* gene by PCR.

Figure 1. Uniplex PCR based detection of mecAgenes among S. aureus



Sources of S. aureus 1. GS 113 Direct finger, 2. GS 114 Direct finger, 3. GS 159 Palm, 4. GS 485 Right nose, 5. GS 276 Direct finger, 6.GS 365 Left nose,7. GS 224 Direct finger,8. GS 376 Direct finger,9. BS 106 Palm,10. GS 371 Left nose11.GS 365 Palm,12. GS 282 Left nose,13. GS 188 Palm,14. GS 78 Direct finger.

In particular, MRSA confirmation by *mec A* gene or by its product- penicillin binding proteins (PBP2a) has been considered to be the gold standard method (Skov *et al.,* 2006). As it has been found in the present study,though Cefoxitin is considered as a surrogate marker for the detection of MRSA (Fernandes *et al.,* 2005), it is currently recommended and an accepted phenotypic method for the detection of MRSA by many reference groups including CLSI (Skov *et al.,* 2003). Similarly, the presence of *mec A* is an important molecular marker to identify MRSA in clinical and environmental samples including carrier derived isolates and the technique inherits added advantages over the conventional approaches. As it has been noted in the study, false negative results of the conventional methods could be picked up by the PCR at a very early stage and could accurately demonstrate *mec A* gene, assisting unequivocal identification of MRSA. While the time taken for diagnosing MRSA by conventional methods extends between 48 and 72 hrs, and PCR based detection needs 18-24 h (Anju *et al.,* 2012). In particular, the study conclude than a correlation between higher number MRSA detection by oxacillin screen agar test and *mec A* gene amplification based MRSA detection by PCR, in needed.

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