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Evaluation for the Novel *mecC* Methicillin Resistance among Methicillin Resistant *Staphylococcal* Isolates in two Egyptian University Hospitals

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Abstract

Methicillin resistance adds to the threat of *staphylococcal* infections in humans. The gene for methicillin resistance in *staphylococci* is not only *mecA* gene as it was thought. The recently discovered *mecC* gene in animal and human isolates also encodes for methicillin resistance. The prevalence of the novel gene may be underestimated. It is discovered so far in Europe with low prevalence. This study evaluates the existence of *mecC* gene in two Egyptian university hospitals. A total of 600 methicillin resistant (520 MRSA and 80 MRCoNS) isolates from two university hospitals were screened for discrepancy in susceptibility between cefoxitin (30 µg) and oxacillin (1 µg) by disc diffusion as a method that could be used to predict the potential existence of *mecC* gene whose protein product has noticeably higher affinity for oxacillin. Discordant isolates included in selected 150 samples (110 MRSA and 40 MR-CoNS) were tested by PBP2a latex agglutination test, and or conventional PCR for *mecA* gene. Samples negative for *mecA* gene were tested for oxacillin and cefoxitin MIC by Vitec II and for *mecC* gene by PCR. All discrepant isolates were positive for PBP2a latex agglutination and *mecA* PCR, while the 6 isolates (5.5%) negative for *mecA* by PCR were resistant to both cefoxitin and oxacillin by disc diffusion and by Vitec II. In addition, those 6 isolates negative for *mecA* were also negative for *mecC* gene. Thus *mecC* gene for methicillin resistance couldn't be detected in this study, though this may be baseline for further prevalence studies.

Keywords: *mecC* gene; Methicillin resistance; S*taphylococci*; PBP2a latex

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Introduction

Methicillin resistant *S. aureus* (MRSA) was first described in 1961 collapsing the treatment options for *staphylococcal* infections as MRSA exhibits resistance to all B lactam antibiotics except 5th generation cephalosporins. Resistance to antibiotics other than ß lactams is common especially in health care associated MRSA (HA-MRSA). The dissemination of MRSA in the community (community associated MRSA [CA-MRSA]) and in animal adapted lineages of *S. aureus* (livestock associated MRSA [LA-MRSA]), besides the originally described HA-MRSA represents a public health problem and a burden to infectious disease medicine. The genetic principle for methicillin resistance was identified more than 20 years after the first description of MRSA. It was due to the acquisition of mobile genetic element called *staphylococcal* cassette chromosome that become integrated into the *staphylococcal* chromosome carrying the gene encoding for resistance [1]. It was thought that the only gene for methicillin resistance in *staphylococci* is *mecA* gene that encodes a transpeptidase PBP2a which has low affinity for ß lactams allowing cell wall synthesis to continue in the presence of the antibiotic. In 2011, whole genome sequencing of *S. aureus* strain LGA251 and other strains that show phenotypically methicillin resistance despite being *mecA* negative, revealed a novel *mecA* homolog that share only 69.7% nucleotide identity with *mecA*. It is carried on novel SCC*mec* type XI which has new *ccr* type 8, divergent *mecA* regulatory genes (*mecI* and *mecR*), and no joining region J3 [2]. The protein product (PBP2a $_{LGA251}$) of *mecC* gene has

63% amino acid identity to that of *mecA* gene and it its affinity towards oxacillin is 4 folds that of PBP2a [3].

Reports from European countries accounted the prevalence of *mecC*-mediated methicillin resistance to be low 0-2.8% among human MRSA isolates in 13 European countries specially Western and Northern Europe. Despite its recent discovery, *mecC* gene was found to be widely distributed in animals carried by isolates of *S. aureus* and other *staphylococcal* species [4-6].

In this study we tried to evaluate the existence of *mecC* gene in methicillin resistant *staphylococcal* isolates obtained from two university hospitals in Egypt.

Materials and Methods

Bacterial isolates

A total of 600 sequential samples of methicillin resistant *staphylococcal* clinical isolates (520 MRSA and 80 MR-CoNS) were collected from clinical microbiology laboratories of two university hospitals in the duration from March 2014 to May 2016. Methicillin sensitive isolates are excluded. Hospitals sharing in this study are presented in **Table 1**. Methicillin resistant *staphylococcal* isolates were identified through routine identification and susceptibility testing. Patients' data were obtained from microbiology request forms.

Identification and susceptibility testing by disc diffusion

Colonies from different clinical samples on different agar media were identified by morphology, gram stain and catalase reaction, then culture on mannitol and DNase agar. Tube coagulase test was performed when discrepancy between mannitol and DNase results observed. Routine susceptibility testing by disc diffusion was followed including cefoxitin 30 µg (Oxoid, UK) and oxacillin 1ug (Oxoid, UK) according to CLSI on Muller Hinton agar plates [7,8]. All 600 isolates were screened for the discrepant susceptibility profile oxacillin sensitive/cefoxitin resistant.

PBP2a latex agglutination

Discrepant isolates along with randomly selected isolates to have a total of 50 isolates were tested for PBP2a by latex agglutination (Mast group Ltd, UK) according to instructions by the manifacturer.

Polymerase chain reaction (PCR)

Isolates tested for *mecA* by conventional PCR included isolates tested by PBP2a latex agglutination and randomly selected isolates From Beni-Suef University Hospital which lies in a semirural area. A total of 150 isolates (110 MRSA + 40 MR-CoNS) were tested for *mecA* by conventional PCR using forward primer (5'- GTA GAA ATG ACT GAA CGT CCG ATA A-3') and reverse primer (5'-CCA ATT CCA CAT TGT TTC GGT CTA A-3') according to McClure et al. [9]. The PCR reaction contained 12.5 µl mastermix (Emerald Amp GT), 1 µl of each primer, and 6 µl of DNA template to reach a total volume of 25 µl. Thermal cycling was adjusted to 5 minute at 94°C for primary denaturation followed by 35 cycles of 30 seconds denaturation at 94°C, 30 seconds at 50°C for annealing, and 30 seconds at 72°C for extension. Finally extension for 7 minutes at 72°C was followed. Agarose gel (1.5%) electrophoresis and ethidium bromide staining was used to separate and visualize the amplified product which was identified at 310 bp using 100 bp DNA ladder. Isolates negative for *mecA* were tested for *mecC* by conventional PCR using forward primer (5'- GCT CCT AAT GCT AAT GCA -3') and reverse primer (5'- TAA GCA ATA ATG ACT ACC-3) according to Cuny et al. [10]. Unlike thermal cycle for *mecA*, annealing temperature for *mecC* was adjusted to 50°C for 40 seconds while extension and final extension were adjusted to 72°C for 40 seconds and 10 minutes respectively. The amplified product was identified at 304 bp. ATCC 33591 MRSA was used as a positive control in PCR for *mecA* and positive control for *mecC* was obtained from Animal Health Research Institute in Giza, Egypt.

Results and Conclusions

Six hundred human clinical cases were included in this study. The gender, mean age, departments sharing and the initial culture site are presented in **Table 2.** All 600 isolates were screened for the susceptibility profile oxacillin sensitive/cefoxitin resistant by disc diffusion method as an easy, widely used, and cheap method that may predict the probable existence of *mecC* gene in isolates that exhibit discrepant susceptibility. **Figure 1** shows the percentage of discrepant susceptibility among both MRSA and MR-CoNS isolates.

All discrepant isolates were positive for PBP2a latex agglutination and were also positive for *mecA* by PCR. Thus discrepant susceptibility by disc diffusion in this study was not due to *mecC* carriage by any of the isolates. Instead discrepancy was due to the inducible character of oxacillin resistance and heterogeneous expression of methicillin resistance in *staphylococci.*

Of 150 isolates tested by PCR for *mecA* gene, 6 isolates tested negative. They were all MRSA isolates (5.5% of MRSA isolates) and were resistant to both cefoxitin and oxacillin by disc diffusion method. Gel electrophoresis for detection of *mecA* amplicon is shown in **Figure 2.**

All isolates negative for *mecA* were also negative for *mecC* by conventional PCR Uniplex PCR for detection of *mecC* amplicon is shown in **Figure 3.**

Table 1 University hospitals sharing in this study.

Hospital	City and Country	Number of isolates	Wards	Duration
Kasr Alainy hospital	Cairo, Egypt	300 MRSA	ICU units	March 2014 – September 2014
Beni-Suef university hospital	Beni- Suef governrate, upper Egypt	220 MRSA and 80 MR-CoNS	Outpatients and different inpatients' wards	April 2015 – May 2016

Table 2 Descriptive data of patients and bacterial isolates.

	Cairo University Hospital [300 MRSA isolates No(%)]	**Beni-Suef University Hospital** [220 MRSA No(%) + 80 MR-CoNS No(%)]
Sex		
Male	180 (60%)	153 (51%)
Female	120 (40%)	147 (49%)
Age groups		
Neonates	-	22 (7%)
Pediatrics	-	74 (25%)
Adults	300 (100%) mean 50	204 (68%) mean 47
Departments		**MRSA** **MR-CoNS**
ICUs	300 (100%)	92 (42%) 28 (35%)
Surgery ward	-	12 (5%)
Medicine ward	-	1 (1.25%)
Pediatrics ward	-	64 (29%)
Outpatient clinic	-	9 (11.25%)
		26 (12%) 34 (42.5)
		26 (12%) 8 (10%)
Sample types		**MRSA MR-CoNS**
Wound swabs and pus	128 (43%)	29 (13%) 1 (1.25%)
Urine	17 (6%)	52 (24%) 5 (6.25%)
Sputum	40 (13%)	31 (14%) -
Blood	115 (38%)	101 (46%) 72 (90%)
CSF	-	4 (2%) 2 (2.5%)
Others	-	3 (1%) -

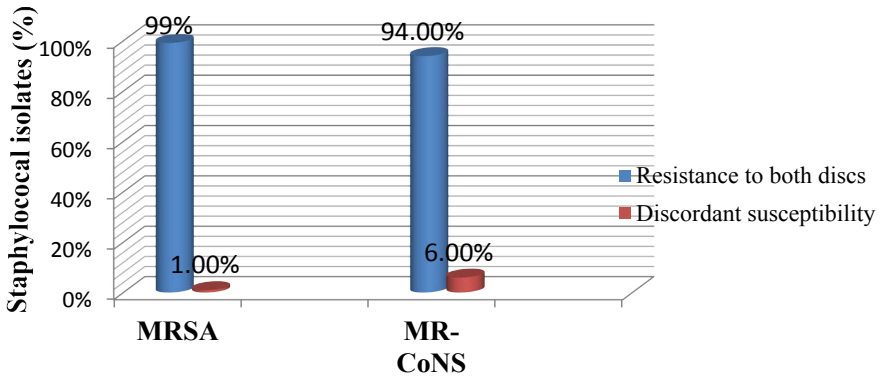
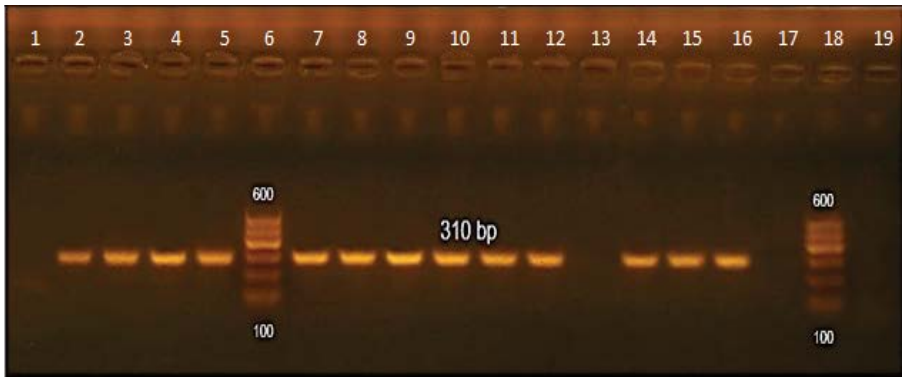
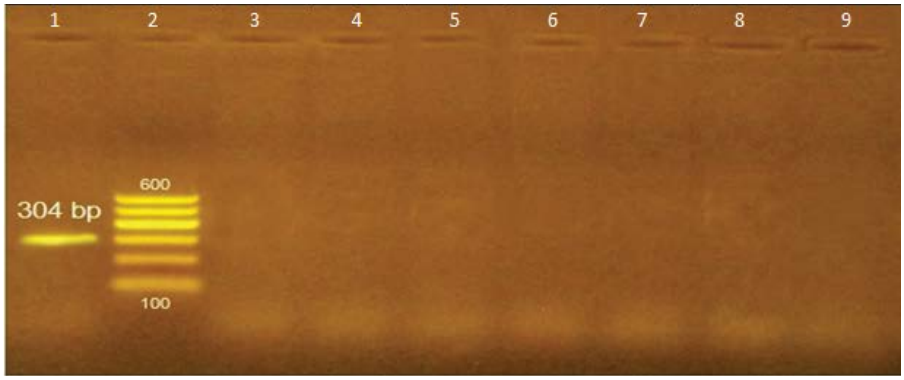


Figure 1 Percentage of susceptibility profiles of MRSA and MR-CoNS isolates.



Lanes 6 and 18 are 100 bp ladder; Las 5 and 19 are positive and negative controls respectively; Lanes 1, 13, and 17 are negative samples while the remaining are positive for *mecA* gene at 310 bp. ne

Figure 2 Uniplex PCR for detection of mecA gene.



Lane 1: Positive control; Lane 2: 100 bp ladder; Lane 3: Negative control; Lanes 4,5,6,7,8, and 9: Test samples negative for *mecC* gene

Figure 3 Uniplex PCR for detection of mecC amplicon at 304 bp.

No *mecC* MRSA isolates were detected in this study. This was in concordance to Ganesan et al. [11] and Basset et al. [12]. Also *mecC* could not be detected among MR-CoNS isolates in this study. This was the case in Nijjar et al. [13]. The *mecA/mecC* negative isolates were all positive by cefoxitin screen by Vitek II and all isolates showed oxacillin MIC ≥ 4 µg/ml. This moderately high resistance may exclude Border Line Oxacillin Resistant *S. aureus* (BORSA) type of resistance to be the cause of methicillin resistance in those isolates. Modified Penicillin binding proteins in *S. aureus* (MODSA) may be the cause of methicillin resistance in *mecA/mecC* negative isolates in this study. This needs further investigations as done by Ba et al. who described amino acid substitutions in penicillin binding proteins on whole genome sequencing for MRSA isolates lacking *mec* genes [14].

In conclusion *mecC* gene was not detected in any of *staphylococcal* isolates in this study, though further studies especially in rural areas testing for *mec* genes in larger number of clinical isolates using multiple primer sets for *mecC*, may increase the probability of detection of *mecC* gene or its homologs.

Funding

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Ethical Approval

No ethical approval was required for this study as the isolates used in the study were collected as part of routine clinical facility.

Conflict of Interest

The authors state that they have no competing interests.

Informed Consent

Informed consent was not applied, though letters were sent to patients to inform them about the study and who to contact if they did not want to share in the study.

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