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Phenotypic and genotypic detection of local MRSA isolates

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ABSTRACT

Methicillin-Resistant *Staphylococcus aureus* (MRSA) infections have become a global health problem particularly in developing countries. MRSA strains are characterized by rapid resistance against different groups of antibiotics.The current study was aimed to identify MRSA isolates phenotypically by disk diffusion method and genoytypically by PCR.

A total of 200 clinical specimens were collected from Zagazig University Hospitals and El- Ahrar Educational Hospital in Zagazig, Egypt, and identified biochemically. Out of these specimens, 117 isolates were identified as MRSA by disk diffusion method (DDM); only 114 of these isolates were identified as MRSA by PCR amplification of *mecA* gene. The study revealed that PCR was more accurate and rapid than other conventional methods and it is considered the gold standard method for MRSA detection to avoid false positive identification of MRSA.

Keywords: *Staphylococcus aureus*, MRSA, disk diffusion method (DDM), PCR, *mecA*.

INTRODUCTION

Methicillin resistant *Staphylococcus aureus* (MRSA) strains have emerged in the 1961 as a major clinical and epidemiological problem in hospitals (Nelson and Gallagher, 2012). In Egypt, the prevalence of nosocomial infections caused by MRSA varies between 55% - 85% (Abd El-Moez *et al*., 2011; Ali, 2015).

MRSA are a serious threat to hospitalised patients who rely on β-lactam antibiotics for decolonisation, prophylaxis and treatment. MRSA shows resistance to all β-lactam antibiotics, which is mediated by the *mecA* gene (Lindsay, 2011).

Nowadays, multidrug resistance (MDR) MRSA isolates become prominent in Egypt due to misuse of antibiotics (Daef *et al*., 2012). Detection of MRSA is based on phenotypic and genotypic characterization of bacterial isolates. Disk diffusion method is the most reliable among all the phenotypic methods that include microdilution, agar dilution, agar screening method, and latex agglutination methods (Farahani *et al*., 2013). Genotypic methods comprises polymerase chain reaction (PCR)-based detection techniques (Anand *et al*., 2009). Methicillin resistance in staphylococci is caused by penicillin-binding protein (PBP2), a protein encoded by *mecA* gene. The *mecA* gene is located on a mobile genetic element, designated as staphylococcal cassette chromosome mec (SCCmec), which contains *mecA* gene and its regulators (Katayama *et al*., 2000).There are many factor effects on both phenotypic and genotypic methods. Factors influence the phenotypic expression of resistance include addition of sodium chloride or sucrose to culture medium, incubation at 30 ^{o}C or culture in the presence of β-lactam antibiotics enhances the expression of resistance (Hartman and Tomasz, 1986). Molecular methods are highly sensitive and gave rapid results which allows rapid infection control practices but it is still more expensive (Baddour *et al*., 1997; Martineau *et al*., 2000). Molecular methods may not be the most appropriate choice for some institutions in the developing countries. Likewise, culture requires a longer turn

around time but can achieve a comparable sensitivity with lower costs (Marlowe and Bankowski, 2011).

For that, seeking for a simple, rapid, more accurate and sensitive method for the detection of MRSA in routine diagnostic laboratories become in need. The aim of the present study was the surveillance of MRSA in Zagazig hospitals with evaluation of both phenotypic and genotypic methods in detection of these isolates.

MATERIALS and METHODS

Specimen collection and bacterial identification

A total of 200 clinical specimens were collected from Zagazig University Hospitals and burn unit of El-Ahrar Educational Hospital in Zagazig, Egypt. Specimens were collected in sterile containers or by sterile swabs depending on type of specimens according to Blair *et al.* (1970). The clinical specimens include: skin (wound infection), blood, sputum, otitis medium, urine and peritoneal fluid. Wound swabbing was performed according to Catherine *et al*. (2002). Blood, Sputum and Urine samples were performed according to Koneman et al. (1997). Middle-ear fluid was obtained by needle tympanocentesis according to Heikkinen *et al. (*1999). The collected specimens were inoculated on nutrient agar as non-selective medium, mannitol salt agar as selective medium and blood agar as enriched medium. Isolates that were suspected to be *S. aureus* were further identified through catalase and coagulase tests. (Koneman *et al*., 1997).

Identification of MRSA isolates by disk diffusion method

The *S. aureus* isolates were screened for antimicrobial susceptibility to Methicillin (ME, 5µg, Oxoid, Hampshire, England) by disk diffusion method (CLSI, 2013). The zones of inhibition of each isolate were tested on Mueller-Hinton agar plates (Oxoid, Hampshire, England), and were interpreted according to the criteria of CLSI (2013).

Identification of MRSA isolates by PCR

PCR was used for detecting *mecA* gene of MRSA isolates. Conserved primer sets, F′ 5′- GTA GAA ATG ACT GAA CGT CCG ATA A-′3 and R′ 5′- CCA ATT CCA CAT TGT TCG GTC TAA-′3 were used to amplify 310 bp of *mecA* (Sun *et al*., 2015). Extracted DNA was subjected to PCR using EconoTaq plus Green 2X Master Mix (Lucigen, USA). Amplification reactions with 25µl reaction mixture (2X Master mix 12.5µl, forward primer 1.5µl, reverse primer1.5µl, Nuclease free water 7.5µl and 2µl of gDNA) were performed in Biometra thermocycler (USA). The cycling conditions were 95°C for 15 min as initial denaturation step; then 30 cycles consisting of denaturation at 94°C for 30 sec followed by annealing at 52ºC for 30 sec, and elongation at 72°C for 1 min, followed by final extension cycle at 72°C for 5 min. The PCR products were analyzed by 1.2% agarose gel electrophoresis in Tris- acetate-EDTA (TAE) buffer followed by staining with ethidium bromide. A single 310 bp band in comparison with HI-LO DNA marker (Bionexus Inc, USA) confirmed the presence of target gene**.**

RESULTS

Identification of *S. aureus* isolates by biochemical tests

S. aureus colonies which appear as golden yellow colonies on nutrient agar medium, ferment mannitol and, β-haemolytic on blood agar and appeared by Gram staining as Gram positive cocci arranged in bunches were further identified biochemically through catalase and coagulase tests and were being catalase

positive and coagulase positive (Koneman *et al*., 1997).

Distribution of *S. aureus* isolates

Out of 200 specimens, 117 were identified as *S. aureus* using colony morphology and biochemical tests. Major numbers of *S. aureus* isolates were obtained from burn infections 52, blood 28 and sputum 24. Other samples include 5 from peritoneal fluid, 4 from urine and 4 from ear discharge. The prevelance of *S. aureus* in the collected specimens is indicated in Table 1.

Table 1: Prevalence of *S. aureus* isolates obtained from different sources

Specimen type	No. of specimens	No. (%) of *S. aureus* isolates
Burn	55	52 (94.5 %)
Blood	30	28 (93.3 %)
Sputum	30	24 (80 %)
P. fluid	25	5 (20 %)
Urine	30	4 (13.3 %)
Ear discharge	30	4 (13.3 %)
Total	200	117 (58.5 %)

MRSA identification

The sensitivity of the 117 *S. aureus* isolates to methicillin (ME) by disk diffusion method revealed that all isolates were resistant to ME (100%).

PCR was used for amplification of *mecA* gene for identification of MRSA. Only 114 of the phenotepically identified MRSA gave a single band at 310 bp matched to the *mecA* gene size (Figure 1).

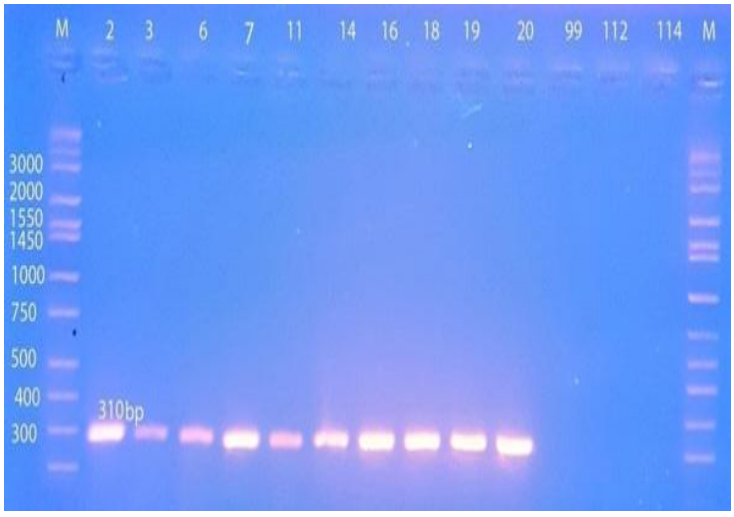

Figure (1): Electrophoretic graph of PCR amplification of *mecA* gene from some representative MRSA isolates. M; Marker; isolates no 2, 3, 6, 7, 11, 14, 16, 18, 19, 22 were positive while, 99, 112, 114 were negative; approximate size of *mecA* gene were 310 bp.

DISCUSSION

Staphylococcus aureus is a nosocomial and community acquired pathogen. Among *S.aureus*, MRSA infection contributes to increase in patient morbidity and mortality (Wolk *et al*., 2009). MRSA isolates are often resistant to other classes of antibiotics (through different mechanisms) making treatment options limited (Stapleton and Taylor, 2002). Screening with accurate and rapid detection of Methicillin resistance in *Staphylococci* is very important for choosing the appropriate antibiotic therapy for patient (Pramodhini *et al*., 2011).The present study was carried out to investigate and compare the detection of Methicillin resistance phenotypically by disk diffusion method and genotypically by PCR in local *S. aureus* isolates.

Our study revealed that, out of 200 specimens, 117 were *S. aureus* (58.5 %). All *S. aureus* isolates were identified as MRSA phenotypically. The major sources were detected in burn, blood and sputum, in agreement with Ahmed *et al*. (2014), Michael *et al*. (2012) and Kulkarni *et al*. (2014), respectively. On the other hand, minor sources were detected in peritoneal fluid, urine and ear discharge in agreement with Rao and Prabhakar, (2011). Previous prevalence was exceeded in studies performed in Benha University Hospital, Ain Shams and Alexandria Universities with 76%, 64% and 60%, respectively (Soliman *et al*., 1997; Ali *et al.*, 2014 ; Alseqely *et al*., 2014)., Whereas, previous prevalence in Mansoura University Hospitals and India was lower 45.6% and 35 %, respectively (El-Daker *et al*., 2008 ; Datta *et al*., 2011).

The development of MRSA is caused by alteration of penicillin-binding protein (PBP2), a protein encoded by *mecA* gene and are involved in the synthesis of peptidoglycan (Stapleton and Taylor, 2002).

Molecular methods that can be used for identification of MRSA in clinical isolates depend on *mecA* gene detection (Salisbury *et al.*, 1997). Detection of *mecA* gene by conventional PCR confirmed 114 (97.4%) MRSA isolates out of 117 MRSA isolates detected by disk diffusion method. One investigation revealed that among 55 *S. aureus* isolates, 20 (36.4%) were positive for the *mecA* gene by PCR while methicillin resistance was detected by oxacillin disc diffusion was 18 (32.7%) (Pramodhini *et al*., 2011). Pournajaf *et al.* (2014) determined the prevalence of methicillin-resistant *S. aureus* by oxacillin disk diffusion method and they found 47.6% (133/ 279) were MRSA; whereas, 45.1% (126/279) of *S. aureus* isolates were mecA- positive in the PCR assay.

The variation of phenotypic and genotypic methods may attribute to many factors. Phenotypic methods could be influenced by temperature, period of incubation, inoculum density, and salt concentration used in culture media; all of which could enhance or suppress growth of MRSA (Kloos and Bannerman, 1999). On the other hand, genotypic methods could cause confusion as *mecA* could be detected in Methicillin sensitive *S. aureus* (MSSA) in case of PBP repression (Mohanasoundaram and Lalitha, 2008). For this reasons, some studies prefer detection of MRSA by both phenotypic and genotypic methods for precise differentiation of MRSA (Pillai et al., 2012). Up to date selection of phenotypic and/or genotypic methods is challenge for any microbiological laboratory due to different advantage and disadvantage for each method. The high sensitivity and

rapid results of molecular methods helps in freeing up isolation beds in isolation rooms with high rate of patients. Culture methods can consume a longer time but its results are acceptable in low financial support in developing countries comparable to that of PCR (Marlowe and Bankowski, 2011).

CONCLUSION

The increasing percentage of detected MRSA in hospitals motives the need for regular active surveillance program against MRSA for high-risk patients (immune deficiency, old age, babies nurseries and intensive care units). This study support the confirmation of phenotypic positive MRSA isolates with PCR especially in non-urgent cases in clinic and surveillance studies for accurate results.

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التعرف على ميكروب الكرييات العنقودية الذهبية المقاومة لمضادالميثيسيلين بالطرق المظهرية والوراثية

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أصبحت العدوى بميكروب الكرييات العنقودية الذهبية المقاومة لمضادالميثيسيلين مشكلة تواجه الصحة العالمية في البلدان النامية. هذة السلالات تتميز بالمقاومة السريعة ضد المجموعات المختلفة من المضادات الحيوية. الدراسة الحالية تهدف إلى التعرف على الميكروب ظاهريا بطريقة اختبار الحساسية ووراثيا بواسطة تفاعل البلمرة المتسلسل.

تم تجميع ٢٠٠ عينة سريرية من مستشفيات جامعة الزقازيق ومستشفى الأحرار التعليمى بالزقازيق، مصر، وتم التعرف عليها من خلال الاختبارات البيو كيميائية وتاكيدها بانها بكتيريا الكرييات العنقودية الذهبية . تم عزل ١١٧ عينة تم التعرف عليها بواسطة طريقة اختبار الحساسية بينما تم تحديد ١١٤عينة وراثيا من هذه العزلات فقط بواسطة تفاعل البلمرة المتسلسل. هذة الدراسة اثبتت ان الطرق الوراثية دقيقة وسريعة عن الطرق التقليدية الأخرى، و تعتبر الطريقة القياسية للكشف عن الميكروب لتجنب النتائج الغير دقيقة عند التعرف على الميكروب.