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International Journal of Current Research
Vol. 5, Issue, 12, pp.4256-4258, December, 2013

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

DETECTION OF (*mecA*)GENE IN METHICILLIN RESISTANT *Staphylococcus aureus* (MRSA) FROM OUTPATIENTS CLINIC IN BAGHDAD HOSPITAL, IRAQ

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ARTICLE INFO

Article History:
Received 14th September, 2013
Received in revised form
26th September, 2013
Accepted 17th October, 2013
Published online 25th December, 2013

Key words:
S.aureus (*mecA*),
Methicillin-resistant *S.aureus.*

ABSTRACT

Total (230) isolates of Staphylococci species were isolated from different clinical samples. They were distributed isolates from urine, wound infections and ear swabs**.** These isolates were diagnosed using different morphological and biochemical test. Out of 100 isolates were *Staphylococcus aureus*. Disk diffusion method antibiotic susceptibility were used, 13 methicillin resistant *S. aureus* isolates was identified, It was found that 13(100%) isolates have positive result for *mec*A gene expressed *S.aureus* (*mecA*)in their PCR products. It was concluded that the polymerase chain reaction appears to offer a time saving and accurate method of detection of methicillin-resistant *S.aureus*. and it can be used to detect a lowlevel of it from different samples without using a selective medium or additional biochemical tests.

INTRODUCTION

*Staphylococcus aureus*is one of the most frequent bacterial pathogens in humans. It causes skin infections, osteoarthritis and respiratory tract infections in the community, as well as postoperative and catheter-related infections in hospitals (Didier *et al*., 2004) Methicillin-resistant *S.aureus* (MRSA) has become one of the most commonly identified antibiotic-resistant pathogens in many parts of the world, including Europe, the Americas, North Africa, the Middle East, and East Asia. In recent decades, MRSA rates have been increasing worldwide, including in the Nordic countries and the Netherlands, where MRSA rates have been low and stable for many years (Fridkin *et al*., 2002). It is a significant pathogen causing both nosocomial, community acquired infections, and its high prevalence in hospitals has been reported from many Countries (Kaya *et al*., 2009) Since most of these bacteria carry multiple resistance genes against commonly used antibiotics, they show multiple antibiotic resistance patterns thus causing important treatment problems (Karami *et al*., 2011). Resistance occurs following the chromosomal acquisition of novel DNA, resulting in the production of a new penicillin-binding protein, termed PBP2a, with a low binding affinity for methicillin (Woodford *et al*., 2005). PBP2a substitutes for all other penicillin-binding proteins, and because of its low affinity for all b-lactam antibiotics it confers resistance to all b-lactam agents, including cephalosporins (Lowy, 2003). The low-affinity to (PBP2a) is encoded by *mecA* gene which represent the main factor responsible for methicillin resistance in *Staphylococcus.*(Chambers, 1997). The *mecA* gene is found on a large mobile genetic element called the staphylococcal chromosomal cassette mec (*SCCmec*) (Weese *et al*., 2005). The mobile *mecA*gene complex is comprised of *mecA* together with its regulator genes, *mecI* and *mecR*, and resides within a genomic island, the staphylococcal cassette chromosome *mec* (SCC*mec*) that constitutes 1%–2% of the ~2.9 million–bp*S. Aureus*chromosome (Lozano *et al*., 2010). The *mecA* gene have never been found in methicillin-susceptible *Staphylococcus aureus* (MSSA), while they have been detected in almost all MRSA isolates (Enright *et al*., 2002). The aims of this research are to isolate *S.aureus* from different infections and detect MRSA using primary screening test followed by genotypic detection for *mecA* gene using PCR technique

METHODS

Specimen's collections and Diagnosis

Total of (230) clinical isolates primary identify as *Staphylococcus* species were obtained from hospitalized and non hospitalized patients from two hospitals: Al-Yarmuk General Teaching Hospital, Al-Kindi General Teaching Hospital in Baghdad city during the period from December, 2012 to April, 2013.clinical isolates were as follows: ear, burn ,skin infections, wound, sputum .These bacterial isolates were identified as *S. aureus* based on their morphology, Gram-staining, positive catalase. Positive Coagulase test and fermentation Mannitol. MRSA isolates were obtained from clinical specimensby using the disk diffusion method.

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Antibiotic susceptibility profile

The antibiotic susceptibility profile for these isolated was tested against Methicilline. Table (1) The disc diffusion (DD) assay was performed using Kirby-Bauer method according to the (CLSI) guideline. All the *S. aureus* isolates that were previously identified by morphological and biochemical tests, plated by streaking on Muller-Hinton agar medium (with a turbidity equivalent to 0.5 McFarland tube; containing approximately 1 to 2 × 10^8cfu/ml). The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye (CLSI) .This gave a profile of drug susceptibility vis-à-vis antibiotic resistance (Chakraborty *et al.*,2011).

Table 1. Antibiotic disks used in the study

Antibiotic	Conc.(mg)	Resistan	Susceptible
Methicillin	5	≥ 15	≤ 14

Isolation of the bacterial chromosome

Genomic DNA Mini Kit was used according to manufacturer's instructions to isolate the DNA from *S. aureus*clinical isolates.

Amplification of *S. aureusmecA* gene

PCR was used to amplify the *S. aureus mecA* gene with the amplicon size of 170 bp using primers described by (Martineau *et al*., 2011).These primers were synthesized by CinnaGen, Iran. Using temperature gradient program starting from 50.7˚ C to 60˚C with 0.5˚C increment each time; annealing temp.was recorded as 58˚C. The oligonucleotide primers specific for the *mec*A genes were diluted using nuclease free water according to the manufacture company information to get primary concentration equal to 100 pmol. thermal cycler and the reaction mixture was prepared according to the procedure that suggested by the manufacture company (Promega, USA). PCR mixture was composed from 12.5 µl of GoTaq®Green Master Mix(1x), 1µl (10 pmol) from each forward and reverse primers, 5 µl of DNA template and 5.5 µl of nuclease free water to get final volume of 25 µl. PCR mixture without DNA template was used as a negative control. A total of 35 cycles were used to amplify 200bp of *mecA*gene . DNA denaturation occur at 95°C for 30 sec primers annealing at 58°C for 1 min extension of the two strands at 72°C for 1 min and a final extension step is 72°C for 5 min. The amplified PCR product were analyzed by agarose gel electrophoresis according to Enright *et al.* (2002) using 1% agarose supplied with 0.5 µg/mL ethidium bromide for 1 hour and a half (7 Volts/ cm²). DNA ladder (100bp) were used to assess PCR product size, then PCR products were visualized by UV light at 336 nm, and photographs were taken using digital camera.

RESULTS AND DISCUSSION

Depending on the conventional cultural procedures, results showed that; 154 samples gave positive culture and many of them showed beta haemolytic activity on blood agar plates. Primary test for species identification was done by Gram staining and standard biochemical tests and results revealed that 61 isolates were detected as *S. aureus*as they appear Gram positive. The clinical isolates ferment mannitol and produced yellow color due to the acid production; they were catalase positive due to the production of catalase enzyme which distinguishes them from *Streptococcus* spp. Isolates were positive for coagulase which distinguished them from other *Staphylococcus* spp. The *mecA* PCR amplification results of thirteen of the *S.aureus* isolates are shown in Figure 1. An amplicon of 200 bp was considered indicative for the presence of *mecA* gene (Murakami *et al*., 1991). The relationships between the results of the PCR assay and the disk diffusion test results of methicillin are summarized. Consequently, the sensitivity and specificity of methicillin disk diffusion test as compared tomecA gene PCR are therefore 100%. This result is close to the result of (Rallapalli *et al*., 2008) who showed that all the 40 isolates determined as MRSA by phenotypic methods, gavepositive results using PCR. Also Kader *et al.* (Kader *et al*., 2011) reported that all the34 strains were harboring *mecA*gene.

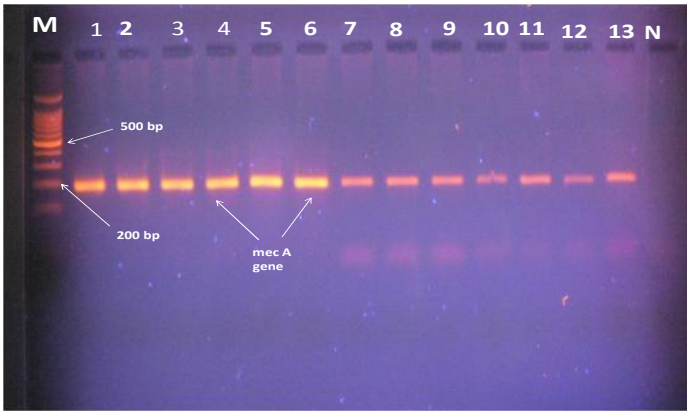

Fig. 1. Agarose gel electrophoresis of PCR amplification products of *S.aureus,mecA*gene (1.5% agarose, 70V, 45min.). M: The DNA molecular wight marker (100pb ladder BIONEER). lanes(1-13) positive amplification of 170 bp for mecA gene.N is a PCR product of negative control

The PCR product appeared as a single DNA band with a (200) bp. in size that was close to that obtained by Martineau *et al*. (2000); Motlagh and Anvari (2010). The results of the present study indicated that the accuracy of methicillin disk diffusion test for detection of MRSA approaches the accuracy of PCR assay and is more accurate than any susceptibility testing method used alone for the detection of MRSA. The presence of the *mecA* gene correlated 100% with the methicillinphenotypic resistance in all MRSA isolates (Martineau *et al*., 2001). In addition, for greater detection rates, molecular methods have the shortest turn around time. Although, molecular testing remains expensive relative to conventional agar based detection, there is an overall cost savings, especially if molecular testing is directed at high-risk populations.

REFERENCES

Chakraborty, S.P., Mahapatra, S.K., Bal, M. and Roy, S. 2011. Isolation and identification of vancomycin resistant *Staphylococcus aureus*from post-operative pus sample. Al Ameen*J. Med. Scie*., 4 (2):152 -168.

Chambers H, F. 1997. Methicillin Resistance in Staphylococci: Molecular and Biochemical Basis and Clinical Implications. Review. *Clin Micro*. 4(10): 781–791.

Clinical Laboratory Standard Institute. 2011. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement, 30(1):1-172

Didier, G; Stephane, B and John, S. 2004. Amoxicillin-Clavulanate Therapy Increases Childhood Nasal Colonization by Methicillin-Susceptible *Staphylo coccusaureus* Strains Producing High Levels of Penicillinase.Antimicrob.*AgentsChemother*., 48:4618-4623.

Enright, M.C.; Robinson, D.C. Randle, G.; Feil, E.J. Grundmann, H. and Spratt, G.E. 2002. The evolutionary history of methicillin-resistant Staphylococcus *aureus (MRSA)*.PNAS. 99(11):7687-7692.

Fridkin, S.K. Hill, H.A. Volkova, N.V. Edwards, J.R. Lawton, R.M. Gaynes, R.P. and McGowan, J.E. 2002. Temporal changes in prevalence of antimicrobial resistance in 23 US hospitals. *Emerg Infect Dis*, 8, 697-701.

Kader, O., Ebid, S., Mostafa, N., El Sayed, S.H and Ghazal, A. (2011). Detection of community acquired methicillin resistance *Staphylococcus aureus* among *staphylococcus aureus*isolates. *J. Americ. Scie.*,7(1).

Karami, S. Rahbar, M. and Yousefi, J.V. 2011. Evaluation of Five Phenotypic Methods for Detection of Methicillin Resistant *Staphylococcus aureus* (MRSA). *Iran. J. Path*. 6 (1):27- 31.

Kaya,E. G. Karakoç, E. Yagci, S. and Yücel, M. 2009. Evaluation of phenotypic and genotypic methods for detection of methicillin resistance in *Staphylococcus aureus. J. Afri. Microbiol. Res*. 3(12): 925-929.

Lowy, F.D. 2003. Antimicrobial resistance: the example of Staphylococcus aureus. *J Clin Invest*; 111: 1265–1273.

Lozano, C. Aspiroz, C. Lasarte, J.J. Gómez-Sanz, E. Zarazaga, M. Torres, C. (2010). Dynamic of nasal colonization by methicillin-resistant *Staphylococcus aureus*ST398 and ST1 after mupirocin treatment in a family in close contact with pigs. *Comp Immunol Microbiol Infect Dis*. Jul 19.

Martineau, F. Picard, F.J and Lansac, N. 2001. Correlation between the mecAgene determined by multiplex PCR assays and the disc diffusion susceptibility patterns of *Staphylococcus aureus*. Chemother., 44: 231-238.

Martineau, F., picard, F. J, Lansac, N., Me'nard, C, Roy, P. H and Ouellette-M.G. 2000. Correlationbetween the resistance genotype determind by multiplex PCR assays and the antibiotic susceptibility patterns of *Staphylococcus aureus* and *staphylococcus epidermidis*.*Antimicrobial Agents and Chemotherapy*. 44(2):231-238.

Motlagh, M.S. and Anvari, M. 2010. Rapid detection of methicillin-resistant staphylococci by multiplex PCR. *Afr. J. Biotech.* 9(45):7629-7631.

Rallapalli, S., Verghese, S and Verma, R.S. 2008. Validation of multiplex pcr strategy for simultaneous detection and identification of methicillin resistant *staphylococcus aureus. India. J. Med.Microbio 26(4): 361-4*.

Weese, JS., Archambault, M., Willey, B.M., Hearn, P., Kreiswirth, B.N, Said-Salim, B., McGeer, A, Likhoshvay., Y., Prescott, J.F, Low, D.E. 2005. Methicillin-resistant *Staphylococcus aureus* in horses and horse personnel, 2000-2002.Emerg*Infect Dis*.;11(3):430-5.

Woodford, N. 2005. Biological counterstrike: antibiotic resistance mechanisms of Gram-positive cocci. *Clin Microbiol Infect*; 11(suppl 3): 2–21.

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