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RESEARCH ARTICLE

Partial Nucleotide Sequencing of the *mecA* Gene of Vancomycin-resistant *Staphylococcus* spp. Isolated in Baghdad, Iraq

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Abstract

Staphylococcus aureus is notorious for its ability to become resistant to antibiotics . In present study , a total of 159 clinical and environmental *Staphylococcus* spp. isolates have been isolated from different samples . Among these isolates , 64.1% of clinical and 53.4% of environmental isolates were identified as methicillin resistant *Staphylococcus spp.* by disc diffusion method . Vancomycin resistance phenotype were determined , and results showed that 21 clinical isolates (24.41%) and 26 environmental isolates (35.61%) were resistance to vancomycin. The MIC values for vancomycin in the clinical and environmental isolates ranged from 2 to 512 µg/ml .

The results of Polymerase Chain Reaction (PCR) showed that *mecA* found in 63.15% of clinical isolates and 76.92% of environmental isolates , one *mecA* isolates had the *van A* gene and 3 isolates had the *vanB* gene . Most vancomycin resistant *S. aureus* (VRSA) isolates were MRSA. PCR amplification of *mecA* gene and sequencing of the amplified product. The amplified product of *mecA* gene shows consistency reaching up to 99 % (in Environmental isolate) and 100% (in clinical isolate) as compared Nitrogen bases sequence of the *mecA* gene present in the *Staphylococcus fleurettii* strain in NCBI.

Introduction

Staphylococcus aureus is a human pathogen that can cause a range of illnesses, from minor skin infections to life-threatening diseases, such as pneumonia, meningitis, endocarditis, toxic shock syndrome (TSS), bacteremia, and sepsis. It is one of the most common causes of nosocomial infections and is often the cause of postsurgical wound infections (Kardas-Sloma *et al*., 2011 ; Okesola , 2011).

Staphylococcus aureus strains resistant to methicillin and many other antibiotics are major causes of nosocomial infections worldwide (Wielders *et al* ., 2002) Methicillin (meticillin)-resistant *Staphylococcus aureus* (MRSA) represent a major problem in human medicine by causing both healthcare-associated and community-associated infections (Grundmann *et al* ., 2006) Resistance to methicillin is determined by the *mecA* gene, which encodes the low-affinity penicillin-binding protein PBP 2A (Wielders *et al* . ,2002). MRSA is generated when methicillin-susceptible *S. aureus* (MSSA) exogenously acquires a staphylococcal cassette chromosome *mec* (SCC*mec*) (Ito *et al* .,1999). Eight major SCC*mec* types (I to VIII) have been described in methicillin-resistant Staphylococcus aureus (MRSA), differing in allotypic combinations of the *mec* and *ccr* gene complexes, with SCC*mec* IVa and V being currently the most prevalent types in community-acquired MRSA (CA-MRSA) strains(IWG-SCC,2009).

Vancomycin has been the cornerstone of treatment of patients with serious MRSA infections. Consequently, vancomycin use has been increasing since the mid-1980's, resulting in the emergence of MRSA with reduced susceptibility to vancomycin (Dhand and Sakoulas , 2012). The emergence of vancomycin resistance in *Staphylococcus aureus*, with 7 of 9 cases worldwide from southeast Michigan (Tenover, 2008), is alarming. The isolation of

enterococci containing the *vanA* gene identical to those of vancomycin-resistant (VR) *S. aureus* (VRSA) strains suggests that the *vanA*-mediated resistance was due to the transfer of an Inc18-type plasmid from VRE containing *traA* and *repR* genes to *S. aureus* (Chang *et al*., 2003).

There are limited reports evaluating sequencing of the *mecA* of VRSA in Baghdad . The aims of this study were to determine the sequencing of the *mecA* gene in vancomycin-resistant *Staphylococcus aureus* (VRSA) isolates in Baghdad.

Materials and Methods

Bacterial isolates and identification. A total of 159 *Staphylococcus* spp. clinical and environmental isolates, collected between 28/8/2012 to 1/1/2013 in Baghdad , were studied. These isolates were identified by conventional biochemical reactions and Gram staining, according to the criteria established by (Forbes *et al* ., 2002).

Antimicrobial susceptibility test:

A) Antimicrobial susceptibility of the isolates were tested by the disc diffusion method for Methicillin(10µg) and Vancomycin (30µg) (Bioanalyse, Turkey) according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI,2009) .

B) The MRSA isolates were subjected to antimicrobial susceptibility testing using Kirby-Bauer disk diffusion method following CLSI guidelines , using commercially available 6mm discs (Bioanalyse /Turkey) The susceptibility of the isolates was determined against 8 antibacterial agents by disc diffusion method, They include: Gentamycin (30µg), Cefotaxime (30µg), Erythromycin (15µg), Azithromycin (30µg), Lincomycin (10µg), Ciprofloxacin (5µg),Teicoplanin(30µg) and Clarithromycin(15µg).

Minimal Inhibitory Concentrations : The MICs of Vancomycin were determined by a broth dilution method. We used Mueller-Hinton broth (Oxoid, England) with vancomycin concentrations (2-512) µg/ml according to the guidelines recommended by the CLSI document.

Plasmid Isolation : Plasmid DNA were isolated using plasmid extraction kit (Promega,USA) , and analysed on 0.8% agarose gel.

DNA Preparation and PCR : A PCR reactions with specific primers were performed to identify *mecA* and *van* genotypes *(vanA* and *vanB*) of each MRSA isolate (Table1). DNA template was prepared as described by (Olsvik and Strockbin,1993) (25µl) of PCR amplification mixture contained deionized sterile water,(12.5)µl Green Go *Taq* Master Mix pH(8) (Promega,USA) contained [(50unit/ml) of Go *Taq* DNA polymerase,(400Mm) of each dNTPs and (3mM) of MgCl**2**],(1pmol) for specific primers(Alpha DNA,Canada) .The PCR cycles for *mecA* were as followed : initial denaturation at (95c◦) for (4 min), 30 cycles of denaturation at (94c◦) for (30sec), annealing at (53c◦) for (45sec) and extension at (72c◦) and final extension at (72c◦) for (4min) , and for *van* genes*(vanA* and *vanB*) were as followed :initial denaturation at (95c◦) for (5 min), 30 cycles of denaturation at (94c◦) for (45sec), annealing at (54c◦) for (45sec) and extension at (72c◦) and final extension at (72c◦) for (7min)using Gradient PCR (TechNet – 500 ,USA)**.**

Table 1. primers used for detecting *mecA* and *van* genes among MRSA isolates.

Genes	Sequence	Product Size	Reference
mecA	5 GTAGAAATGACTGAACGTCCGATAA 5 CCAATTCCACATTGTTTCGGTCTAA	310 bp	(Zhang *et al*.,2004)
vanA	5 GGGAAAACGACAATTGC 5 GTACAATGCGGCCGTTA	732 bp	(Dutka-Malen *et al*.,1995)
vanB	5 AAGCTATGCAAGAAGCCATG 5 CCGACAATCAAATCATCCTC	536 bp	(Elsayed *et al*.,2001)

DNA sequence analysis . The DNA fragments for sequencing were obtained by PCR amplification of each chromosomal DNA as the template, the fragments of each PCR products were sequenced with the set of primers by Macrogen , USA). The program (BioEdit Pro.version: 7.0.0) was used for bioinformatic analysis of nucleotide sequences.

Results and Discussion

Staphylococcus aureus is notorious for its ability to become resistant to antibiotics. Infections that are caused by antibiotic-resistant strains often occur in epidemic waves that are initiated by one or a few successful clones. Methicillin-resistant *S. aureus* (MRSA) features prominently in these epidemics. Historically associated with hospitals and other health care settings, MRSA has now emerged as a widespread cause of community infections (Chambers and DeLeo ,2009) .

In present study , a total of 159 clinical and environmental *Staphylococcus* spp. isolates have been isolated from different samples including : 86 clinical isolates from urine, blood and swaps of: (otitis media, burns and wounds) from different hospitals in Baghdad , and 73 environmental isolates from poultry , sewage and food during the period from 28/8/2012 to 1/1/2013 . All isolates were identified through morphological, cultural and biochemical tests .

Among the 159 isolates of *Staphylococcus spp.*, 64.1% of clinical isolates and 53.4% of environmental isolates were identified as methicillin resistant *Staphylococcus spp.* by disc diffusion method . The most effective antibiotic aganist clinical isolates were clarithromycin and gentamicin ,while cefotaxime and clarthromycin were more effective against environmental isolates (Table 2), these results disagreement with results of Al-Maliki (2009) which showed that the rate of MRSA was 80.3% ,but in the study of (Jamaluddin et al.,2008) the resistance was lower the current study.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major nosocomial pathogen in India, and up to 70% methicillin resistance has been reported from hospitals in various parts of India (Arakere et al.,2005).

Vancomycin resistance phenotype were determined , and results showed that 21 clinical isolates (24.41%) and 26 environmental isolates (35.61%) were resistance to vancomycin. The MIC values for vancomycin in the clinical and environmental isolates ranged from 2 to 512 µg/ml .

In 1997, first strain of *Staphylococcus aureus* reduced susceptibility to vancomycin was reported from Japan, after that ,two more cases were reported from united state . In 2002 ,the first clinical isolate of vancomycin –resistant *Staphylococcus aureus* was reported by workers from Brazil and Jordan (Ng *et al*. 2011). In recent years, the widespread use of antibiotics has undoubtedly accelerated the evolution of *S.aureus*, and led to the emergence of strains that have systematically acquired multiple resistance genes (Hope *et al*., 2008).

Table 2. Susceptibility of *Staphylococcus* spp. isolates to 9 Antibiotics

Antibiotic used	Resistant %	
	Clinical isolates	environmental isolates
Methicillin	64.1	53.4
Cefotaxime	60	19.17
Erythromycin	53.73	60.27
Azithromycin	50.74	61.64
Ciprofloxacin	50.74	39.72
Lincomycin	38.88	60
Gentamycin	35.82	42.46
Clarithromycin	33.33	20
Teicoplanin	4.65	10.95

The detection of plasmid DNA in vancomycin resistant isolates by gel electrophoresis showed that some isolates carried plasmid band of □ □ 10kbp. (Fig 1).

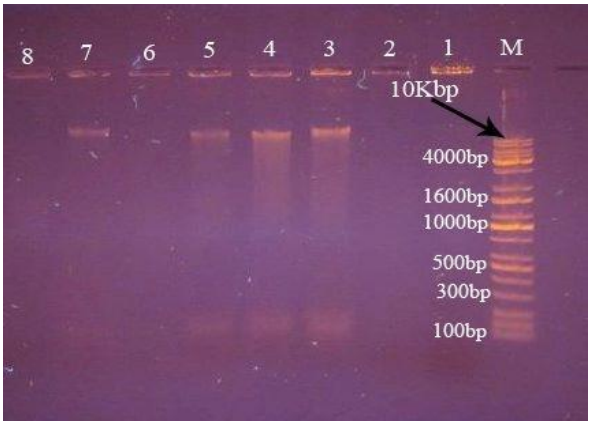

Fig. 1 Plasmid contents of some vancomycin-resistant *Staphylococcus* spp isolates. M: 10kp DNA ladder, Lane 3 plasmid content of environmental isolate, 4,5 and 7 plasmid content of clinical isolates.

Polymerase Chain Reaction (PCR) technique was performed done using specific primers targeting the specific sequences of the *mecA* , *van A* and *van B* genes , the results showed that *mecA* found in 63.15% of clinical isolates and 76.92% of environmental isolates , one *mecA* isolates had the *van A* gene and 3 isolates had the *vanB* gene(Fig. 2) (Fig. 3) . Most vancomycin resistant *S. aureus* (VRSA) isolates were MRSA. Ghazal *et al.*, (2011) reported that all the 34 isolates were harbouring *mecA* gene. Possessing *mecA* gene will reflect an altered PBP2 as mode of resistance to methicillin, oxacillin, and probably to all other currently available β-lactam antibiotics (DeGiusti *et al* ., 1999).

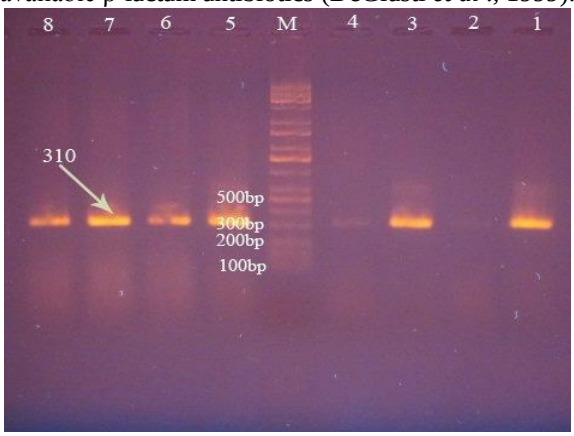

Fig. 2 : PCR amplification of the *mec*A gene in methicillin resistant *Staphylococcus spp.*, Lanes 1, 3 *mec*A positive environment isolates ; Lane 5-8 *mec*A positive clinical isolates, M-10kbp ladder.

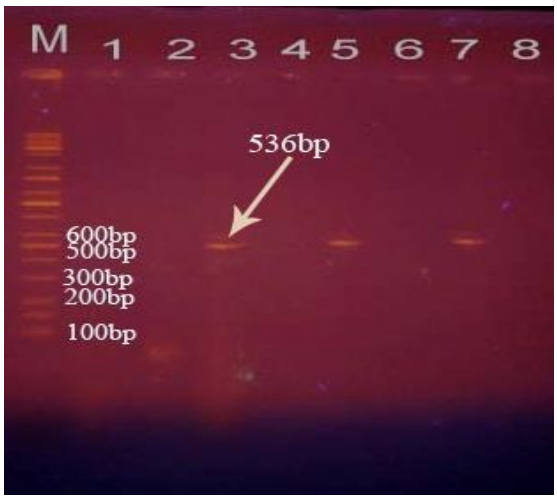

Fig.3 : PCR amplification of the *vanB* gene for vancomycin resistance in methicillin resistant *Staphylococcus spp*. Lanes 3,5 and 7 *vanB* positive .

Results of the Nitrogen bases sequencing for PCR product of one clinical and environmental samples revealed consistency reaching up to 99 % (in Environmental isolate) and 100% (in clinical isolate) as compared Nitrogen bases sequence of the *mecA* gene present in the *Staphylococcus fleurettii* strain in NCBI (Fig.4) . Amplification of specific gene and sequencing of *mec*A gene gives insight into pharmaceutical aspects to design new effective drugs for treatment of methicillin resistance *S. aureus.* The development of antibiotic resistance in developing countries like ours seems to be very much related to the irrational antibiotic usage due to it's easy availability at the drug store without prescription, injudicious use in hospitals and uncontrolled use in agriculture, animal husbandry and fisheries.

Conclusions:

The increase in vancomycin resistance among MRSA and excessive use of antimicrobial agents have worsened the sensitivity. Larger studies need to be done in various geographical regions of the country to better define the epidemiology.

Score **Expect** **Identities** **Gaps** **Strand** **Frame**

507 bits(274) 3e-140() 277/278(99%) 1/278(0%) Plus/Plus

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Sbjct  210  TAAAAGTTTAGGCGTTAAAGATATAAACATTCAGGATCGTAAAATAAAAAAAGTATCTAA  269

Query  64   aaataaaaaacgagtagatgctcaatataaaattaaaacaaaCTACGGTAACATTGATCG  123
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Sbjct  270  AAATAAAAAACGAGTAGATGCTCAATATAAAATTAAAACAAACTACGGTAACATTGATCG  329

Query  124  CAACGTTCAATTTAATTTTGTTAAAGAAGATGGTATGTGGAAGTTAGATTGGGATCATAG  183
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Sbjct  330  CAACGTTCAATTTAATTTTGTTAAAGAAGATGGTATGTGGAAGTTAGATTGGGATCATAG  389

Query  184  CGTCATTATTCCAGGAATGCAGAAAGACCAAAGCATACATATTGAAAATTTAAAATCAGA  243
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Sbjct  390  CGTCATTATTCCAGGAATGCAGAAAGACCAAAGCATACATATTGAAAATTTAAAATCAGA  449

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Sbjct  450  ACGTGGTAAAATTTTAGACCGAAACAATGTGGAATTGG  487
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A) **Environmental isolate**

Score **Expect** **Identities** **Gaps** **Strand** **Frame**

512 bits(277) 6e-142() 277/277(100%) 0/277(0%) Plus/Plus

Features

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Sbjct  451  CGTGGTAAAATTTTAGACCGAAACAATGTGGAATTGG  487
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B)clinical isolate

Fig.4: Nitrogen bases sequencing of the *mecA* gene in A) Environmental isolate and B)clinical isolate of *Staphylococcus* sp

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