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Molecular Detection of Methicillin and Vancomycin Resistance in *Staphylococcus aureus* Isolated From Burn and Wound Infection Patients

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Abstract:

Methicillin-resistant *Staphylococcus aureus* is the major cause of healthcare-associated bacteremia in most of Hospital and it increased risk of infection, morbidity and mortality especially, when associated vancomycin resistance in same infection. In this study 42 *S. aureus* were isolated from burn and wound infection patients in Al-Qadisiyah teaching hospital and *S. aureus* was isolated by selective medium out form 50 swab samples. The PCR assay was used for direct detection of methicillin (*mecA*) and vancomycin (*van*) antibiotics resistance gene in 42 *S. aureus* isolates. The results show 8 isolates (19%) were have *mecA gene* methicillin resistance and 3 isolates (7.1%) were have *van* gene vancomycin resistance in all isolates. In conclusion, PCR assay as highly sensitive and specific in detection of methicillin and vancomycin resistance gene, and Vancomycin-resistant can be associated with Methicillin resistance in *S. aureus* isolated from wound infection.

Key word: *S. aureus*, PCR assay, *mecA* , *van*.

Introduction

Staphylococcus aureus is one of important cause of nosocomial infections, including bacteremia, surgical wound infections, as well as pneumonia [1,2,3]. Methicillin-resistant *Staphylococcus aureus* (MRSA) is usually acquired during exposure to hospitals and other healthcare facilities and causes a variety of serious healthcare-associated infections [4]. MRSA is determined by the availability of weak patients, selective pressure exerted by antimicrobial use, increased potential for transmission from larger numbers of colonized or infected patients ("colonization pressure"), and the impact of implementation and adherence to prevention efforts [5]. Methicillin resistance in staphylococci is determined by *mecA* gene, composed of 50 kb of

DNA chromosome found only in methicillin-resistant strains. Methicillin resistance is defined as the strains of *S. aureus* that are resistant to the isoxazoyl penicillins such as oxacillin and flucloxacillin. MRSA are cross-resistant to all currently licensed β-lactam antibiotics [6]. The MRSA infections are usually treated by vancomycin, linezolid, daptomycin, teicoplanin, quinupristine-dalfopristine and tigecycline. The glycopeptide vancomycin has been regarded as the drug of choice for the treatment of infections due to methicillin-resistant strains [7]. Extensive use of vancomycin creates a selective pressure that favors the outgrowth of rare, vancomycin-resistant clones leading to heterogenous vancomycin intermediate *S. aureus* clones, and eventually, with continued exposure, to a uniform population of vancomycin-intermediate *S. aureus* (VISA) clones [8]. There are different breakpoints used in defining vancomycin susceptibilities which include the Predisposing factors and clinical significance, among the clinical factors, exposure to glycopeptides or vancomycin is the biggest risk factor for vancomycin-resistant *S. aureus* (VRSA) and vancomycin-resistant coagulase negative staphylococci [9]. Peritoneal dialysis and renal failure may also be risk factors [10]. In present study aimed to use polymerase chain reaction (PCR) to direct detection of antibiotics resistance genes for methicillin and vancomycin in *S. aureus* and explain the association between them that isolated from wound and burn infection patients.

Materials and Methods

Sample collection:

Staphylococcal isolates obtained during the period from March 2015 to June 2015 from burn patients in Diwaniyah Teaching Hospital, 50 swab samples were collected from burn infection patients in Burns Division. The samples directly transferred into microbiology Laboratory and store in refrigerator until bacterial isolation.

Identification of an Organism

Samples were cultured (BHI broth/blood agar) and after 24-h incubation the plates were examined for colony characteristics. Isolates were identified by colony characteristics, Gram stain, catalase test and oxidase. Bacitracin (0.04 U) and Novobiocin (5μg)

susceptibilities were determined to exclude *Micrococcus*, *Planococcus*, and *Stomatococcus spp*. Coagulase test and mannitol fermentation were done to exclude *Staphylococcus aureus* and other coagulase-positive species. These tests were performed on all samples of *staphylococcus* as per standard procedures. The isolates were identified to species level by using Vitek-2 system (bioMerieux) according to the manufacturer's instructions.

Genomic DNA Extraction:

Bacterial genomic DNA was extracted from *Staphylococcus aureus* isolates by using (PrestoTM Mini gDNA Bacteria Kit. Geneaid. USA). One ml of overnight bacterial growth on BHI broth was placed in 1.5ml microcentrifuge tubes and then transferred in centrifuge at 10000 rpm for 1 minute. After that, the supernatant was discarded and the bacterial cells pellets were used in genomic DNA extraction and the extraction was done according to company instruction. After that, the extracted gDNA was checked by Nanodrop spectrophotometer, then store in -20C at refrigerator until perform PCR assay.

Amplification by Polymerase Chain Reaction (PCR):

PCR assay was performed by using specific primer for detection methicillin (*mec A*) and vancomycin (*van*) antibiotics resistance gene. These primes were designed in this study by using NCBI-GenBank recorded sequence for *mecA* gene (Genbank: KC243783.1) and *van* gene (Genbank: GQ273978.1) and by using primer3 plus design online. These primers were provided by (Bioneer company . Korea) as show in the following table:

Table 1: Sequence and size of forward and reverse primers

Primer		Sequence	Product size
vanA	F	AGCTGTACTCTCGCCGGATA	284bp
	R	CCACCGGCCTATCATCTTTA	
mecA	F	GGCCAATACAGGAACAGCAT	421bp
	R	AACGATTGTGACACGATAGCC	

Then PCR master mix was prepared by using (AccuPower® PCR PreMix kit. Bioneer. Korea). The PCR premix tube contains freeze-dried pellet of (Taq DNA polymerase 1U, dNTPs 250µM, Tris-HCl (pH 9.0) 10mM, KCl 30mM, MgCl2 1.5mM, stabilizer, and tracking dye) and the PCR master mix reaction was prepared according to kit instructions in 20µl total volume by added 5µl of purified genomic DNA and 1.5µl of 10pmole of forward primer and 1.5µl of 10pmole of reverse primer, then complete the PCR premix tube by deionizer PCR water into 20µl and briefly mixed by Exispin vortex centrifuge (Bioneer. Korea). The reaction was performed in a thermocycler (Mygene Bioneer. Korea) by set up the following thermocycler conditions; initial denaturation temperature of 95 °C for 5 min; followed by 30 cycles at denaturation 95 °C for 30 sec, annealing 58 °C for 30 sec, and extension 72 °C for 1min and then final extension at 72 °C for 10 min. The PCR products were examined by electrophoresis in a 1% agarose gel, stained with ethidium bromide, and visualized under UV transilluminator.

Results and Discussion

Out of 50 wound infection samples only 42 samples were given positive as *S. aureus* isolates in bacterial isolation. The direct PCR was done on *S. aureus* isolates without performed antibiotics resistances test, and the result show as following table:

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Table 2: Number and Percentage resistance isolation for Methicillin and Vancomycin.

Type of resistance	No resistance isolate	Percentage
mecA	8/42	19 %
van	3/42	7.1 %

In this study 19% of *S. aureus* isolates were resistant to methicillin. This result is more than 13.1% reported from Abuja Nigeria [11]. And less than 47.8% reported from Southwestern Nigeria [12]. The low occurrence of MRSA in this study may be due to low level of mistreatment of antibiotics in this locality by both health practitioners and in the community since emergence of resistant strains has been largely due to antibiotic mistreatment. Moreover MRSA strains were largely susceptible to other antibiotics and none was resistant to vancomycin [13].

PCR was amplified methicillin resistance gene (*mecA*) at 421bp PCR product whereas, vancomycin resistance gene (*van)* at 284bp PCR product. These PCR products bands were show in agarose gel electrophoresis at under UV transilluminator. Figure (1) and (2).

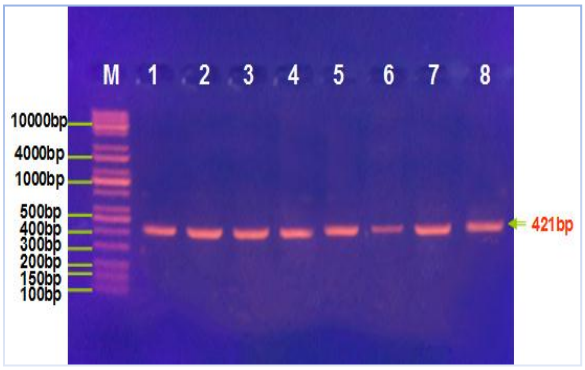

Figure (1): Ethidium bromide-stained agarose gel of PCR products results from amplification of extracted DNA with the primer of *mecA* . Lane M= marker(Ladder 10000bp), Lane 1-8=positive methicillin gene (1% agarose, 100 volt ; 1 hour).

In this study we used the PCR technique for detection of *S. aureus* antibiotics resistances rather than other methods, such Disk-diffusion test on agar or chromogenic agar-based culture method; because the PCR assay is molecular method based gene detection and was appeared highly sensitive and specific in detection of methicillin and vancomycin resistance gene. This technique also used by [14].

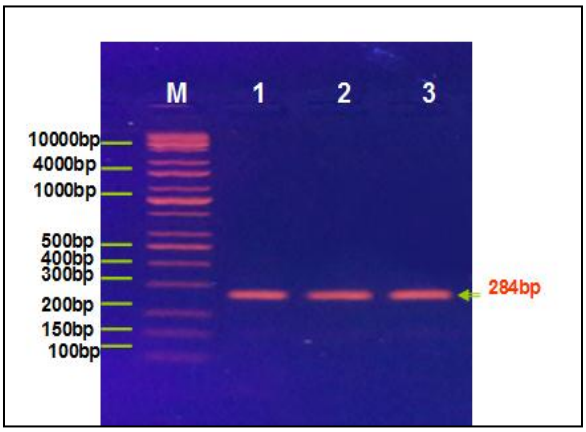

Figure (2): Figure (1): Ethidium bromide-stained agarose gel of PCR products results from amplification of extracted DNA with the primer of *van* . Lane M= marker(Ladder 10000bp), Lane 1-3=positive vancomycin gene (1% agarose, 100 volt ; 1 hour).

Who performed a prospective study of vancomycin resistance VRE screening tests to compare the performance of PCR to that of a chromogenic agar-based culture method, and explain that PCR could be an alternative or supportive method for effective control of nosocomial VRE infection. The MRSA has caused problems in most hospitals worldwide and increasing numbers have been reported in a number of countries. There have been significant increases in methicillin resistance in clinical strains of *S. aureus* isolates between 1999 and 2002 in European countries [15]. Most of the MRSA isolates were also resistant to other antibiotics. The presence of *mec A* gene complex which specifies the production of an abnormal penicillin binding protein PBP2a that has a decreased affinity for binding β- lactam antibiotics results in resistance to methicillin and also to all β-lactams including penicillins and cephalosporins also contains insertion sites for plasmids and transposons that facilitate acquisition of resistance to other antibiotics[16].

Thus, cross-resistance to non-β-lactam antibiotics such as erythromycin, clindamycin, and ciprofloxacin is common [17]. Vancomycin resistance was observed in 3 isolates that associated with Methicillin resistance *Staphylococcus aureus* MRSA. The reported prevalence rate of VRSA in United States range from 0% to 10% among clinical isolates in agreement with the present study [18]. Vancomycin-resistant *S. aureus* (VRSA) infections, which are always methicillin-resistant, are a rare but serious public health concern [19]. In conclusion we conclude that PCR assay can be used as highly sensitive and specific in detection of methicillin and vancomycin resistance gene, and Vancomycin-resistant can be associated with Methicillin resistance in *Staphylococcus aureus* isolated from wound infection.

Recommendations

Using more advance techniques such as real time PCR, DNA sequencing, and restriction enzymes for detection and characterization the gene expression of *mecA* and *van.*

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