Association of methicillin and vancomycin antibiotics resistance in *Staphylococcus aureus* isolated from 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 wound infection patients in Diwaniya 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 only 5 isolates (11%) were have single methicillin resistance and there no single vancomycin resistance in all isolates. While the PCR results show there found 3 isolates (7.1%) were have association resistance methicillin and vancomycin resistance. But all methicillin resistance was 12 isolates (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.

Keywords: Methicillin; Vancomycin; Wound; *Staphylococcus aureus*

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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 methicillin, 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 percent study aimed to used polymerase chain reaction (PCR) to direct detection of methicillin and vancomycin antibiotics resistance gene in *Staphylococcus aureus* and explain the association between them that isolated from wound infection patients.

Materials and Methods

Sample collection: 50 swab samples were collected from wound infection patients in Diwaniya hospital. The samples directly transferred into microbiology Laboratory and store in refrigerator until bacterial isolation.

Bacterial Isolation

Staphylococcus aureus was isolated from wound infection samples by inoculation on Brain Heart Infusion Broth medium at 37°C overnight for primary enrichment culture and then the bacterial growth were inoculated on manetol salt agar (MSA) at 37°C overnight for selective isolation of pure culture *Staphylococcus aureus* isolates.

Bacterial genomic DNA extraction

Bacterial genomic DNA was extracted from *Staphylococcus aureus* isolates by using (PrestoTM Mini gDNA Bacteria Kit. Geneaid. USA). 1ml 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.

Polymerase chain reaction (PCR)

PCR assay was performed by using specific primer for detection methicillin (mec) 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 primer 3 plus design online. These primers were provided by (Bioneer company. Korea) as show in the table below:

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 s, annealing 58 °C for 30 s, 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:

Type of resistance	No. of single resistance isolate	No. of association resistance isolate	Total isolate	Percentage
Methicillin	5	3	8/42	19 %
Vancomycin	0	3	3/42	7.1 %

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 Figure 2.

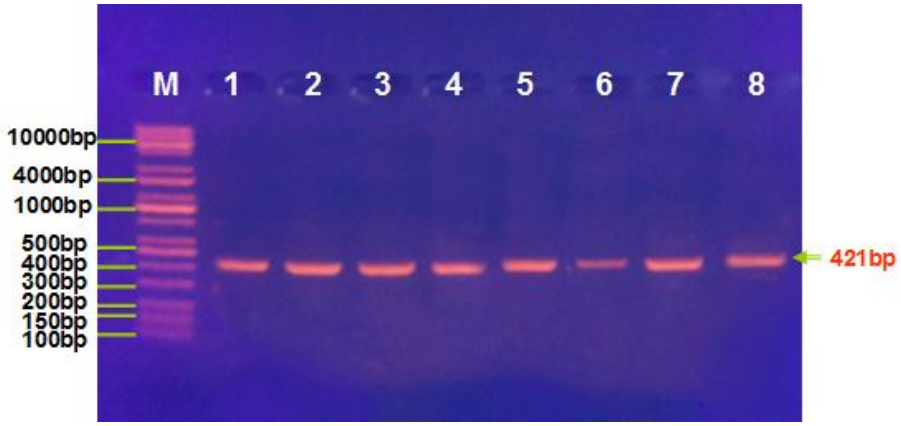

Figure 1.

Agarose gel electrophoresis of methicillin resistance gene (mecA) PCR products, where, Lane (M) DNA marker (100bp), lane (1-7) methicillin resistance isolates, Lane (8-12) association resistance isolates.

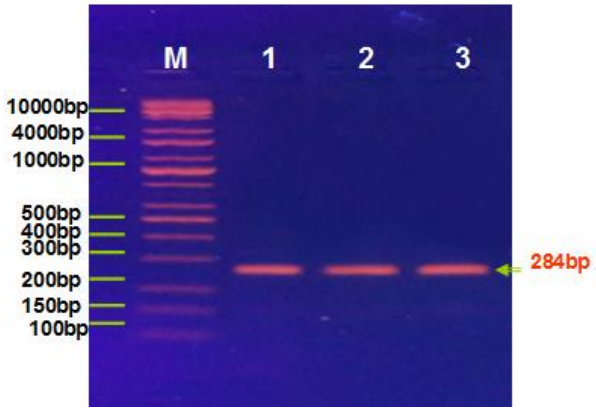

Figure 2.

Agarose gel electrophoresis of vancomycin resistance gene (van) PCR products, where, Lane (M) DNA marker (100bp), lane (1-3) vancomycin resistance isolates, Lane (5-8) association resistance isolates.

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 Seo1 [11] 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. Methicillin resistance *Staphylococcus aureus* 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 [12].

In this study 19% of S. aureus isolates were resistant to methicillin. This result is more than 13.1% reported from Abuja Nigeria [13]. And less than 47.8% reported from Southwestern Nigeria [14]. 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. 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. Thus, cross-resistance to non-β-lactam antibiotics such as erythromycin, clindamycin, and ciprofloxacin is common [15].

Vancomycin resistance was observed in 3 isolates that associated with Methicillin resistance *Staphylococcus aureus* MRSA. The reported prevalence rate of VRSA in united state range from 0% to 10% among clinical isolates in agreement with the present study [16]. Vancomycin-resistant *Staphylococcus aureus* (VRSA) infections, which are always methicillin-resistant, are a rare but serious public health concern [17]. 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.

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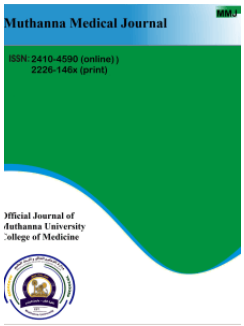

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