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Detection of Van B gene in Staphylococcus spp. Isolated from Food in Baghdad/Iraq

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الكشف عن جين مقاومة الفانكومايسين في المكورات العنقودية المعزولة من الاغذية في بغداد ـ العراق

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Abstract

Seventy three Staphylococcus spp. isolates were recovered from bacterial sample included: poultry , foods , sewage and soil .The antibiotic susceptibility test to all environment isolate to Methicillin and Vancomycin were prepared , and the result showed that 39 Staphylococcus spp. isolates (53.42%) were resistant to Methicillin while 26 isolates (35.61%) were resistant to Vancomycin. The range of the minimum inhibitory concentration of Vancomycin were (2-512 µg\ml). Results of plasmid DNA extraction from Methicillin and Vancomycin resistant isolates showed that some isolates have one plasmid band. The results of the detection of Vancomycin resistance genes (van A and van B) showed the presence of van B gene in isolate No. ESf44 with MIC ≥ 64 µg\m that isolated from raw milk , while didn't notice the presence of van A gene in other isolates were resistant to Vancomycin that have been studied . This study was conducted to investigate the van B gene within the isolated MRSA strains from food, sewage water and soil in Baghdad.

Keywords: Staphylococcus spp. , Vancomycin resistance genes and Bacterial isolates .

المستخلص

حصل على 73 عزلة تعود لجنس *Staphylococcus* spp. من عينات بيئية مختلفة شملت الدواجن والأغذية ومياه المجاري والتربة . اختبرت حساسية جميع العزلات البيئية لمضادي المثيسيلين و الفانكومايسين و أظهرت النتائج أن 39عزلة كانت مقاومة لمضاد المثيسيلين و بنسبة 53.42% في حين كانت 26عزلة مقاومة لمضاد الفانكومايسين وبنسبة 35.61% . حدد التركيز المثبط الادنى (MIC) لمضاد الفانكومايسين للعزلات قيد الدراسة التي اظهرت مقاومة تجاه هذا المضاد في فحص الحساسية بطريقة انتشار الاقراص وقد بينت النتائج قيمة الـ MIC تراوحت ما بين 512-2 مايكرو غرام /مل. أظهرت نتائج ترحيل الدنا البلازميدي المستخلص من العزلات المقاومة لمضادي المثيسيلين والفانكومايسين احتواء بعض العزلات على حزمة بلازميدية واحدة .بينما العزلات الاخرى بينت نتائج التحري عن جينات مقاومة الفانكومايسين (van A و van B) وجود جين van B في العزلة رقم ESf44 المعزولة من الحليب الخام والتي كانت ذات MIC 64 مايكرو غرام /مل ولم يلحظ وجود van A في جميع العزلات المقاومة للفانكومايسين التي درست. هذه الدراسة ركزت على البحث عن جين المقاومة المعزولة من سلالات المقاومة للمثيسيلسن من الاغذية ومياه المجاري والتربة في مدينة بغداد .

كلمات مفتاحية: Staphylococcus spp, جينات مقاومة الفانكومايسين وعزلات بكتيرية .

Introduction

The *Staphylococcus* genus belongs to the family Staphylococcaceae, a common species of Commensal, which is present naturally in the nose and pharynx of the human. It may be pathogenic due to its rapid adaptations to the selective pressures of the host and the length of their survival on the surfaces, even if they are not alive (Al-camo,2001 ; Malachowa and Delo ,2010).

Staphylococcus aureus is one of the causes of food-borne diseases, which are usually associated with unprocessed raw milk produced from cows infected with *Staphylococcal mastitis* (Morgan,2008) and have an important role in the formation of taste and odor in some foods such as cheese and sausages (Pietti and Verschaegen, 2009).

Infectious bacteria may cause serious infections to newborns, children, elderly people, diabetic patients or persons suffer from cancer. These bacteria will cause dangerous injuries such as a deep skin injury or they may be carried out to the blood and other organs causing septicemia, failure for heart valves and other diseases (Benson, 2002).

The first strain of methicillin resistant *Staphylococcus aureus* was isolated in 1961, but it did not become a major problem until late 1970 and early 1980 and is believed to have an animal origin, i.e. it can be transmitted from animals to humans and vice versa (Yamamoto, *et al.*, 2010).

The risk of infection of these bacterial strains is their resistance to multiple antibiotics. Vancomycin is considered to be the best treatment to Methicillin-resistant *Staphylococcus aureus* (MRSA) strains, but due to the selection pressure, many strains of *Staphylococcus aureus* showed resistant to Vancomycin and Teicoplanin specifically in the MRSA strains, which

reduces the possible treatment options and increases the risk of bacterial infection (Hawkey, 2009)

The emergence of strains resistant to Vancomycin Resistant *Staphylococcus aureus* (VRSA) has become one of the major medical problems.

The first recorded appearance of these strains was in Michigan 2002, after which there were infections in many hospitals in the United States of America(Cui, *et al*., 2003).

Several types of Vancomycin resistance have been described which were distinguished from each other, depending on the sequence of the synthetic gene of the Vancomycin-resistant Ligase (van A, van B, van C, van E and van G). These types were investigated by PCR technique.

PCR technique can identify between each of these genes and will thus determine the genotype as well as the phenotype of each gene, especially types van A and van B, which is known for their high levels of resistance to Vancomycin as well as their transmission between different bacterial species, making them dangerous sources for the spread of resistance between these species (Kolar, *et al*., 2006).

This study aim to detection on methicillin and vancomycin resistance isolates from clinical samples(Al-Kindy hospital and Baghdad teaching hospital) and environmental samples (poultry, food, sewage and soil) increase vancomycin resistance isolate is alarm because this drug of choice for patient that suffered from multi-resistance of antibiotic and this alarm threat on community in Baghdad and this case must be need production new generation of antibiotic that treat VRSA and MRSA.

Therefore, this study was conducted to investigate the van B gene within the isolated MRSA strains from food sewage water and soil in Baghdad.

Materials and Methods

Bacterial Isolates

Various environmental samples, including poultry, food, sewage and soil, were collected to investigate the presence of *Staphylococcus* spp. in them.

The samples were cultured on blood-agar medium and then transferred to selective cultures. The isolates were identified on the basis of their microscopic and cultural characteristics on the Mannitol salty medium.

A number of biochemical tests were performed, also, including Coagulase, Urease and Catalase according to (Brooks, *et al.*, 2007).

Sensitivity testing of Methicillin and Vancomycin

The sensitivity of isolates under study was tested for Methicillin (10 µg / ml) and Vancomycin (30 µg / ml) according to the method of the spread of tablets on Muler-Hinton solid culture, while resistance and sensitivity were determined based on the standard diameters method according to (CLSI,2011).

Determination of the minimal inhibitory concentration (MIC) of Vancomycin

The minimum inhibitory concentration (MIC) of Vancomycin was determined by the method of double-stranded dilution in the solid-root culture as indicated by (Merello, *et al.*, 2003).

Plasmid DNA Isolation

Plasmid DNA was isolated from bacterial isolates resistant to methicillin and vancomycin by using several ready-made kits and according to the instructions of the Geneaid company. The results of the extraction were carried out using agarose gel (0.8%).

Detection of van A and van B genes using PCR technique

The primers of gene van A and gene van B in the study isolates according to Table (1).

The solution was prepared according to the instructions of Certificate of Analysis (USA) Company with the use of sterile distilled water to obtain a concentration of 100 picomole /microliter.

Table 1. The primers used in the study

No.	Primer	Sequence of the primer 5' → 3'	No. of bases bp	Conc. Pmol	Size µD.w1	Size of the product Bp	Reference
1	vanA-F	GGGAAAACGACAATTGC	17	229900	2299	732	Dutka malen et al.(1995)
	R	GTACAATGCGGCCGTTA	17	252800	2528		
2	vanB-F	AAGCTATGCAAGAAGCCATG	20	63300	653	536	Elsayed et al. (2001); Jackson et al.(2004)
	R	CCGACAATCAAATCATCCTC	20	216200	21628		

Electrophoresis was carried out in the agarose gel. The gel was examined after the end of the run by exposure it to a source of ultraviolet radiation. The molecular size of the multiplicated piece was determined by comparison with the location of the used run volumetric guide with the conjugated products.

Results and Discussion

Out of 73 isolates of *Staphylococcus* spp. were obtained from various environmental samples including poultry, food, sewage and soil, these isolates were: 43 isolates from poultry, 15 isolates from sewage, 9 isolates from food and 6 isolates from soil.

The bacterial isolates were identified initially as *Staphylococcus* spp. according to their phenotype properties after were cultured on blood-agar at aerobic conditions, at 37 °C for 24 h. The size of the colonies were medium to large with a diameter of 1-3mm, regular smooth, convex, shiny dark with buttery edges.

These colonies were surrounded by a narrow decomposition zone which corresponds with what mentioned by (Merello, *et al.*, 2006).

The sensitivity of all isolates was tested for Methicillin and Vancomycin, and the results showed that 39 isolates were resistant to Methicillin (53.42%), while 26 isolates were resistant to Vancomycin (35.61%).

The minimal inhibitory concentration (MIC) of Vancomycin was determined for the isolates as above with the value of MIC ranged 2-512 µg /ml.

Staphylococcus bacteria have the ability to acquire the van operon genes, and these acquired genes have the ability to migrate to other species such as *Enterococcus* spp., *Streptococcus* spp. and anaerobic bacilli (Guardabassi, *et al.*, 2005).

The results of the investigation of the van B gene showed that the isolate ESf44 from food has van B gene after running the replication product through agarose gel and compared it with the size of the evidence of approximately 536 base pairs (Fig. 1).

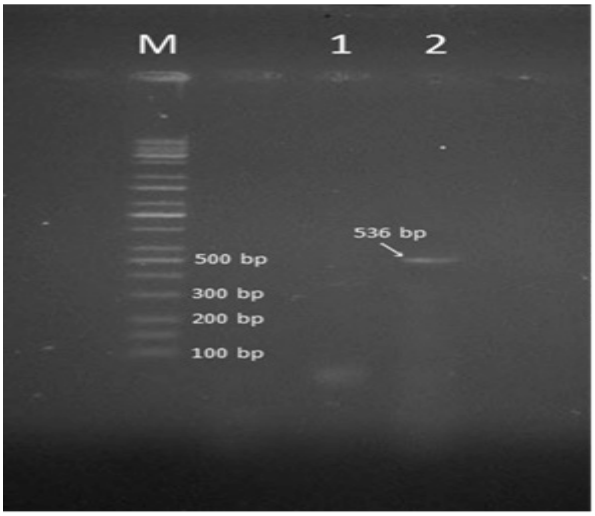

Figure 1: Electrical run of the PCR reaction product of *Staphylococcus* spp using the primaries of van B gene when dyed with Ethidium bromide and exposed to ultraviolet radiation.
Gel concentration, voltages (75) volts for 45 minutes Path (M), Volumetric guide
Path (1): isolate (ESF47)
Path (2): Isolate (ESF44)

This study (Figure 1) is important because van B gene located on the plasmid and to improve that this gene located on it.

When compared with the results of (Elsayed, *et al.*, 2001), this isolate has the genotype and the phenotype of the gene van B, which means it resist Vancomycin and is sensitive to teicoplanin. A study conducted by (Guardabassi, *et al.*, 2005).

showed that the isolated strains are resist to Vancomycin and could contribute to the spread of this resistance to human-pathogenic bacteria isolates.

The presence of van A gene was not observed in all Vancomycin-resistant isolates that was studied.

Studies show that resistance genes can be rapidly transferred from one bacterial cell to another. Studies also show that MRSA and VRSA strains can be passed from field animals to humans. also transmitted through contaminated food and water, which shows the importance of surveys on the spread of resistance genes of methicillin and vancomycin in the environment

Conclusion

Out of 73 Staphylococcus spp. isolates were recovered from environmental samples included: poultry , foods , sewage and soil .The antibiotic susceptibility test to all environment isolate to Methicillin and Vancomycin were done, one isolates that have MIC 64 mg/ml showed that Van B gene which isolated from raw milk. While Van A gene did not appear in any other isolates.

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