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Van A &B in Vancomycin-resistant Staphylococcus Aureus Isolated in Samarra City

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Abstract

A total of 110 isolates belonging to *Staphylococcus aureus* were obtained from different clinical samples of patients who were sleeping and visiting the Samarra General Hospital. From 25/4/2017 to 1/5/2017, a number of phenotypic and biochemical tests were adopted to distinguish bacteria from other bacterial species , The results showed that 25 isolates (22.7%) were resistant to vancomycin, . The putative presence of vanA gene was examined by PCR, using specific primers. Positive PCR amplifications were obtained in 3 isolates(12%) for vanA gene and the absence of the Van B gene. Vancomycin resistant staphylococcus aureus increased gradually in Samarra General Hospital and high dissemination of vanA gene, which encoded high resistance level to vancomycin. Continued surveillance is required to prevent further spread of these serious resistances. Van A & B genes were detected using PCR technique. This is evidence of high resistance to vancomycin in Samarra General Hospital.

Keywords: Staphylococcus aureus.

1. Introduction

S*taphylococcus aureus* is a is agram positive round-shaped bacterium. It is the main human pathogen. Primarily as a colonizer in one-third of general population, S. aureus can also cause life-threatening infections both in the community and healthcare settings. Staphylococcal infections range in severity from uncomplicated skin and soft tissue infections (such as folliculitis) to the more severe infections like necrotizing pneumonia and endocarditis [1,2].

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Resistance of *staphylococci* to antimicrobial agents is an issue of worldwide concern with a history of almost 70 years. Penicillin was successfully used to treat *S. aureus* until 1942, when penicillin-resistant *S. aureus* appeared [3]. In 1961, methicillin-resistant *S. aureus* (MRSA) was reported from England and is now a common cause of hospital-acquired infections [4]. Vancomycin is the main antimicrobial agent available to treat serious infections caused by S.aureus. In May 1996, the first documented clinical infection due to S. aureus with the intermediate resistance to vancomycin (minimum inhibitory concentration [MIC] equal to 8 µg/ml) was reported from Japan [5]. Later, vancomycin-intermediate S. aureus (VISA) strains were isolated in USA, Australia, Europe and other Asian countries [2]. The first clinical MRSA isolate exhibiting high-level resistance to glycopeptides (vancomycin MIC>256 µg/ml) due to acquisition of the vanA operon was detected in 2002 from Michigan(6) Although there are few reports of vancomycin-resistant Staphylcoccus aureus (VRSA). The risk of infection of these bacteria in their resistance to multiple antibiotics such as resistance Vancomycin and the acquisition of genes from other bacteria such as Enterococcus spp by coupling and therefore difficult to treat, which makes the need to find new alternatives to treat the resulting injuries [7,8]. The present study aimed at detecting the genes responsible for the resistance of vancomycin-resistant staphylococcus aureus (VRSA].

2. Materials and methods

Bacterial isolates:

Various clinical samples were collected from different patients in the patients who were sleeping and visiting the Samarra General Hospital for the period from 25/2/2017 to 1/5/2017. The isolates of *Staphylococcus aureus* were identified on microscopic and agricultural characteristics and a number of biochemical tests. '(2)

Bacterial identification All isolates have been identified by morphological, cultural and biochemical tests such as API 20 STREP test (bioMérieux, France) and Rapid ID-32STREP system (bioMérieux, France]. All isolates were identified using the agar supplemented by potassium tellurite reduction, motility, and pigment production tests [9]. Antimicrobial susceptibility testing For susceptibility testing, the agar diffusion method was used, performed, and interpreted according to the Clinical Laboratory Standards Institute guidelines [10].

DNA extraction and PCR Extraction of bacterial DNABacterial DNA Isolation

DNA isolates were isolated from the anti-vancomycin antibody by using several processed DNA extracts from Bromica, USA, from the bacterial isolates. The results of the extraction were extracted using acarose gel (0.8%].Genes encoding the vancomycin resistance determinants, *vanA* and *vanB*, were investigated by PCR using specific primers (Table 1) [11]. PCR amplification was carried out in a 20µl reaction mixture with each primer as the following steps: an initial denaturation step at 98°C for 2 min; followed by 35 cycles of 98°C for 10 sec, 50°C for 1 min and 72°C for 90 sec for *vanA* gene, and an initial denaturation step at 94°C for 10 min; followed by 30 cycles of 94°C for 30 sec, 50°C for 45 sec and 72°C for 30 sec for *vanB* gene, then finally elongation step at 72°C for 10 min. The PCR products were electrophoresed in a 1.5% agarose gel which was stained with ethidium bromide and visualized by using UV transillumination [11].

Table1: Primers used in this study

Target	Primer	Sequence 5 to 3	Product size	Refrence
VanA	Forward	GGGAAAACGACAATTGC	732bp	
	Reverse	GTACAATGCGGCCGTTA		
Van A	Forward	GATGAATAACGCTAATACGATCAA	1030bp	Devriese and his colleagues 1996
	Reverse	CCCCTTTAACGCTAATACGATCAA		
Van B	Forward	AAGCTATGCAAGAAGCCATG	536bp	Lopez and his colleagues 2011
	Reverse	CCGACAATCAAATCATCCTC		

3. Results and discussion

A total of 110 isolates of *Staphylococcus aureus* were obtained from patients who were sleeping and visiting the Samarra General Hospital. A number of phenotypic and biochemical tests were used to differentiate the bacteria from other bacterial species according to [9]. All local isolates of anthrax Vancomycin showed that 25 isolates and 22.7% were resistant to vancomycin, During the past decade VRSA did not spread rapidly and there were only a few reports of this superbug. Until the end of 2012, 33 cases of vanA-type VRSA have been reported worldwide: 13 from the United States, 16 from India, 3 from Iran (2 from Tehran, 1 from Mashhad) and 1 from Pakistan.7 Limited spread of VRSA is attributed to the highly-costly vanA operon for S. aureus, which can be acquired from enterococcal conjugation [12).

Recent articles show that VRSA is now reported in at least four continents (i.e. Asia, Europe, North and South America) [7,13,14]. The European VRSA was isolated in May 2013 from pus of the toe amputation wound of a 74-year-old female in a Portuguese hospital. The patient had multiple co-morbidities and her culture grew *Pseudomonas aeruginosa*, vancomycin-resistant *Enterococcus faecalis*, and methicillin-resistant VRSA. There is also another report from Brazil which describes a 35-year-old male with a history of diabetes mellitus and Sezary syndrome who had blood culture positive for methicillin-resistant VRSA. Vancomycin resistant *E. faecalis* was also isolated from the patient and he died despite vancomycin therapy [14]. Unfortunately, we do not have enough clinical information regarding our.

The widespread use of anticoagulant antibiotics, including vancomycin, resulted in the emergence of resistant strains of *Staphylococcous* and *Enterococcous* bacteria. Two methods were observed for the proliferation of Vancomycin resistance genes: the first was vertical transduction; the second was transient by the bacterial coupling; Tn 1546 between strains of intestinal bacterial strains, especially high-level vancomycin-resistant *Staphylococcus aureus.* (15)

The present study showed the presence of the gene A in 3 isolates and(12%) As shown in Figure 1

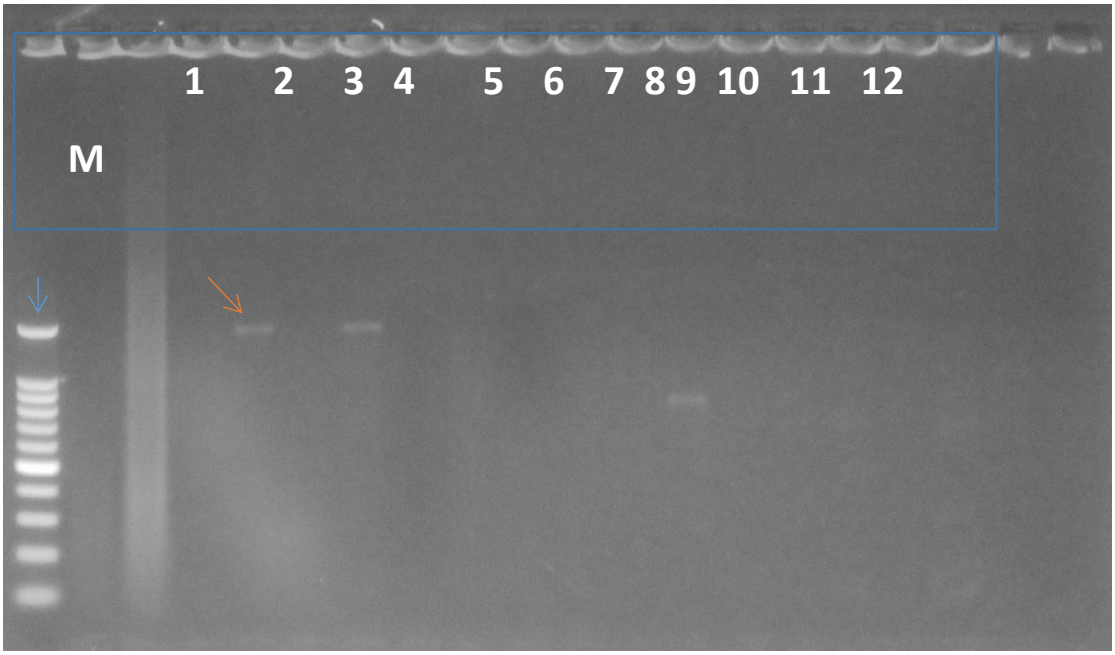

Figure1: Gel electrophoresis image of different vancomycin resistance genes, M:100bp DNA Marker,(4,6) Van A 1030bp ,(12) van A 732bp

(this result corresponds to the conclusion of the (16) mechanism, which found only two isolates showing the Van A gene, but it is contrary to(17), which found that 40% of the resistant vancomycin isolates showed The VanA gene.

The Van B gene showed no association with the resistance of Vaccumycin *Staphylococcus aureus* and these results correspond to (18), which found that all Vancomycin-resistant genes carry the Van A gene, but only three bear the Van B gene. This may open the door to researchers In this area to look for other factors may be responsible for the phenomenon of VRSA instead of Van genes

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