How to Cite:
Mahmood, S. H., Mohamed, A., & Khalaf, I. A. H. (2022). Detection of acquired resistance genes for vancomycin in local Iraqi isolates of Klebsiella pneumoniae. *International Journal of Health Sciences*, *6*(S6), 8083–8090. https://doi.org/10.53730/ijhs.v6nS6.12204

Detection of acquired resistance genes for vancomycin in local Iraqi isolates of Klebsiella pneumoniae

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***Abstract*---**The species were collected from 3 main hospitals in different regions in Baghdad, and presented by one hundred and seventy-four for various genders and ages. The collected sources were 60 samples of urine, 33 samples of wounds, 36 samples of burns, 45 sample of fecal material. A total 60 isolates were isolated and diagnosed according to microscopic, cultural and biochemical tests as well as Api – 20 E, VITEK2 Compact systems were performed as well as specific 16SrRNA gene was applied for molecular identification of Klebsiella pneumoniae isolates deposited in the National Center for Biotechnology Information (NCBI) (From LC314482.1 - LC314486.1). As a result of these tests a total of 13 Klebsiella pneumoniae isolates were isolated and identified. For the precise detection of studied isolates in molecular techniques, chromosomal DNA was extracted. It was clear that the percentage of VanA gene appearance was 40% and 6.66% VanB gene for the selected isolates. Vancomycin-resistant genes were investigated for these bacterial isolates using specific primers for the resistant genes (VanA & VanB). Results of gel electrophoresis of the PCR products for Klebsiella pneumoniae that they have the resistant genes (VanA & VanB) appeared on chromosomal DNA.

***Keywords*---**Klebsiella pneumonia, VanA, VanB, PCR, molecular study.

Introduction

Klebsiella pneumonia is one of the most important of the *Enterobacteraiecae* family and one of the opportunistic bacteria which is considered one of the causes of contamination of wounds, urinary tract infection, nosocomial infection and contamination of operating theaters. These bacteria are characterized by their resistance to β-lactam antibiotics to produce β-lactamase enzyme as well as having animportant virulence factor (lipopolysaccharide and capsule) that resists phagocytosis (Jasim *et al.*, 2020). A team of researchers were making explorative *in vitro* study for the optimization of an initial design and PCR methods for the detection of the main bacteria that cause neonatal sepsis in Indonesia, mainly *Klebsiella pneumonia* (Suryani *et al.*, 2020). In a Dutch study for VRE cases, the dissemination of the *VanA* gene clusterin their hospitals between 2012 and 2015 was dominated by clonal spread, they also identified plasmid dissemination in a high frequencies with the spread of resistance which mainly driven by horizontal gene transfer (HGT).Their study clarified the feasibility of differentiate between modes of dissemination with short-read data and provides a new review to determine the relative contribution of nested genomic elements in the distribution of *VanA*-type resistance (Alonso *et al.*, 2021).

The researcher (Sivertsen *et al.*, 2016) indicated that the spread of variable resistance of *Enterobacteriaceae* due to elements that inhibit the *VanA* gene, and that it cannot be diagnosed through the phenotype and endogenously induced molecular determinationsthat are notable for its change to a vancomyce-resistant isolate are spent by the allele of the *VanA* gene. On the other hand, the Iranian study found twenty-seven isolates ofvancomycin-resistant *Enterococci* tested, eight clinical isolates of *VanA* and *VanB* detected by TaqMan Real Time PCR assay which indicated the presence of both *VanA* and *VanB* genotypes in the three hospitals (Mirzai *et al.*, 2015). Based on what was mentioned in the scientific references, there is a necessity for continuous monitoring of the presence of *Van* genes is very important to avoid developing a reservoir for antimicrobial resistances. The aim of this article is molecular study of the genetic correlations of *Klebsiella pneumonia* species isolated from local sources and detection of the proliferation of vancomycin resistance genes.

Material and Methods

Samples collection

The collected sample were taken from 3 main hospitals in Baghdad from different regions about one hundred and seventy-four specimens including blood, urine, stool and burns. These specimens were taken under sterile condition.

Isolation and Identification of *Klebsiella*

These clinical samples were cultured on Macconkey agar, incubated at 37 ˚C for overnight, the positive cultures were undergoing biochemical tests after checking the colonies morphology, appearance and color.

Investigation of *Klebsiella pneumoniae* species by Vit. 2 compact

Vitek 2 compact auto analyzer was implied to investigate the positivecultures after finishing all the manual tests.

Chromosomal DNA extraction

Chromosomal DNA extraction Chromosomal DNA extraction achieved, according to the procedure of the company supplied (presto Mini gDNA bacteria kit of Geneaid company).

PCR amplification analysis

For confirming diagnosis of *Klebsiella pneumonia 16SrRNA* gene identified by PCR using primers pairs *16SrRNA* forward (F) and revers(R)with (331 pb size product) as described previously (Mahmood *et al.*, 2017)[DOI: 10.22401/ANJS.00.1.11] . The extracted genomic DNA and universal primers were used to amplify the *VanA* and *VanB* genes by polymerase chain reaction for their detection, and forward primer F (GGGAAAACGACAATTGC) and reverse primer R (GTACAATGCGGCCGTTA) whereas *Van B* F (ATGGGAAGCCGATAGTC) R (GATTTCGTTCCTCGACC).

The reactions were performed of each isolate for both *VanA* and *VanB* genes in 25 µl containing 3 µl (100 ng) template DNA solution,12.5µl master mix (GoTaq®Green Master Mix), 5.5 µl DDH_2O, 2 µl (10 Pmol) forward primer and 2 µl (10 Pmol) reverse primer. and *VanB* 25 µl containing 3 µl (100 ng) template DNA solution,12.5µl master mix (GoTaq®Green Master Mix), 5.5 µl DDH_2O, 2 µl (10 Pmol) forward primer and 2 µl (10 Pmol) reverse primer. The negative control was represented by the use of nuclease-free water. The PCR program was implemented for both genes according to the protocol of the manufacturingcompany (Integrated DNA Technologies), gel electrophorese was done for each 10µl of PCR product in 1.5 % (w**/**v) agarose gel with marker of 100 bp and performed at 90 volts for 1.5 hour.5 µl (0.05%) of the gel red was used to stain the gel and photographed by using Gel photo documentationsystem.

Results

As mentioned in table No.1 ,the samples presented by wounds (33) , urine (60), stool (45) and burns (36) of a total positive samples not exceed 174 Table No.1.

Table 1
The distribution of *Klebseilla pneumonia* and other *Enterobacteriaceae*

sample		% sample	Other genus of *Enterobacteriaceae*	*Klebseilla pneumonia*	*Total number*	
					N	%
Urine	60	34.4%	26	8	34	56.66%
Stool	45	25.86%	12	1	13	21.66%

Wounds	33	18.96%	3	4	7	10.93%
burns	36	20.68%	6	0	6	10.0%
174			47 78.33%	13 21.66%	60	

Investigation of *Klebseilla pneumoniae* species by Vitek 2 compact

Investigation of *Klebseilla pneumonia* cultures were confirmed by Vit.2 compact system after conducting the traditional methods Tab.1 represents the distribution results in various positive samples and isolates, whereas the percentage of positive samples and the clinical sample were as follows, urine 34.4%, stool 25.86%, wound 18.96% and burns 20.68%. Also, the diagnosis of isolates was confirmed by polymerase chain reaction technology using the *16SrRNA* gene, as well as the results of the analysis of the sequences of this gene, which were previously published (Mahmood*et al.*, 2017).

Detection of *Van* genes in *Klebseilla pneumoniae*

The polymerase chain reaction of the chromosomal DNA was carried out by using the primers *VanA*, *VanB* and with the reaction mixture using their own kit. The results of electrophoresis for 13 isolates of this bacterium showed that the total percentage of appearance of *Van* genes was 46.6%, of which *VanA* was 40% and *VanB* was 6.66%,(Figure 1).

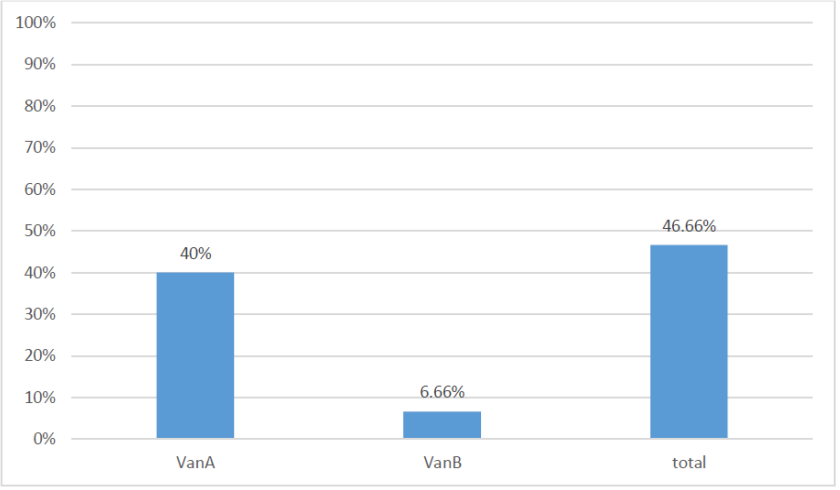

Figure 1. The percentages of *Van* genes appearing in *Klebsiella pneumoniae*

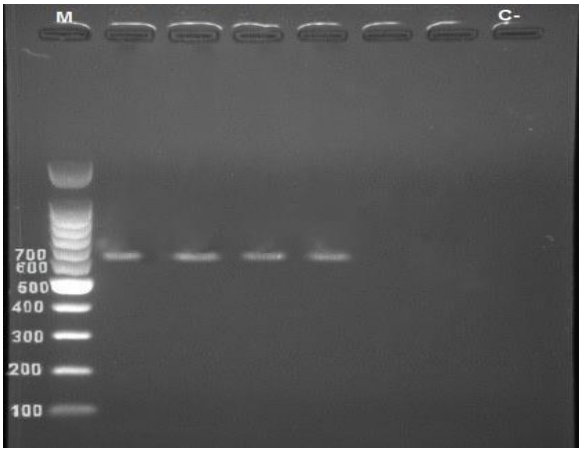

Figure 2. Gel electrophoresis of PCR product of *VanA* gene (731pb) for *K. pneumoniae* using agarose gel at a concentration of 1.5%, at 5 Volt/cm for two hours, Lane (CH30,CH39,CH7, CH8) PCR product positive for *VanA* gene, M: 100 bp DNA marker, C-: negative control

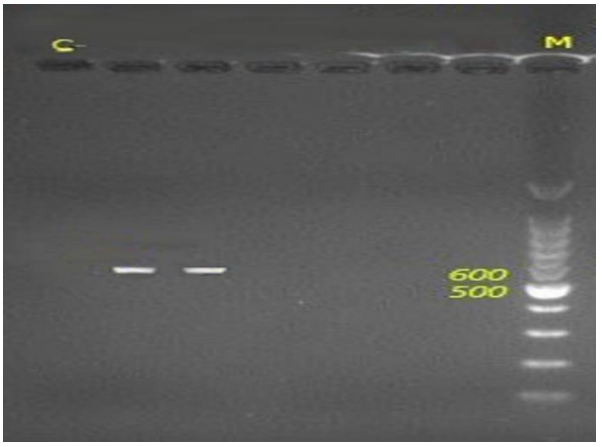

Figure 3. Gel electrophoresis of PCR product of *VanB* gene (634pb) for *K. pneumonia* using agarose gel at a concentration of 1.5%, at 5 Volt/cmfor two hours, M: 100 bp DNA marker, C-: negative control

Discussion

Vancomycin-resistant *Enterococci* (VREs) have become one of the most important nosocomial pathogens worldwide, associated with prolonged hospital stays increased treatment costs and high mortality. In light of the results obtained in this study, many studies have been published in this regard and their results may be close to or different from ours. So , a study conducted by a group of researchers (Filho *et al.*, 2003) who specializes in the control of pathological infections that occur in hospitals about the fact that VRE spread out through intestinal bacteria and prefers the environment of the intestine because it is very suitable for conjugation with the possibility of detecting, they selected 43 clinical isolates of *Enterococcus faecalis*, 5 isolates of *Enterococcus faceium*, where the results showed that the *VanA* gene appeared in all studied isolates and the

appearance of the *VanB* and *VanC* genes. Thus, the percentage of the appearance of the *VanA* gene in their study became 100% and the percentage that was obtained in our study was 40%. In other local study by a group of researchers (Saleh *et al.*, 2015) where their study was about the coding *VanA* gene of vancomycin resistant on plasmids and chromosomal DNA in *Staphylococcus aureus* isolated from infected wounds, where they indicated in their results that the *VanA* gene is present at 50% on the chromosomal DNA and this result is close to the percentage of the presence of the *VanA* gene in our study (40%).

Whilst the Saudi research (Buyukyoruk *et al.*, 2014) whose study included two types of*enterococci* the percentage of appearance of the *VanB* gene was 7.3%while the percentage of the appearance of this gene in this study was 6.66%. The two researchers (Mirani and Jamil, 2013) they selected two isolates of *Staphylococcus aureus* the first from pus and the second from the blood of a patient after a heart operation, his results showed that the bacterial isolate from the blood was harboring the *VanA* cluster which is present on the plasmid DNA as for the second isolated from pus it was intermediate resistant and the results of the genetic examination by PCR technique showed that it did not contain the genes *VanA*, *VanB* and *VanC*, Thus through its results the percentage of appearance of the *VanA* gene in their study became 50%which is very close to the percentage of appearance of the same gene in this study (40%).As for the researcher (Dahl *et al.*, 2007) where their study was about *Enterococcus* ssp bacteria and the presence of *VanA*, *VanB* and *VanC* genes on chromosomal and plasmid DNA, fourteen strains were selected 9 of them clinically human and five isolates from poultry all of which are faecal samples and the most important results were the presence of two isolates in which no gene appeared and three isolates in which the *VanB* gene was found on the chromosomal DNA with a percentage of 21.42% and only one isolate was found in which the *VanB* gene was found on the chromosomal and plasmid DNA with a percentage of 7.14% and this percentage is very close to the percentage that was obtained in our study (6.66%).

Whereas the researcher (Sivertsen *et al.*, 2016) also indicated that the spread of variable resistance *Enterobacteriaceae* due to elements thatinhibit the *VanA* gene and It was not possible to diagnose it by phenotype and to identify the endogenous molecular mechanisms thatled to changed it to vancomycin-resistant isolate through horizontal transfer of the *VanA* gene. Another study (Simjee *et al.*, 2002) reported about the anti-vancomycin resistance genes in bacteria *Enterococcus spp* isolated from human subjects showed matches withgenes found in VER bacteria isolated from infected dogs with UTI in the United States of America, which indicates that there is an exchange of resistance factors between strains even if the organism from which they were isolated differs this led to the belief that animals close to humans may play a role in spreading resistance factors and thus reaching humans.

A group of Turkish researchers (Ture *et al.*, 2017) performed a study on the presence of vancomycin-resistant genes in the bacterial DNA of *Enterococcus faecium* isolated from fish cage sediments, vancomycin resistance gene was found in 64.7% of the *E. faecium* strains the *VanA* and *VanB* genes were determined in 29.41% and 31.76% of the bacteria respectively. Recent article (Oueslati *et al.*, 2021) developed a rapid detection of *VanA* and *VanB* to prevent infections and

outbreaks, they used a new technique it's called a lateral flow immunoassay NG-Test *VanB* (NG Biotech) which was evaluated for the rapid detection of *VanB*-producing vancomycin-resistant *Enterococci* (*VanB*-VREs), together with the NG-Test *VanA*, that could replace the existing tests available for the confirmation of acquired vancomycin resistance in *Enterococci*.

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