Frontiers in Health Informatics
ISSN-Online: 2676-7104
www.healthinformaticsjournal.com
2024; Vol 13: Issue 3
Open Access

Molecular detection of vanA, vanB genes and identification of 16SrRNA gene in *E. coli* isolated clinically and from water samples in Baqubah city

Zahraa Deyaa Sagban [1] , Saba Jassim Jawad Al-Zubaidi [2]
[1] Biology department, Faculty of education for pure science, University of Diyala, Baaquba, Iraq
[2] Biology department, Faculty of education for pure science, University of Diyala, Baaquba, Iraq
[1] zahrabaioo179@gmail.com, [2]saba.jasim@uodiyala.edu.iq
Orchid :https://orcid.org/0009-0006-1500-1913

Cite this paper as: Zahraa Deyaa Sagban, Saba Jassim Jawad Al-Zubaidi (2024) Molecular detection of vanA, vanB genes and identification of 16SrRNA gene in E. coli isolated clinically and from water samples in Baqubah city. *Frontiers in Health Informatics, 13* (3), 3339-3349

Abstract

This study included collecting 211 samples, including 140 clinical samples (urine, swab burns, swab wounds, urine of patients with renal failure) from Baquba Teaching Hospital and Baladrooz General Hospital for both genders and different ages, and 71 water samples from the Public Health Laboratory of the Food Department in Diyala Governorate to check for the presence of E. coli bacteria. 44 samples were isolated and diagnosed at a rate of 20.85% based on cultural, microscopic, biochemical and Vitek tests. All bacterial samples were tested for sensitivity to 11 antibiotics, and the highest resistance rate was to vancomycin (100%), amoxicillin and ampicillin (86.84%), cefotaxime (84.21%), and the highest sensitivity rate was to meropenem and imipenem (100%). For the purpose of accurate diagnosis of isolates by molecular methods, E. coli bacteria were diagnosed using a specific primer for the 16S rRNA gene. The results showed that the occurrence rate of this gene was 100%. An evolutionary tree was built for the new isolates, in addition to registering (3) isolates in the National Center for Biotechnology Information (two clinical isolates and one aquatic isolate). As for molecular detection, the occurrence rate of the Van A gene was (36%) (n = 9 isolates) and the occurrence rate of the Van B gene was (16%) (n = 4 isolates).

Keyword : E.coli , Van A , Van B , 16SrRNA , antibiotic resistance

Introduction

Escherichia coli (*E. coli*) is a Gram-negative, bacillus bacterium that inhabits the intestines of people and animals. While *E. coli* is typically regarded as a benign commensal bacterium, many strains are unequivocally harmful and responsible for several illnesses, including urinary tract infections, peritonitis, septicemia, diarrhea, pneumonia, and meningitis in neonates(1). Certain strains of E. coli can induce many diseases, particularly urinary tract infections, as around 90% of these infections can enter the bloodstream and result in septicemia. These infections advance to affect the bladder and kidneys due to virulence factors that promote bacterial adhesion and invasion, posing a risk since they can lead to mortality in the elderly, children, or anyone with compromised immunity(2) (3). Antibiotic resistance is one of the major problems responsible for thousands of deaths worldwide (4) and is estimated by some to result in 10 million deaths annually by 2050 (5). The indiscriminate use of these antibiotics has led to the discovery of bacterial strains that are resistant to one or more antibiotics (6) , consists of the ultimate therapeutic agent (vancomycin). The rapid dissemination of

resistance genes globally highlights the emergence of this issue as a significant public health concern [7]. One of the difficulties is determining why new antibiotic resistance genes emerge in different environments to prevent the emergence and spread of new antibiotic resistance genes [8]. Vancomycin resistance has been documented in Gram-positive bacteria; however, recent reports indicate that Gram-negative bacteria, including Escherichia coli, have also exhibited vancomycin resistance and sensitivity, acquiring this resistance via cell membrane impermeability. The emergence of this resistance is attributable to their adaptation to the external environment. Lipopolysaccharides (LPS) are modified by Gram-negative bacteria in response to stressful environmental conditions in order to maintain the integrity of their membranes [9] . The observed vancomycin resistance was attributed to a synergistic interaction among two or three medication combinations [10] . A minor fraction of exogenous vancomycin penetrates E. coli, exerting minimal impact on growth or lethality. The presence of additional antibiotics in the environment exhibits a synergistic effect on wild-type E. coli. External variables and stresses in the bacterial environment can induce E. coli to exhibit either vancomycin sensitivity or resistance [9] .16S rRNA gene sequencing is a highly sensitive and extensively utilized diagnostic technique. The 16SrRNA gene has great specificity for each bacterial species, rendering it an optimal target for bacterial identification. This method involves the amplification of the 16SrRNA gene, followed by the determination of the resultant nucleotide sequences through comparison with database entries[11] .

The objective of the study is to identify the 16SrRNA gene in *E.coli* that has been isolated from clinical samples as well as water samples. Molecular detection of van A and van B genes is of particular interest.

Material and Methods

Bacterial Specimens Collection

140 clinical samples were collected from (Baquba Teaching Hospital and Baladruz General Hospital) from different sources (urine, burns, wounds, kidney failure) for the period from2023\12\1 to 2024\3\30 for both sexes and of different ages. Also, 71 environmental samples (water) were collected from the Public Health Laboratory, Food Department, in Diyala Governorate. Then the samples were planted on different media and then incubated for 24 hours at a temperature of 37 °C for the purpose of isolating bacteria and their initial diagnosis.

Bacterial isolation and diagnosis

The bacterial isolates were isolated and diagnosed by growing them on culture media suitable for their growth, studying the phenotypic characteristics of the colonies and bacterial cells, and performing microscopic examination and biochemical tests. The clinical bacterial isolates were plotted on MacConkey agar medium in order to diagnose the isolates phenotypically [12]. As for the water isolates, they were grown on MacConkey broth medium by taking (1) ml of a water sample and adding it to (10) ml of sterile medium [13] . For the purpose of confirmation, the samples were grown on Eosin Methylene Blue medium (EMB) and Hichrome E. coli medium [14] The bacterial isolates were incubated for 24 hours at a temperature of 37°C, as well as biochemical diagnosis through several tests such as the catalase test [15] Oxidase enzyme testing [16] . as well as diagnostics using the Vitek system As in Figure (1) .

Antibiotic sensitivity test

Antibiotic susceptibility testing of *E. coli* isolates was performed using Mueller-Hinton-Agar medium using Kirby-Bauer method based on (CLSI, 2023), [17] .The antibiotics in this study were (norfloxacin (30 µg),

amikacin (30 µg), gentamicin (10 µg), cefotaxime (10 µg), cefoxitin (30 µg), ciprofloxacin (10 µg), ampicillin (25 µg), amoxicillin (10 µg), meropenem (10 µg), imipenem (10 µg), vancomycin (30 µg) (as in Figure (3

Molecular identification of Van A and Van B genes and detection of 16S rRNA genes

DNA Extraction

Bacterial DNA was extracted from clinical and environmental (water) isolates according to the ABIO Pure protocol.

Molecular identifying of Van A and Van B genes

The use of PCR primers in the investigation of the vancomycin resistance genes Van A and Van B is demonstrated in Table 1[18]. To begin, do one cycle of denaturation at 95 degrees Celsius for five minutes. This is followed by ten cycles, each consisting of sixty seconds of denaturation, forty seconds of annealing at 58 degrees Celsius, and sixty seconds of extension at 72 degrees Celsius. Following this, 25 cycles of denaturation at 95 degrees Celsius for sixty seconds, annealing at 54 degrees Celsius for forty seconds, and extension at 72 degrees Celsius for sixty seconds are carried out for the Van A gene. This is then followed by a final extension at 72 degrees Celsius for five minutes and a hold at 10 degrees Celsius for ten minutes. Following a single denaturation cycle at 95 degrees Celsius for five minutes, there were thirty cycles of denaturation, annealing, and extension at 72 degrees Celsius for thirty seconds each. One last extension was performed at 72 degrees Celsius for seven minutes, and then a Van B gene hold was performed at 10 degrees Celsius for ten minutes.

Identification of 16SrRNA

The polymerase chain reaction (PCR) amplification was carried out by employing particular primers, as shown in Table (1) [19], in a reaction mixture of 25 µl, as indicated in Table (2), in accordance with the following: Initial denaturation at 95 degrees Celsius for five minutes for one cycle, followed by thirty cycles of denaturation at 95 degrees Celsius for thirty seconds, annealing at sixty degrees Celsius for thirty seconds, extension at seventy degrees Celsius for thirty seconds, and one cycle of final extension at seventy degrees Celsius for seven minutes, followed by a ten-second hold at ten minutes.

Table (1) primers sequence used in the study

Primer name	Primer sequence ('5-'3)	Product size ◌̊C	Reference
Van A-F	GGGAAAACGACAATTGC	732	18
Van A-R	GTACAATGCGGCCGTTA		
Van B-F	ATGGGAAGCCGATAGTC	634	18
Van B-R	GATTTCGTTCCTCGACC		
27-F	AGAGTTTGATCCTGGCTCAG	1500	19
1492-R	TACGGTTACCTTGTTACGACTT		

***F: Forward** **R: Reverse**

Table (2) : Optimal reaction mixture for DNA replication of the Van A and Van B and 16SrRNA genes

Master mix components	Volume
Master Mix	12.5
Forward primer	1
Reverse primer	1
Nuclease Free Water	8.2
DNA	2
Total volume	25

DNA Sequencing of 16SrRNA gene

Macrogen Corporation in Korea dispatched PCR products for Sanger sequencing using the ABI3730XL automated DNA sequencer. The results were acquired via email and subsequently analyzed using Geneious software.

Constructing of the phylogenetic tree

The genetic relationship between all *E.coli* clinical isolates and those isolated from the 16S rRNA gene water under study was found by creating a genetic tree, converting the results that appeared into a descriptive tree, then entering this data into the Geneious software program in order to obtain a genetic tree for the isolates .

Results and Discussion

Isolation and identification of *Escherichia coli*

The study included the collection of 211 samples (140) clinical samples, at a rate of (66.35%), and (71) water samples, at a rate of (33.64%), and for the period from (December 1, 2023 to April 30, 2024). There were (44) positive cases, with a rate of (20.85%). (44) isolates of *E. coli* bacteria were obtained (38 clinical isolates and 6 water isolates).

A study [20] showed that E. coli bacteria were isolated from clinical sources, with the percentage of bacteria isolated from urine samples reaching 22 (34.4%) and wounds 10 (15.6%).A study by [21] in which *E.coli* bacteria were isolated from drinking water sources and (97) isolates of *E.coli* bacteria were obtained at a rate of (18.7%).

Table (3): Number of samples and percentage and number of isolates and percentage

Insulation source	Number of samples and percentage %	Number of isolates and percentage %
Urine	93 (66.42%)	37 (39.78%)
Swab burns	11 (7.85%)	1 (9.09%)

Swab wounds	15 (10.71%)	0
Urine of patients with renal failure	21 (15%)	0
Water	71 (33.64%)	6 (8.45%)
Total	211	44

A **B** **C**

Figure (1) A: *E.coli* on MacConkey agar. B: *E. coli* bacteria cultured on eosin methylene blue media. C: *E. coli* bacteria on the HiChrome

Sensitivity Antibiotics

The sensitivity of *E. coli* bacterial isolates to 11 antibiotics was tested :(Norfloxacin (NOR) , Amikacin (AK) , Gentamycin (CN) , Cefotaxime (CEF), Cefoxitin (CX) , Ciprofloxacin (CIP) , Ampicillin (AM) , Amoxicillin(AX) , Imipenem (IMP) , Meropenem (MEM) , Vancomycin (VA))

The results of the isolates showed a clear difference in the extent of the response of the clinical and environmental isolates to the antibiotics used. The percentage of resistance of the bacterial isolates to antibiotics was as follows: VA(100%) (n = 38 clinical isolates, n = 6 water isolates),AX (86.84%) (n = 33 clinical isolate) (83.33%) (n=5 water isolate) AM (86.84%) (n=33 clinical isolate)(100%) (n=6 water isolate, CEF (84.21%)(n=32 clinical isolate)(66.66%)(n=4 water isolate), NOR(50%)(n=19clinical isolate)(33.33%)(n=2water isolate), CIP(44.73%(n=17clinical isolate) (33.33%(n=2water isolate), CX(34.21%)(n=13clinical isolate)(There are no resistant water isolates), CN(21.05%)(n=8clinical isolate)(16.66%)(n=1water isolate), amikacin(7.89 %) (n=3clinical isolate)(There are no resistant water isolates), MEM and IMP did not show any resistance rate in clinical and water isolates As in Figure (2) .

A study [18] in which E. coli was isolated from clinical and aquatic sources showed that the resistance to vancomycin was (100%), a study [22] in which the highest resistance to the antibiotics ampicillin (84%) and amoxicillin (86%), a study [23] reported that the highest sensitivity to the antibiotics meropenem was (100%) and imipenem (95%), and a study [24] in which the highest resistance rate to cefotaxime was (86%), and these results of studies are consistent with the results of the current study.

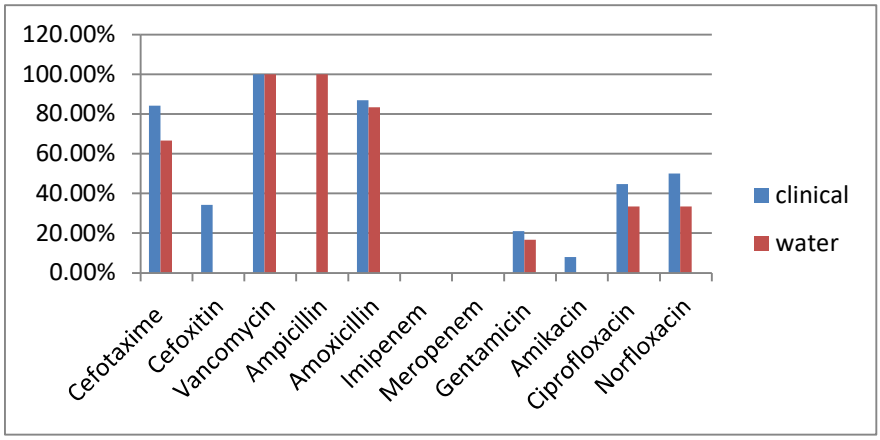
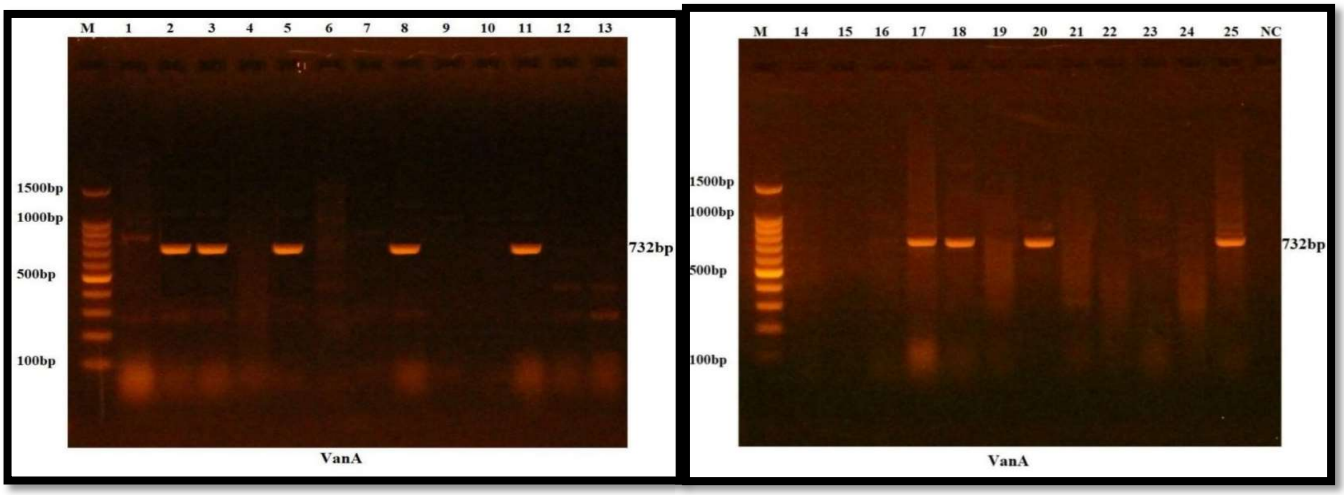


Figure (2) : Percentage of antibiotics used in the study

Detection of Van A and Van B genes in *Escherichia coli*



Polymerase chain reaction (PCR) was performed using VanA and VanB primers individually with conventional PCR mixture for 25 DNA samples selected based on their vancomycin resistance. After transferring the PCR results of the DNA samples to 1.5% agarose gel and examining them under UV, the bands resulting from repetition were shown, with the percentage of VanA gene expression being (36%), with 9 isolates showing positive results as shown in Figure (3) .

Figure (3) : The amplification results of Van A genes from Escherichia coli were separated using 1.5% agarose gel electrophoresis and stained with ethidium bromide. M: 100 bp ladder marker. Lanes 1-19 contain clinical isolates, while lanes 20-25 consist of water isolates, both exhibiting 732 bp PCR products.

The total percentage of appearance of the Van B gene is (16%) and the number of isolates in which the gene appeared is 4 isolates, as in Figure (4).

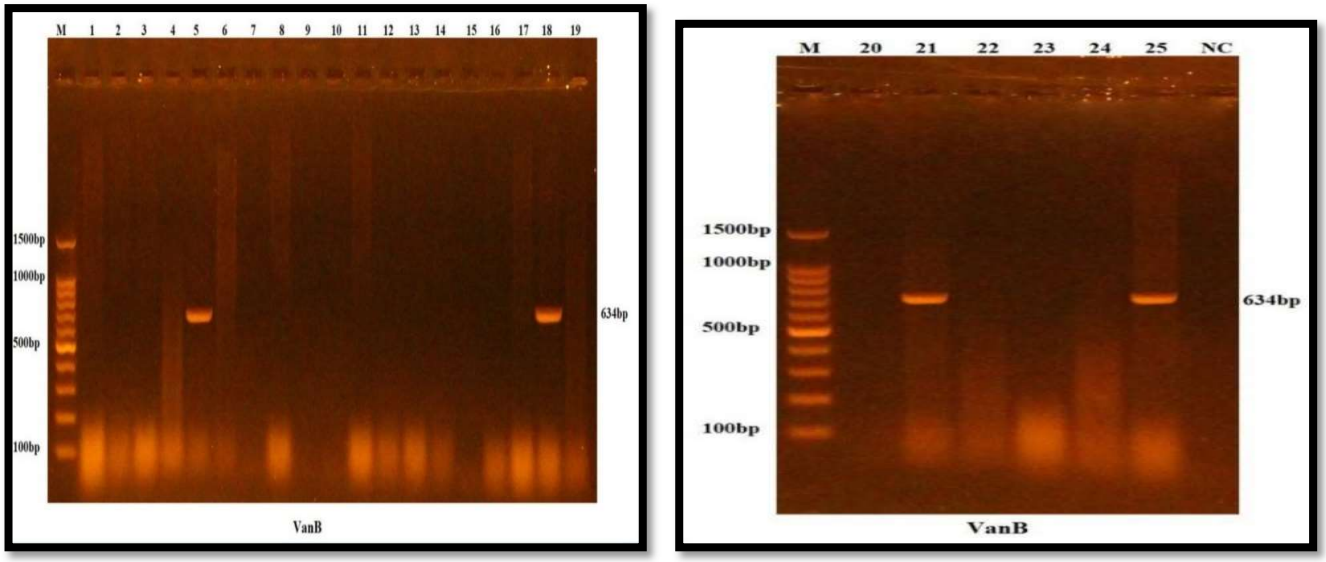
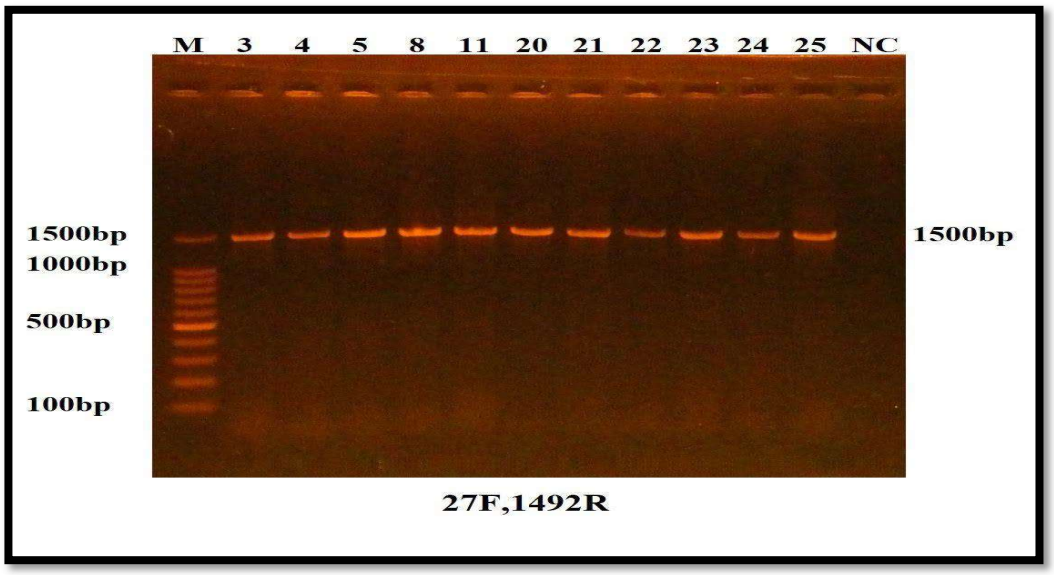


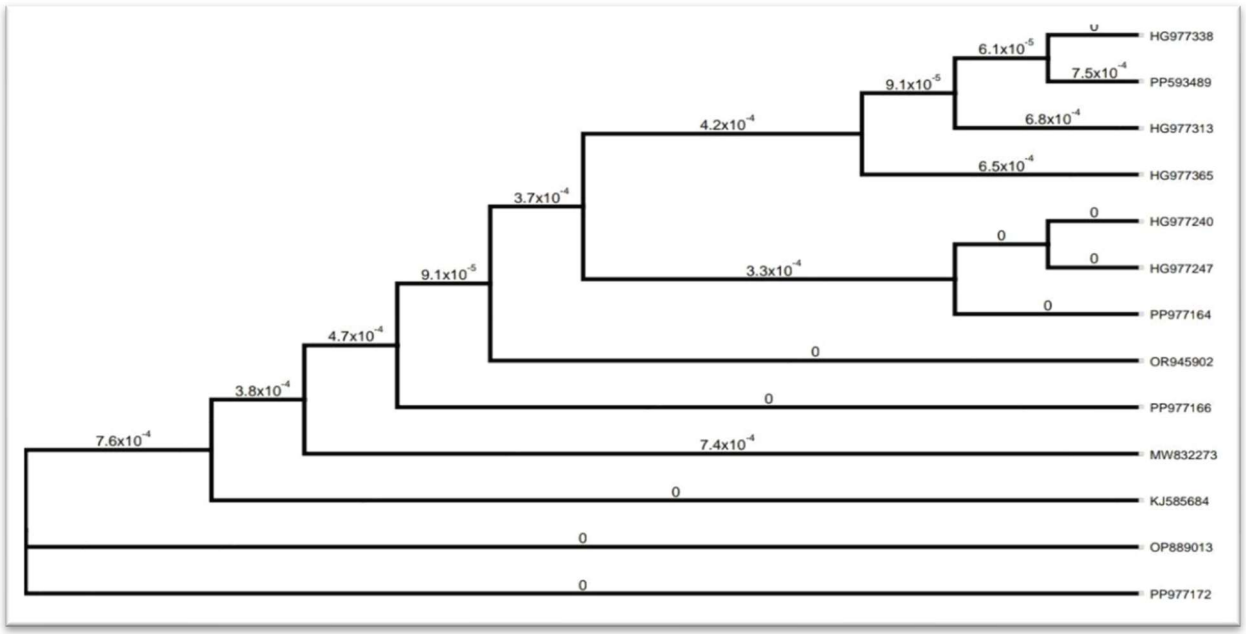
Figure (4): The amplification results of VanB genes from Escherichia coli were separated using 1.5% agarose gel electrophoresis and stained with Ethidium Bromide. M: 100 bp ladder marker. Lanes 1-19 exhibit clinical isolates, while lanes 20-25 display water isolates, both resembling 634 bp PCR products.

The results of the current study were consistent with the results of the study [25], as 8 isolates showed a positive result for the Van A gene, while they were close to the Van B gene, as only one isolate showed a positive result. The results of the current study differed from the results of the study [18], as the percentage of appearance of the Van A gene reached 45.16%, as 14 isolates showed a positive result, while with regard to the Van B gene, the percentage of its appearance was 38.70%, as 12 isolates showed a positive result. A group of researchers [26].who isolated vancomycin-resistant Enterococci bacteria showed that the percentage of appearance of the VanA and VanB genes was (34%), which is consistent with the results of the current study for the VanA gene, but differs for the VanB gene.

Identification based on 16SrRNA*Escherichia coli* bacteria were identified by molecular methods using the 16S rRNA gene for clinical and aquatic isolates. Polymerase chain reaction (PCR) was performed for 11 bacterial isolates, numbered (3, 4, 5, 8, 11) clinical and (20-25) aquatic, where the gene occurrence rate was (100%) as shown in Figure (5). Then the PCR results were sent to the National Center for Biotechnology Information (NCBI) for the purpose of sequencing the isolates. Three new isolates were detected at a rate of (99%), two clinical isolates (3, 4) and one aquatic isolate (20). They were registered in the National Center for Bacterial and Virus Information, and each sample has its own access number, where the access number is specific to sample No. 3 (PP977164), No. 4 (PP977166), and No. 20 (PP977172). Based on the sequencing results, an evolutionary tree was created for the new recorded isolates using the geneious program, as shown in Figure (6).

Figure(5) : Results of Escherichia coli 16SrRNA gene amplification were fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lanes 3-4-5-8-11 clinical isolate and 20-25 water isolate resemble 1500bp PCR products.





Figure(6) : Phylogenetic tree showing the evolution of *Escherichia coli* based on the sequence of *Escherichia coli* strains for the 16S rRNA gene, 5 new isolates. Phylogenetic tree was constructed using the geneious program.

Polymerase chain reaction (PCR) was the most accurate method for identifying E.coli bacteria by detecting the 16S rRNA gene, so it can be considered an effective diagnostic method. This method was used after traditional techniques such as culture, morphological, microscopic and biochemical tests and Vitek device.

Conclusion

According to the results of this study to prevent Escherichia coli bacteria resistant to antibiotics, especially vancomycin, in clinical isolates and environmental isolates (water), and the emergence of Gram-negative bacteria carrying genes resistant to both vancomycin A and van B, or one of them, this resistance may be due to the acquisition of resistance genes through horizontal gene transfer of those genes.

Reference

1. Gaibani, P., Mazzariol, A., Secci, B., Diani, E., & Gibellini, D. (2024). Genomic characterization of a high pathogenic *Escherichia coli* causing bacterial meningitis in a newborn in Italy, 2022: *E. coli* neonatal meningitis. Diagnostic Microbiology and Infectious Disease, 110(3), 116397.
2. Farrokh, C., Jordan, K., Auvray, F., Glass, K., Oppegaard, H. and Raynaud, S. (2013). Review of Shiga-toxin-producing *Escherichia coli*(STEC) and their significance in dairy production. Int .J. .Food .Microbiol. 162: 190-212.
3. Zeadan, A. M., Saleem, A. J., & Abbas, S. M. (2022). Detection and Analysis of Resistance Genes in *Escherichia coli* Bacteria Isolated from Children in Baghdad. Advanced Gut & Microbiome Research, 2022(1), 1450019.

4. Adzitey, F. (2020). Incidence and antimicrobial susceptibility of *Escherichia coli* isolated from beef (meat muscle, liver and kidney) samples in Wa Abattoir, Ghana. Cogent Food Agricult. 6:1718269. DOI: 10.1080/ 23311932.2020.171 8269.
5. Praveenkumarreddy, Y., Akiba, M., Guruge, K. S., Balakrishna, K., Vandana, K. E., and Kumar, V. (2020). Occurrence of antimicrobial-resistant *Escherichia coli* in sewage treatment plants of south India. J. Water Sanit. Hygiene Devel. 10, 48–55. DOI: 10.2166/washdev.2020.051.
6. Abdolmaleki, Z., Mashak, Z., and Dehkordi, F. S. (2019). Phenotypic and genotypic characterization of antibiotic resistance in the methicillinresistant *Staphylococcus aureus* strains isolated from hospital cockroaches. Antimicrobial Resistance and Infection Control, 8(1),1-14.
7. Urban-Chmiel, R., Marek, A., Stępień-Pyśniak, D., Wieczorek, K., Dec, M., Nowaczek, A., & Osek, J. (2022). Antibiotic resistance in bacteria—A review. Antibiotics, 11(8), 1079.
8. Al-Kareem, A. (2023). Isolation and Diagnosis of Vancomycin-Resistant *Escherichia coli* from Different Water Sources. Egyptian Journal of Aquatic Biology and Fisheries, 27(2), 585-590.
9. Nithiya, P., Alagarsamy, G., Sathish, P. B., Rajarathnam, D., Li, X., Jeyaraj, S., … & Selvakumar, R. (2024). Impact of effluent parameters and vancomycin concentration on vancomycin resistant *Escherichia coli* and its host specific bacteriophage lytic activity in hospital effluent. Environmental Research, 247, 118334.
10. Hamoud, R., Zimmermann, S., Reichling, J., & Wink, M. (2014). Synergistic interactions in two-drug and three-drug combinations (thymol, EDTA and vancomycin) against multi drug resistant bacteria including *E. coli*. Phytomedicine, 21(4), 443-447.
11. Osińska, A., Korzeniewska, E., Korzeniowska-Kowal, A., Wzorek, A., Harnisz, M., Jachimowicz, P., … & Zieliński, W. (2023). The challenges in the identification of *Escherichia coli* from environmental samples and their genetic characterization. Environmental Science and Pollution Research, 30(5), 11572-11583.
12. Forbes, B. A.; Saham, D. F. and Weissfeld, A. S. (2007). Baily and Scott's Diagnostic Microbiology. 12thed. Mosby, Inc., an anffilliate of Elsevier, Inc. 1031pp.
13. Al_Blue, Musab Obaid Hamad. And Al-Numan, writer Younis Sharif. Investigating the pollution of drinking water on the right coast of the city of Mosul using the multiple fermentation tube method. Journal of Education and Science.2018; Volume 28. Issue 2. 167-184.
14. Salfinger, Y., & Tortorello, M. L. Fifth (Ed.) , 2015, Compendium of methods for the microbiological examination of foods, 5 th Ed., American Public Health Association, Washington, D.C.
15. Cappuccino, J. G. and Welsh, C.T. (2018). Microbiology: A Laboratory Manual. 11th ed. Pearson Education. England.
16. Procop, G.; Church, D.; Hall, G.; Janda,W.; Koneman, E.; Schreckenberger, P. and Woods, G.(2017). Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 7th ed. Wolters Kluwer Health, Philadelphia.
17. Magiorakos, A.P., Srinivasan, A., Carey, R.B., Carmeli, Y., Falagas, M.E., Giske, C.G., Harbarth, S., Hindler, J.F., Kahlmeter, G., OlssonLiljequist, B. and Paterson, D.L., 2012. Multidrugresistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical microbiology and infection, 18(3):268-281.
18. Mahmoud, Shahed Hisham, Turki, Ahmed Mohammed and Khalaf, Elham Abdel Hadi. (2017). Detection of vancomycin resistance genes in local *Escherichia coli* isolates and their relationship with

other resistance patterns. University of Anbar, College of Science. Journal of Anbar University for Pure Sciences. Volume (11), Issue 3.

19. Sadeq, A.M. and Ismail, Z.Z., 2024. Microalgae Growth in a Biocathode-Photosynthesis Microbial Desalination Cell: Molecular Characterization, Modeling Study, and Performance Evaluation. Iraqi Journal of Chemical and Petroleum Engineering, 25(1), pp.1-12.
20. Abd El-Baky, R. M., Ibrahim, R. A., Mohamed, D. S., Ahmed, E. F., & Hashem, Z. S. (2020). Prevalence of virulence genes and their association with antimicrobial resistance among pathogenic *E. coli* isolated from Egyptian patients with different clinical infections. Infection and Drug Resistance, 1221-1236.
21. Odonkor, S. T., & Addo, K. K. (2018). Prevalence of multidrug-resistant *Escherichia coli* isolated from drinking water sources. International journal of microbiology, 2018(1), 7204013.
22. Kazemnia A, Ahmadi M, Dilmaghani M. Antibiotic resistance pattern of different *Escherichia coli* phylogenetic groups isolated from human urinary tract infection and avian colibacillosis. Iran Biomed J. 2014;18(4):219-24. Doi: 10.6091/ibj.1394.2014. PMID: 25326020; PMCID: PMC4225061.
23. Khulaif, M. J., & Al-Charrakh, A. H. (2023). Detection of class 1 integron and antibiotic resistance of β-lactamase-producing *Escherichia coli* isolated from four hospitals in Babylon, Iraq. Medical Journal of Babylon, 20(2), 375-382.
24. Atif, M., Al-Rubaye, D. S., & Al-Hraishawi, H. R. (2023). PLASMID PROFILING OF EXTENDED SPECTRUM B-LACTAMASES PRODUCING *ESCHERICHIA COLI* IN SOME HOSPITALS IN BAGHDAD. Iraqi Journal of Agricultural Sciences, 54(2), 360-368.
25. Al-Kareem, A. (2023). Isolation and Diagnosis of Vancomycin-Resistant *Escherichia coli* from Different Water Sources. Egyptian Journal of Aquatic Biology and Fisheries, 27(2), 585-590.
26. Ateba, C. N., Lekoma, K. P., & Kawadza, D. T. (2013). Detection of vanA and vanB genes in vancomycin-resistant enterococci (VRE) from groundwater using multiplex PCR analysis. Journal of water and health, 11(4), 684-691.