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Isolation and Diagnosis of Vancomycin –Resistant *Escherichia coli* from Different Water Sources

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ABSTRACT

The overuse of antibiotics for the remedy of humans and animals causes antimicrobial residues released into water surfaces and wastewater. Wastewater treatment plants have been used to remove chemical and biological pollutants; however, this environment can be considered a suitable medium for the spread of resistant genes via horizontal transfer. Thus, twenty water samples were collected from the water surface of different places in the Tigris River in March 2022 in Baghdad city. Samples were analyzed to detect the resistance of *Escherichia coli* against vancomycin antibiotics. Based on phenotype detection, among 25 *E. coli* isolates, 15 isolates were resistant to vancomycin antibiotics. While, PCR analysis showed that, among 15 vancomycin-resistant *E.coli*, only 8 isolates included the van-A gene, and one isolate recorded a van-B gene. Vancomycin resistance of *Escherichia coli* has been recorded in the environment associated with the presence of antibiotic residues released in various water environments due to the increased use of human and animal medicine, as well as farming and aquaculture for the sake of preventing and treating infections.

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INTRODUCTION

Antibiotics are effective tools used to treat infectious diseases through inhibiting the growth of bacterial cells. Antibiotics are frequently given to normal farm animals to encourage growth and stop the spread of illness; they are provided for the benefit of patients and farm animals **(O'Neill, 2016)**. Antibiotic resistance (AR) may be intrinsic genes or acquired resistance genes (ARG) **(Wright, 2010)**.

The risks they pose should be considered in the context of integrating human health and the environment, with an emphasis on the recombinant DNA mechanisms that lead to proliferation. Addressing health issues such as antibiotic resistance in the context of integrating environmental, animal and human factors is the One Health principle endorsed by the World Health Organization, and AR has a significant impact in half. Medical. 10 Sustainable Development Goals of the United Nations **(WHO, 2020)**.

One of the difficulties is to determine the cause of the appearance of new antibiotics resistant genes in different water environments to block the emergence and spread of novel antibiotic resistance genes. *Escherichia coli* is used as an indicator of fecal surface water contamination for its abundance and its potential to be cultured, in addition to the fact that it is usually associated with the gut of mammals. Although faecal coliforms bacteria cannot cause disease for humans, they have been linked to a higher risk of acute gastroenteritis **(Scott *et al*., 2017)**.

The increase of *Escherichia coli* species multidrug resistance, especially vancomycin-resistant *Escherichia coli* complicates the treatment of *E. coli* infections **(Von Baum & Marre, 2005)**.

Therefore, this study focused on vancomycin-resistant *Escherichia coli* in the surface of water.

MATERIALS AND METHODS

In March 2022, twenty samples of Tigris River water in Baghdad city were collected from different points (Figure 1), and samples were remotely collected from wastewater treatment plants and hospitals to extract 500ml of sterilized water (Duran Short – Glass). For the standard coliform analysis and *E. coli* isolation, standard methods were used to determine *E. coli* counts by using presumptive test **(Harley & Prescott 2002)**. Positive tests were subjected to morphological studies to confirm the presence of faecal bacilli. For further confirmation, eosin-methylene blue (EMB) agar plates were streaked and performed according to the method of **Cappuccino and Sherman (1999)**.

Escherichia coli colonies were selected; additional biochemical tests were performed to detect *Escherichia coli* **(Coyle *et al*., 1985)**. Catalase, gelatin dilution (M. Cheesbough, 2006), and other biochemical tests were performed (**Aneja, 2003)**.

Van gene amplification

The amplification of van A gene with specific primer sets is presented in Table (1) **(Depardieu *et al*. 2004)**. The amplified specific DNA fragment of 732 bp and 647 bp were respectively recorded. PCR analysis was achieved by adding the following reaction mixture to a PCR tube. Amplification was performed in thermal cycles depending on the reaction protocol of **Dutka-Malen (1995)**. A volume of 5ml of amplified PCR product was mixed with loading buffer. A 1.0% agarose gel acted as a cast. Samples were loaded on a 511 kb scale (Bioline, UK) as a DNA marker. To visualize the bands, the gel was run and observed under a UV transmission illuminator.

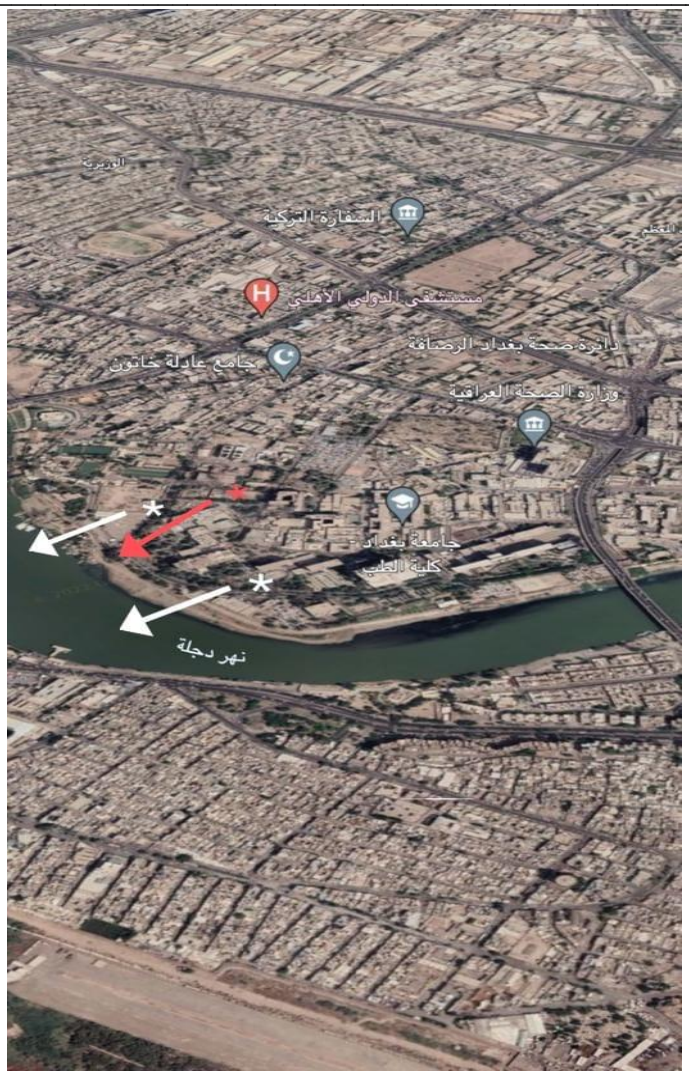

Fig. 1. A map showing the modeling site points from Tigris River near medical city hospital; discharge site

Table 1. Primers sequences used in this study

Primers name	Sequences	Product size (bp)
vanA- F	GGGAAAACGACAATTGC	732
vanA- R	GTACAATGCGGCCGTTA	
van B-F	ACGGAATGGGAAGCCGA	647
van B-R	TGCACCCGATTTCGTTC	

RESULTS AND DISCUSSION

Among the water samples collected for the current study, 20 samples were examined to confirm the presence of coliforms. For coliforms, 15 out of the 20 river water samples recorded a definite positive result. The highest detection rates of *E. coli* were observed in water samples obtained from raw and treated wastewater. All coliform-

positive samples were tested for *E. coli* isolates in the presumptive tube. Confirmatory tests for coliforms on EMB agar showed only *E. coli* with a typical green metallic sheen. The completed test showed acids and gases in the broth tube, Gram-stained bacilli, indicating that *E. coli* was isolated from each of the 35 water samples and maintained as stock cultures on nutrient agar gradients, and one of these isolates was selected for further qualitative biochemical analysis.

According to the World Health Organization, inadequate drinking water and poor sanitation are responsible for 80% of all diseases in the world **(Pant, 2004)** as reported from PWS.

From 25 *E.coli* isolates, 15 isolates were resistant to vancomycin antibiotic depending on phenotype detection , and from 15 vancomycin resistant *E.coli*, 8 isolates included vanA gene, while only one isolate had a vanB gene.

WHO estimated that, 80% of all diseases in the world can be attributed to inadequate drinking water supplies and poor sanitation **(Pant, 2004)**. Moreover, in the case of waterborne diseases, the outbreaks of *E. coli* are caused by the consumption of contaminated water, as reported from a public water supply system **(Keldreich *et al*., 1992)**. Among the 25 isolates of *E.coli*, 15 isolates were dependent on the vancomycin antibiotic phenotype, and of the 15 vancomycin-resistant isolates, 8 isolates contained the vanA gene and only one isolate was resistant to the vanB gene.

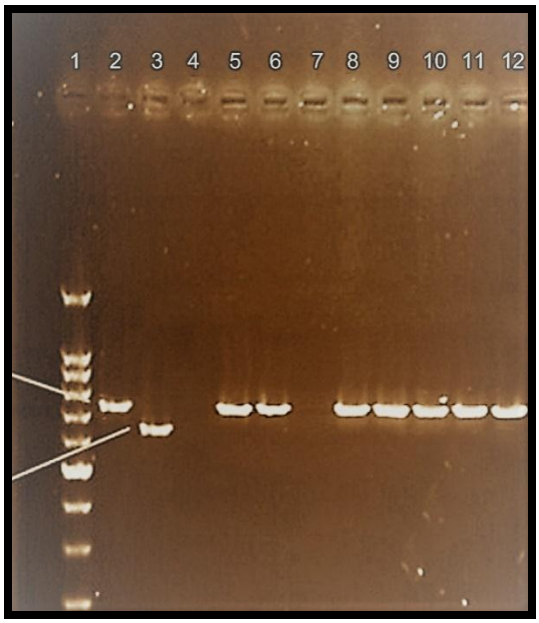

Fig. 2. Analysis by DNA amplification of vancomycin-resistant *E.coli*. Lanes: 2, 5,6,8,9,10,11,12, *E. coli* (*vanA*) - Lane: 3 , *E. coli* (*vanB*). Lane: 1,M, molecular size marker (100-bp DNA ladder).

In the case of waterborne diseases, the outbreaks of *E. coli* are caused by consumption of contaminated water. Reported by a public water supply system **(Keldreich, *et al*., 1992)**.

Escherichia coli is a common bacterial species with numerous strains that can be very dangerous **(Tareen *et al*., 2022)**. Infections with antibiotic-resistant *Escherichia coli* prolong hospitalizations and impose direct and indirect economic pressures on

populations and health systems **(MacKinnon *et al*., 2020)**. Resistant bacteria are the occurrence in which a particular subpopulation of bacteria becomes resistant to the treatment of more than one antibiotic or pathogens that exhibit multidrug resistance or "superbugs" **(Chowdhari *et al*., 2014)**.

Numerous studies have shown that the inappropriate use of antibiotics can cause resistant in many bacteria, making treatment of dangerous pathogenic bacteria more difficult **(Ventola, 2015)**.

It is a critical problem since it faces both human and animals and can make infections more difficult to treat.

The prevalence of vancomycin resistance in *E. coli* in the river water may be associated to antimicrobial residues since antibiotic presence in the aquatic environment is widely used to prevent or treat infections, mainly in humans and veterinary medicine, aquaculture and agriculture **(Kümmerer, 2009)**. Antimicrobials are released into the water and soil through urine and faeces or waste discharge such as household waste. In addition, several chemicals including antibiotics can be released into the water in the form of industry wastewater **(Polianciuc *et al*., 2020)**.

CONCLUSION

The prevalence of vancomycin resistant *Escherichis coli* (15/25) recorded a percentage of 60 in the surface water, depending on phenotype test. Detection of vanA gene in (8/15) recorded a value of 53%, and only one isolate had vanB gene. Further studies are recommended regarding the prevalence of vancomycin resistant *Escherichis coli* in drinking & ground water. In addition, a relationship should be conducted between vancomycin resistant genes and MIC vancomycin antibiotic in water surface.

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