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RESEARCH PAPER

Molecular characteristics and antimicrobial resistance patterns of *E. coli* O157:H7 Isolated from hospitals wastewaters in Erbil City

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A B S T R A C T:

The current study was conducted to detect virulence genes and perform antimicrobial susceptibility testing of *Escherichia coli* serotype O157:H7 in 100 wastewater Samples collected from seven hospitals in Erbil city, including (Hawler Teaching Hospital, West Emergency Hospital, Central Emergency Hospital, Raparin Teaching Hospital, Rizgari Teaching Hospital, Surgical Specialty Hospital/Cardiac Center and East Emergency Hospital). As a result of the present study, four samples of *E. coli* serotype O157:H7 were isolated from the total number of wastewater Samples collected. Furthermore, the isolated samples were confronted with fourteen antimicrobials, including Ceftazidime (CAZ30), Rifampicin (RD5), Amoxicillin/Clavulanic Acid (AMC30), Ceftriaxone (CRO30), Cefotaxime (SXT30), Piperacillin-Tazobactam (TZP110), Aztreonam (ATM30), Tetracycline (TE30), Amikacin (AK30), Ferodantine (F300), Tobramycin (NN10), Amoxicillin (AML25), Imipenem (IPM10), and Ciprofloxacin (CIP5) to conduct an antimicrobial susceptibility tests for each isolated sample, and The results demonstrated that the isolates had strong resistance levels to Rifampicin (RD5) and Amoxicillin (AML25), but were susceptible to Piperacillin-Tazobactam (TZP110), Aztreonam (ATM30), and Ciprofloxacin (CIP5). Further four virulence genes were investigated by PCR, and these genes are (*stx1*, *stx2*, *eae*, and *uidA*). According to the results of the current study, the stx1 and stx2 genes were not detected in the isolated *E. coli* O157:H7 serotype. It is concluded that the incidence of *Escherichia coli* O157:H7 is low in hospitals.

KEYWORDS: Virulence gene, *Escherichia coli* O157:H7, Antimicrobial susceptibility, Shiga toxin.
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1.INTRODUCTION :

Sewage water is comprised of human excreta, wash water, industrial waste, and agricultural waste. It contains bacteria, fungi, viruses, parasites, and other microorganisms from the detritus of the intestines of humans, animals, birds, and other creatures (Aleisa, 2019). The discharge of untreated sewage water has healthcare implications for those who utilize this water for recreational purposes. *Escherichia coli* dominates the facultative anaerobic flora of the feces, it's also used as a water contamination guide by micro-organisms (Al-Sarawi et al., 2022).

Inflammations of the digestive and intestinal tracts are brought on via *Escherichia coli*, a member symbiotic of the gut microbiota of people and other blood warm mammals (Srinivasan et al., 2007, Pitout, 2012).

Escherichia coli is responsible for community acquired infections in the world with comorbidity diseases and associated drug burden (Orole et al., 2022). itis forming biofilm, exogenous enzymes, and harmful toxins, which are virulence factors that are used to facilitate colonization, invasions, pathogenicity, resistance to antimicrobials, and the severity of infections (Sedighi et al., 2015). Additionally, *Escherichia coli* reflects an increase in resistance genes that have been seen in *Escherichia coli* isolates during the previous generation, finding a large number of

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resistant genes in *Escherichia coli* isolates, considering that the acquisition of resistant genes was through horizontal gene transfer in the intestinal gene pool, *Escherichia coli* may acquire resistance genes from other bacteria and pass along its own resistance genes to new generations of bacteria because it is both a donor and a recipient of resistance genes (Poirel et al., 2018).

Among of the *E. coli* strains, including the majority toxigenic strains, comprising over four hundred subspecies of *E. coli* strains that generate Shiga toxins (stxs), and was coined so the toxin produced by *Escherichia coli* species is essentially similar to that produced by shigellosis type I watery diarrhea (Amézquita-López et al., 2018). *Escherichia coli*, that produces this Shiga toxin is a potentially harmful foodborne pathogen, can infect humans and cause harmful side effects. Despite the fact that human samples have been the source of most O157 serotype isolations (Kholdi et al., 2021).

Shiga toxins that are produced by *E. coli* has a wide range of virulent agents. These agents can be coded by the chromosomal genes and are frequently found in enteric pathogen islands or plasmids. *E. coli* toxins are damages endothelium cells and has the potential to result in many dangerous significant complications, including haemolytic-uremic syndrome, hemorrhagic colitis and severe colitis. Stx1 and Stx2 are two sub-divided of shiga toxin, which dominates the lethality of E.coli, and it is recognized as a crucial parameter in determining the degree of sickness. (Bai et al., 2013).

Some strains of *Escherichia coli* also may possess eae gene, a gene that produces the protein called intimin, eae gene encodes an increases polymorphic outer membrane protein, that is responsible for bacteria's close attachment to the intestinal epithelium and the effacement of the microvilli enterocytes. When associated with the (stx) gene, it serves an essential function in the capacity to cause enteric pathogens (Rosauer et al., 2022) and is characterized by enterohemorrhagic *Escherichia coli* (Fröhlicher et al., 2008). The detection of *E. coli* remains an essential aspect of establishing the microbiological safety of wastewater systems (Naidoo and Olaniran, 2014). Furthermore, the presence and activity of beta-D-glucuronidase encoded by the gene uidA in *Escherichia coli* strains such as serotype O157:H7 is routinely used to identify *Escherichia coli* (Iqbal et al., 1997).

Because of the increasing utilization of antimicrobials in both human and veterinary medicine, antimicrobial-resistant bacteria have been widely spread over the world (Reinthaler et al., 2003), and untreated waste water and manure, as well as human excretions, are two ways that antimicrobial-resistant bacteria enter the ecosystem (Ranjbar et al., 2017). This study was designed to isolate and identify *E. coli* serotype O157:H7 from Erbil local hospitals to check its presence in sewage water, determine antimicrobial susceptibility, and detect the presence of some virulence characteristics of *E. coli* serotype O157:H7.

2. MATERIALS AND METHODS

2.1 Sewage Collection Area:

In this study, 100 sewage water samples have been collected in local Erbil hospitals from June 2021 to June 2022, from Hawler Teaching Hospital, West Emergency Hospital, Central Emergency Hospital, Raparin Teaching Hospital, Rizgari Teaching Hospital, Surgical Specialty Hospital/Cardiac Center, and East Emergency Hospital. The specimens were gathered in sterile 1 L glass bottles and transferred in a cold box to the Department of Biology's research laboratory, Salahaddin University within 1 hour. To obtain bacterial identification, culture all of the samples on agar and the samples that obey the criteria of *Escherichia coli* were sent to VITEK 2 systems (version 9.02). Then, all positive samples were tested for antimicrobial susceptibility using 14 antimicrobials.

2.2 Isolation and Identification of *Escherichia coli* O157:H7:

An effort was made to isolate *Escherichia coli* serotype O157:H7, as per (Weldeselassie et al., 2021) just slightly modified. one milliliter of each sample was added to ten milliliters of peptone broth (HiMedia, India), and the mixture was then incubated at 37 °C for 24 hours. the MacConkey Agar (HiMedia, India) plates were incubated at 37°C for 24 hours after being infected with enriched samples. colonies that were pink were assumed to be *E. coli* colonies. to identify the size, shape, and arrangement of the

bacteria, gram staining was next used. *Escherichia coli* was suspected to be the organisms that were discovered because they were gram-negative, pink in color, and rod-shaped. they were also found arranged singly or in pairs.

From MacConkey Agar, one well-isolated colony was selected, streaked on Eosin Methylene Blue Agar (HiMedia, India), and incubated for 24 hours at 37°C. *Escherichia coli* may be identified based on the formation of colonies that have the distinctive green metallic sheen. For further biochemical analysis, such colonies were transferred from Eosin Methylene Blue Agar to Moller-Hinton agar. conventional biochemical tests (Indole test, Catalase test, Citrate utilization test, Urease production test, and Triple Sugar Iron test) they were utilized a proof of identifying, to detect and identify *Escherichia coli* serotype O157:H7, colonies that were identified as *Escherichia coli* were further sub cultured onto Sorbitol MacConkey Agar. the hemolysis test Following bacterial inoculation on blood agar plates and a 24-hour incubation period at 37°C, the medium around the bacteria growing there was tested for changes in transparency that indicate complete hemolysis (beta hemolysis). However, the Vitek-2 system was used for the final confirmation, and it was set up according to the manufacturer's instructions (BioMerieux, Marcy L'Eyoily, France) by utilizing a gram-negative card.

2.3 Antimicrobial Susceptibility Test:

Four *E. coli* O157:H7 isolates were examined for susceptibility to 14 antimicrobes using the disc diffusion method (Dos Santos et al., 2021). with antimicrobial disks (Liofilchem, Roseto degli Abruzzi, Italy) as follows: ceftazidime (CAZ30), Rifampicin (RD5), Amoxicillin/Clavulanic Acid (AMC30), Ceftriaxone (CRO30), Cefotaxime (SXT30), Piperacillin-Tazobactam (TZP110), Aztreonam (ATM30), Tetracycline (TE30), Amikacin (AK30), Ferodantine (F300), Tobramycin (NN10), Amoxicillin (AML25), Imipenem (IPM10), and Ciprofloxacin (CIP5). The examined *E. coli* O157:H7 isolates were grown on Müller Hinton agar plates (Biokar Diagnostics, Beauvais, France) and incubated at 37 °C for 24 hours. According to the National Committee for Clinical Laboratory Standards (CLSI 2020), isolates were categorized as being either susceptible, intermediate, or resistant to each antimicrobe. (Weinstein and Lewis, 2020).

2.4 Molecular Study of Isolated *E. coli* O157:H7:

All *E. coli* O157:H7 isolates were screened for the presence of Shiga toxin-producing *Escherichia coli* virulence genes that include stx1, stx2, eae, and uidA, by PCR.

2.4.1 Bacterial Genomic DNA extraction:

Bacterial strains were sub-cultured in peptone broth (Merck, Germany) and further incubated for 48 h at 37 ºC. The DNA was extracted from *Escherichia coli* serotype O157:H7 colonies using the DNA extraction kit (Geneaid/Korea) according to the manufacturer's instructions. Then the concentration and purity of genomic DNA extracted from each bacterial suspension samples were determined using Nano-Drop ™ (Thermo Scientific, USA) spectrophotometer by recording the concentration ranged (288.04 ng/µl -600.94 ng/µl) and purity (1.70-2.34) for each sample (Psifidi et al., 2015).

2.4.2 Molecular identification for Shiga toxins, *eae and uidA* virulence genes:

The targeted Shiga toxins (stx1and stex2) were amplified via Multiplex-PCR using Thermocycler machine (Techne/UK). The PCR reaction was performed in the total volume of 25µl (12.5 µl Gotaq Green Master Mix (Promega/USA), 3µl of genomic DNA, 1 µl for each stx1 forward primer:5'-CAACACTGGATGATCTCAG-3', stx1 reverse primer:5'-CCCCCTCAACTGCTAATA-3', stx2 forward primer: 5'-ATCAGTCGTCACTCACTGGT-3', stx2 reverse primer: 5'-CTGCTGCTGTCACAGTGACAAA-3' (Hessain et al., 2015) and the volume completed with 5.5µl nuclease free water. The program cycles were as follows: initial denaturation: 5 min at 95°C, followed by 35 cycles at 95°C for 45 sec, 55°C for 45 sec, 72°C for 45 sec and final extension 72°C for 7min. The molecular technique was used for detection of eae and uidA genes separately. The PCR amplification was done in the total volume of 25µl (12.5 µl Gotaq Green Master Mix (Promega/USA), 3µl of genomic DNA, 1 µl for each eae forward, 5′ GTTCACTGGACTTCTTATTACCG 3′ and reverse, 5′ ATCGTCACCAGAGGAATC

3′(Beneduce et al., 2008) and uidA forward, 5′ TGGTAATTACCGACGAAAACGGC 3′ and reverse, 5′ ACGCGTGGTTACAGTCTTGCG 3′ (Molina Rodríguez et al., 2015). The PCR condition for eae gene: denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 30sec, 52°C for 30 sec and 72°C for 50 s and the final extension at 72°C for 8 min while the program cycle for uidA as follow 95°C for 5 min, followed by 35 cycles of 95°C for 30sec, 59°C for 30 sec and 72°C for 50 s and the final extension at 72°C for 10 min.

2.5. Statistical Analysis

The statistical analysis was performed using GraphPad Prism 8.0.1 software (GraphPad Software, Inc.). D'Agostino & Pearson test and Shapiro-Wilk test were applied to determine normality and lognormality. Because the data did pass the normality test, statistical differences were determined using One -way ANOVA with post-hoc analysis by Tukey test. The data were expressed as Mean±S.E, and the significance level was set at $P<0.05$.

3.RESULTS AND DISCUSSION

3.1. RESULTS

3.1.1 Occurrence of *E. coli* serotype O157:H7 in swage water samples:

Four *Escherichia coli* O157:H7 serotypes were detected in a total of 100 samples of sewage water from various hospitals (24.91%). Out of these four O157:H7 serotypes of *Escherichia coli* one sample (6.6 %) was isolated from Rizgari teaching hospital, one samples from Raparin teaching hospital (7.14 %), and two samples (11.11 %) from surgical specialty hospital (Table 1), Over all samples collected, the result was 4%, without mentioning the sites of sample collection.

The isolates were identified as gram-negative rods using the Gram stain, the colonies on agar medium were analysed based on their colony morphology, cell morphology, and biochemical assays.

Escherichia coli were identified by lactose fermentation and thus grew as smooth, shiny, pink colonies on MacConkey agar. In contrast to other microbiota, *Escherichia coli* serotype O157:H7 does not degrade sorbitol and produces a colorless colony. Therefore, sorbitol MacConkey agar instead of lactose used as a differential medium for the detection of *Escherichia coli* serotype O157:H7. Moreover, on blood and Eosin Methylene Blue agar, it generates a distinct zone of hemolysis with a greenish metallic sheen, respectively. The isolates tested positive for indole while testing negative for citrate utilization, urease, and oxidase, according to biochemical assays. With a high degree of probability, *Escherichia coli* serotype O157:H7 was confirmed by VITEK 2 system.

3.1.2 Assay of Antimicrobial Activity against Pathogenic Organisms

The antibiograms of the isolates were examined against fourteen antimicrobe. The outcomes were recorded after incubation. The zone's diameter was matched to the interpreted zone size Table2. In the present study, the result statistically demonstrates that, Rifampin (RD5) and Amoxicillin (AML25) are the most resistant antibiotics, while Piperacillin-Tazobactam (TZP110), Aztreonam (ATM30), Imipenem (IPM10), and Ciprofloxacin (CIP5) are the most sensitive antimicrobials against *Escherichia coli* serotype 0157:H7 as shown in Table2 and figure1.

3.1.3 PCR Amplification of Virulence Genes of *E. coli* serotype O157:H7 A total of four *Escherichia coli* serotype O157:H7 isolates and one ATCC (43888) were analyzed by PCR. It has been shown to not have the stx1 and stx2 genes. Also, through the same current study to perform the identification and detection of two other genes, it was found that three of the isolated samples and ATCC (43888) possess the genes eae and uidA, which are among two samples isolated from the Surgical Specialty Hospital/Cardiac Center, one of which possesses these genes, and the other two samples were obtained from Rizgari Teaching Hospital and Raparin Teaching Hospital, respectively, as shown in Figures 2 and 3.

Table 1. Isolation and Detection of *E. coli* O157:H7 from Different Hospitals Samples in Sewage Water**.**

	Hospitals	No. of Collected	No. of Positive	Percentage
study Areas	Rizgari teaching hospital	15	1	6.66%
	Hawler teaching hospital	14	0	0
	west emergency	15	0	0
	east emergency	13	0	0
	Raparin teaching hospital	14	1	7.14%
	surgical specialty hospital	18	2	11.11%
	central emergency hospital	11	0	0
	Total	100	4	24.91%

Table 2. Antimicrobial susceptibility testing of *E. coli* O157:H7 isolates.

Antimicrobial agents	Result				Zone Diameter Break Points nearest mm		
	Surgical Specialty Hospital/Cardiac Center 1	Surgical Specialty Hospital/Cardiac Center 2	Raparin Teaching Hospital	Rizgari Teaching Hospital	Sensitive	Intermediate	Resistant
Ceftazidime (CAZ30)	22.5mm	24.5mm	25.5mm	28mm	≥ 18	15 - 17 ^	≤ 14
Rifampin (RD5)	11mm	7.5mm	6.5mm	14.5mm	≥ 20	17 - 19 ^	≤ 16
Amoxicillin/Clavulanic Acid (AMC30)	16mm	17mm	14.5mm	17.5mm	≥ 18	14 - 17 ^	≤ 13
Ceftriaxone (CRO30)	28.5mm	30mm	33mm	24mm	≥ 23	20 - 22 ^	≤ 19
Cefotaxime (SXT30)	27.5mm	31.5mm	30mm	27.5mm	≥ 26	23 - 25 ^	≤ 25
Piperacillin - Tazobactam (TZP110)	29mm	31.5mm	31mm	29mm	≥ 21	18 - 20 ^	≤ 17
Aztreonam (ATM30)	29mm	31.5mm	29.5mm	30mm	≥ 21	18 - 20 ^	≤ 17
Tetracycline (TE30)	22.5mm	24.5mm	25mm	22.5mm	≥ 15	12 - 14 ^	≤ 11
Amikacin (AK30)	19.5mm	22.5mm	20mm	25mm	≥ 17	15 - 16 ^	≤ 14
Nitrofurantoin (F300)	21.5mm	24mm	22.5mm	25mm	≥ 17	15 - 16 ^	≤ 14
Tobramycin (NN10)	18mm	22mm	17.5mm	23mm	≥ 15	13 - 14 ^	≤ 12
Amoxicillin (AML25)	12.5mm	12.5mm	11mm	13mm	≥ 17	14 - 16 ^	≤ 13
Imipenem (IPM10)	28mm	30mm	30.5mm	29.5mm	≥ 23	20 - 22 ^	≤ 19
Ciprofloxacin (CIP5)	29mm	32mm	30mm	28.5mm	≥ 26	22 - 25 ^	≤ 21

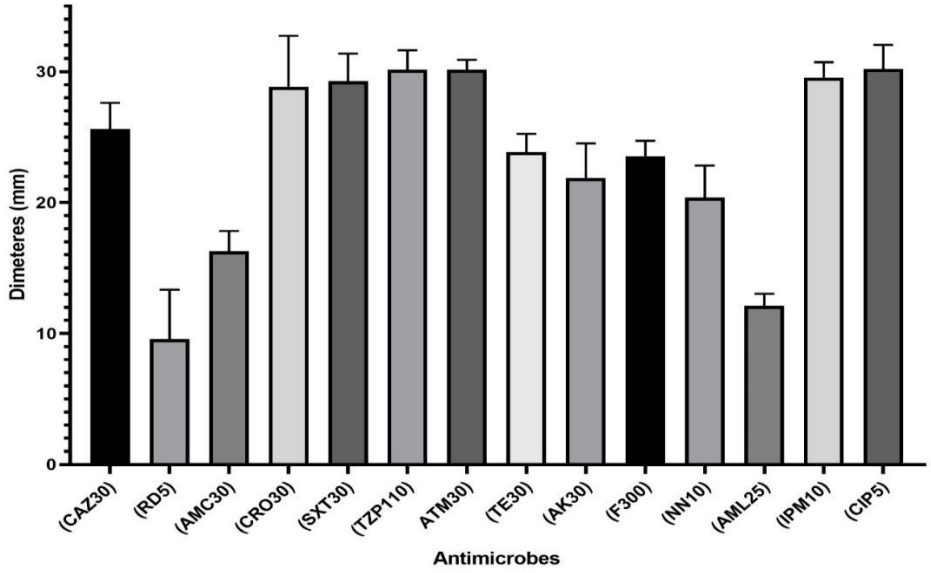

Figure 1. Comparison of AST results for antimicrobics (table of statistics).

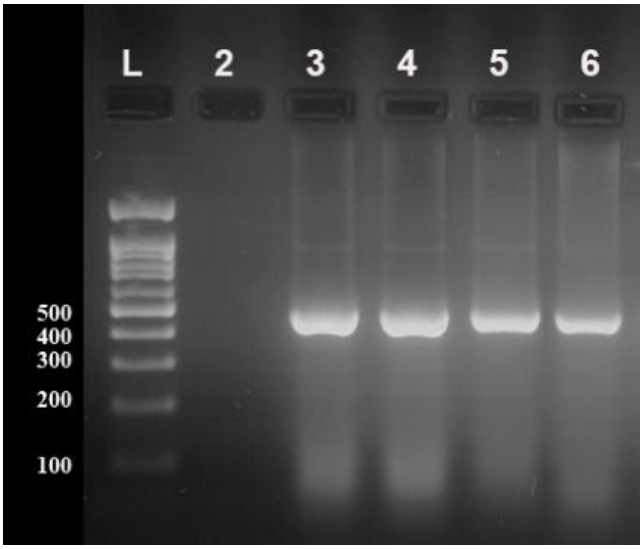

Figure 2. Agarose gel (2%) of DNA extracted from the *E. coli* O157:H7 isolated from sewage samples and *E. coli* control strains (ATCC 43888). Lane 1 DNA marker, Lanes 2and 3 fragments amplified from DNA of *E. coli* O157:H7 isolates obtained from surgical specialty hospital samples; Lanes 4 fragments amplified from DNA of *E. coli* O157:H7 isolates obtained from Rizgari teaching hospital samples; Lanes 5 fragments amplified from DNA of *E. coli* O157:H7 isolates obtained from Raparin teaching hospital samples; Lanes 6 fragments amplified from DNA of obtained from *E. coli* O157:H7 (ATCC 43888), respectively for detecting *eae* gene using PCR size 482 bp.

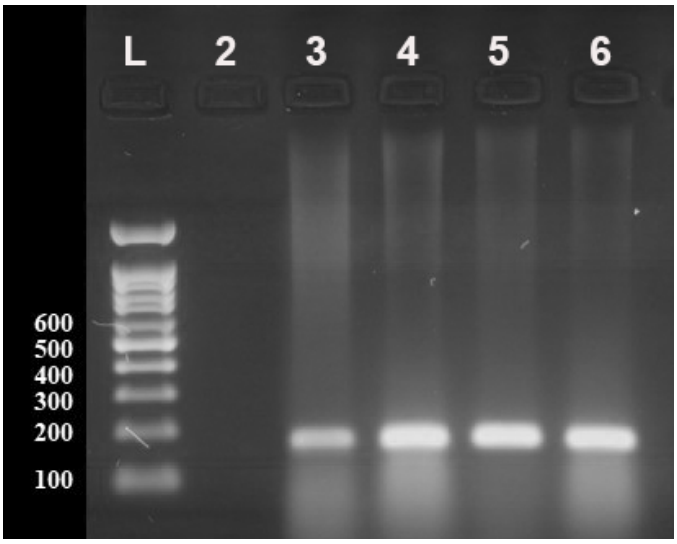

Figure 3. Agarose gel (2%) of DNA extracted from the *E. coli* O157:H7 isolated from sewage samples and *E. coli* control strains (ATCC 43888). Lane 1 DNA marker, Lanes 2and 3 fragments amplified from DNA of *E. coli* O157:H7 isolates obtained from surgical specialty hospital samples; Lanes 4 fragments amplified from DNA of *E. coli* O157:H7 isolates obtained from Rizgari teaching hospital samples; Lanes 5 fragments amplified from DNA of *E. coli* O157:H7 isolates obtained from Raparin teaching hospital samples; Lanes 6 fragments amplified from DNA of obtained from *E. coli* O157:H7 (ATCC 43888), respectively for detecting *uidA* gene using PCR size 162 bp.

3.2. DISCUSSION

The present study demonstrates that the numerous reports of *Escherichia coli* serotype O157:H7, which has mostly emerged in recent years as a causative agent of bloody diarrhea in humans and sometimes leads to further complications such as hemolytic-uremic syndrome (Sibanyoni et al., 2017, Ateba and Akindolire, 2019).

The presence of *Escherichia coli* serotype O157:H7 in or around materials and tools in

hospitals poses a great danger to human life, especially in operating theater rooms as well as sterilization tools (Dexter et al., 2020) The presence of *E. coli* serotype O157:H7 in or around supplies and equipment in hospitals poses a serious risk to patient safety, particularly in operating rooms and with sterilization equipment and kitchens (Ekici and Dümen, 2019). To highlight this topic, one hundred wastewater samples were collected from Erbil city hospitals. Only four *Escherichia coli* serotype O157:H7 have been isolated. This is considered a good thing according to the isolated percentage due to the excessive use and application of different kinds of detergents, disinfectants, and sterilization materials by hospital staff. The bacteria are killed by alkaline detergents containing sodium hydroxide, or KOH, and sodium hypochlorite (Sharma and Beuchat, 2004) or killed by hydrogen peroxide, the potential disinfectant application (Rutala and Weber, 2015).

Antimicrobial resistance is critical since it's possible for bacteria that environmental bacteria may play a key role as antibiotic resistance reservoirs. In this study, 14 antimicrobials were examined for antimicrobial susceptibility tests. The *E. coli* O157:H7 has the highest resistance rate for each of Rifampin (RD5) and Amoxicillin (AML25). These findings were in agreement with the previous reports by (Iweriebor et al., 2015, Mashak, 2018). This resistance may arise from the fact that bacteria from various water sources and habitats exchange resistance genes. As a result, Antimicrobial medications for humans are consequently rendered useless, raising the price of medical services (Manyi-Loh et al., 2018, Mudryk et al., 2010). However, they showed more sensitivity to Piperacillin-Tazobactam (TZP110), Aztreonam (ATM30), Imipenem (IPM10), and Ciprofloxacin (CIP5) This is consistent with the results (El-Shatoury et al., 2015).

In the current study focusing on virulence factors of Shiga toxin-producing *Escherichia coli*, especially the stx1, stx2, eae, and uidA genes for the detection of the pathogenic *E. coli* serotype O157:H7, the genes coding for the Stx1 and Stx2 *E. coli* shiga-like toxins were examined. These virulent factors negatively affect the host and cause many health problems in the human body (LeBlanc, 2003). The results showed that the absence of both Stx1 and Stx2 genes in all of the isolated samples was also not found in the control strain ATCC (43888) which agrees with the findings reported by (Mellmann et al., 2008) which stx loss is connected to serotype or stx subtype is a theory that is supported by the majority of the currently available data (Senthakumaran et al., 2018). In seven distinct patients infected with Shiga toxin-producing *Escherichia coli* O26:H11 and O157: NM, there is observed progressive stx loss. Another study from the same group discovered that by the time of testing, 5% of hemolytic uremic syndrome patients infected with Shiga toxin-producing *Escherichia coli* O26:H11, O103:H2, O145:H28, and O157:H7 had lost stx (Bielaszewska et al., 2007). This findings are conflicted as other studies have displayed that stx genes are more consistently kept up in *Escherichia coli* O157:H7 strains than in non-O157 strains (Joris et al., 2011).

Most, though not all, *E. coli* serotype O157:H7 isolates express intimin, as do three of four sewage isolates and ATCC (43888). The result is agreed upon (Law, 2000). The intimin encoded by eae gene has a strong association with diarrhea (Sandhu et al., 1996). eae gene encodes 17 antigenically different intimin related with several illnesses (Etcheverría and Padola, 2013). The O group was correlated with the presence or absence of the eae gene. The eae gene was shown to be associated with several serotypes that all linked to livestock or human illness, such as O5: NM, O26:H11, O69:H11, O103:H2, O111:NM, O145:NM, and O157:H7. In addition, the O5, O26, O111, and O157 are the most prevalent O serogroups among typed eae positive isolates (Adak et al., 2022). Several findings, however, contradict the current study, stating a low rate of eae gene isolates from more *E. coli* O157 samples, particularly those associated with human feces or animal studies (Schmidt et al., 1994).

According to the several study for detecting and isolation uidA gene and their spatiality from *E. coli* serotype O157:H7 by many ways but more accurate is PCR as Also followed in our study that from four isolated O157:H7 serotype samples three of them were positive it’s contrast with (Saxena et al., 2015). The sequence variability of uid in O157:H7 isolates was higher than in other *E. coli* isolates, indicating that the gene is polymorphic. There are two nucleotide changes between O157:H7 and other *E. coli* that cause the gene to be inactive; as a result, O157:H7 is often unable to ferment sorbitol. Several system for distinguishing O157:H7 from other *E. coli* are based on this inability to digest sorbitol (Feng and Lampel, 1994).

3.3 CONCLUSION

In the current study we concluded that the occurrence of *Escherichia coli* O157:H7 are few in the hospitals due to excessive use of detergent, disinfectant and sterilizers. Furthermore, the antimicrobial test proved that the *Escherichia coli* O157:H7 have more resistance against rifampin and amoxicillin in contrast, highly sensitive to Piperacillin-Tazobactam, Aztreonam, Imipenem, and Ciprofloxacin. It is remarkable in this investigation because neither the isolated samples nor the ATCC have the stx1 or stx2 genes. Additionally, the eae and uidA genes' molecular detection may aid in the diagnosis of *E. coli* O157:H7.

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