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Isolation of ferric Yersinia bactin A (*fyuA*) as virulence gene and biofilm forming in *Escherichia coli* was Collected from patient with UTI

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ABSTRACT: The focus of the current investigation was applied on determining the presence of ferric yersiniabactin uptake A (*fyuA*) virulence gene and identifying the antibiotic resistance properties in biofilm forming *Escherichia coli* isolated from recurrent urinary tract infection patients and healthy individuals.
In the Microbiology laboratory at Al Yarmouk hospital in Baghdad, Iraq, 20 (16 females and 4 males) UTI-diagnosed patient urine and stool samples, as well as 20 (10 females and 10 males) healthy individual stool samples, were collected. The samples were exposure to series test including O and H antigen based identification using an antibody (polyvalent and monovalent)-directed agglutination test, antibiotic sensitivity testing using the Kirby Bauer disc diffusion method, Congo-red agar method-based biofilm formation identification, and virulence gene identification using polymerase chain reaction (PCR).Results: 40 and 20 strains were discovered from UTI patients and healthy persons, respectively, in which O serogroups were found in 7(35%) and 3(15%) of those strains, respectively. Furthermore, only two strains of enterohemorrhagic E. coli (O157 H7) and enteroaggregative E. coli (O44 H7) were found in the UTI-based urine samples. Multidrug resistance (MDR) was detected in 60% of the biofilm positive producers (BPPs) isolated from UTI urine samples; however, only 20% of the biofilm negative producers (BNPs) isolated from UTI urine samples displayed MDR features. In the instance of virulence genes, a link was shown between fyuA (a gene that codes for an outer-membrane protein called ferric yersiniabactin uptake) and biofilm in 75% and 60% of UTI urine and stool strains, respectively, and in 55% of healthy individual stool strains. The findings of the antibiotic sensitivity test revealed that the UTI strains were 90 percent, 100 percent, 65 percent, and 40 percent sensitive to imipenem, amikacin, gentamicin, and tobramycin, respectively.
Conclusion:The present research offers detailed information on the biofilm, virulence gene, and antibiotic resistance features of *Escherichia coli* from recurrent urinary tract infection patients and healthy people.

Keywords: Biofilm formation, *E. coli*, *fyuA* gene, UTIs. antibiotic resistance, gene expert

1. INTRODUCTION

The gastrointestinal system of both humans and animals is known to contain Escherichia coli. Together with its environment, E. coli creates a major symbiotic relationship and makes a considerable contribution to the stabilization and maintenance of the normal gut microbiota homeostasis. Due to its commensal nature, E. coli only occasionally

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causes sickness in the gastrointestinal system. However, even non-pathogenic commensal E. coli can cause disease in immunocompromised or those with GIT-barrier defects. (Yan and Polk, 2004). These strains pick up virulence factors, which are crucial in giving the bacteria the unique abilities to adapt to their specialized intestinal niches and cause disease. The bacteria acquires these traits by the transmission of genetic material to various bacteria or the environment, like plasmids, horizontal gene transfer, and/or bacteriophages. The bacteria of E. coli was often divided into four type which are caused deferent human diseases [18][12].

Urinary tract infections (UTI), which comprise cystitis and pyelonephritis, are one of the urologic pathogenic Escherichia coli (UPEC) infections that affect people most often. Numerous variables, including host immunological responses, anatomical characteristics, and bacterial pathogenicity, might contribute to UTIs. E. coli must be capable of attachment to host cells and intracellular penetration (in certain bacteria), bacterial multiplication, and several organ systems via blood-dependent dispersion to spread an illness. Asymptomatic bacteriuria refers to the presence of bacteria without any indications of discomfort in the urine as a result of colonization (ABU)[6][24]. ABU-based infections might not need medical treatment; in fact, they might give the immune system the tools it needs to protect itself from other strains of E. coli. [9][4][23]. Antibiotics can successfully cure UTIs, but the issue of bacterial resistance to antibiotics is growing, making UTIs more likely to reoccur, especially when biofilm development and virulence factors are present. [13]. The current study was done in the Al-Yarmouk Hospital in Baghdad –Iraq.

2. MATERIAL AND METHODS

2.1 Patient and sample

According to the mentioned abstract, 20 (16 females and 4 males) UTI-diagnosed patient urine and stool samples and 20 (10 females and 10 males) healthy individual stool samples were collected in the Microbiology laboratory in Al_Yarmouk hospital Baghdad -Iraq. The samples were exposed to series of tests that included H and O antigenic depend upon identification using Kirby Bauer disc diffusion method, Congo-red agar method-dependent identification of biofilm formation, and polymerase chain reaction (PCR)-relied virulence gene identification.

2.2 Phenotype tests

- **Serotyping**

The test includes an antibody (polyvalent and monovalent)-directed agglutination test for O and H antigenic identification. The test was carried out utilizing procedures derived from [14].

- **Test MIC TEST**

The antibiotic sensitivity test, which was based on the Kirby Bauer disc diffusion method, was induced using methods developed by [21].

- **Biofilm formation**

Congo-red agar method-dependent identification of biofilm formation was done using methods by [19]. in which the cultivation process was processed by placing the inoculated agar in an incubator at 37°C for overnight. The results were scanned as black colonies with weak pink slime.

2.3 Detection of virulence genes

- **DNA extraction**

The extraction of the genomic DNA was done using methods by [16] in which colonies were suspended in TE solution for the initiation of the extraction processes.

- **Polymerase chain reaction**

fyuA gene was targeted using a pair of primers; F: TGATTAACCCCGCGACGGGAA and R: CGCAGTAGGCACGATGTTGTA to amplify a region of 880bp. The primers were followed from [11] and purchased from (IDT integrated DNA Technologies, Coralville, Iowa USA). My TaqTMHS Red Mix kit (BioLine, UK) was used. Each PCR reaction tube had the ingredients of master mix at 6µl, each primer at 1µL10pmoles, DNA template at 1µl, and water for PCR at 12µl. The reaction conditions were used from [11][3] PCR products were 1.7%-agarose electrophoresed and visualized using a UV-light-based imager.

3. STATISYICAL ANALYSIS

BM SPSS software v 20.0. (Armonk, NY: IBM Corp) Data was occasionally presented as numbers and percentages (percent). When p is less than 5%, the null hypothesis is not followed.

4. RESULT AND DISCUSSION

4.1 Serotyping

40 and 20 strains were discovered from UTI patients and healthy persons, respectively, with O serogrpoups showing (35%) and 3 (15%) incidence in those strains. Furthermore, only two strains of enterohemorrhagic E. coli (O157 H7) and enteroaggregative E. coli (O44 H7) were found in the UTI-based urine samples., figure 1, 2, and 3.

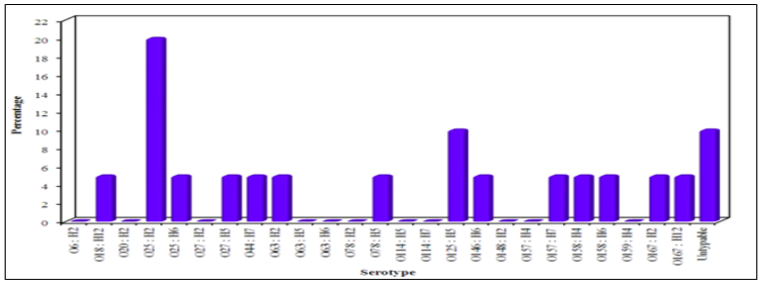

FIGURE 1. - Serogroups of UTI E. coli strains from urine samples

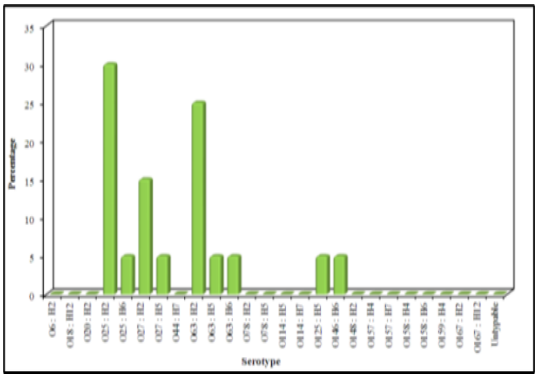

FIGURE 2. - Serogroups of UTI patients of E. coli strains from stool

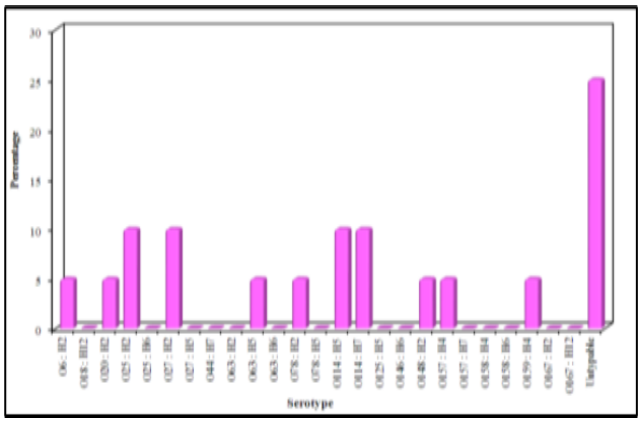

FIGURE 3. - Serogroups of E. coli strains from stool samples of healthy individuals

4.2 Test for antibiotic sensitivity

Multidrug resistance (MDR) was found in 60% of the biofilm positive producers, but only 20% of the biofilm negative consumers (BNPs) collected from UTI urine samples. The antibiotic sensitivity test findings showed that the UTI strains, the sensitivity rates to imipenem, amikacin, gentamicin, and tobramycin were 90%, 100%, 65%, and 40%, respectively.

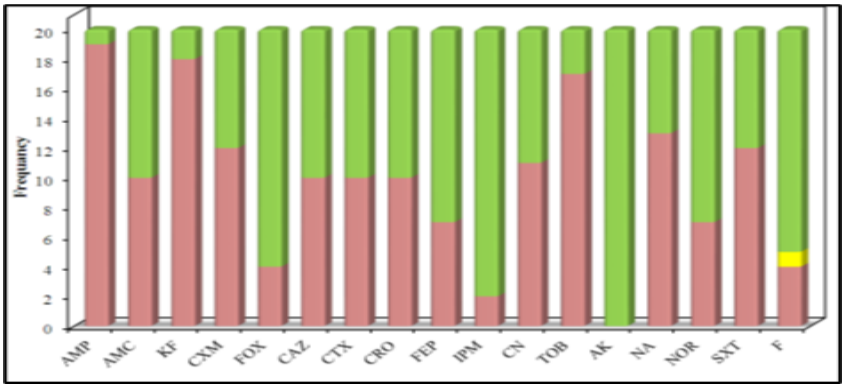

FIGURE 4. - Antibiotic sensitivity test results

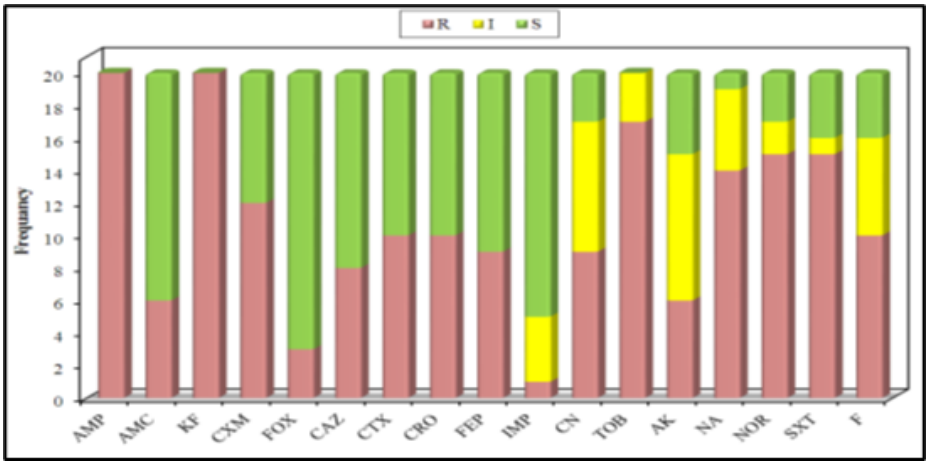

FIGURE 5. - Antibiotic sensitivity of E. coli strains from stool samples of healthy individuals

4.3 FyuA virulence gene

In the case of the virulence genes, a correlation between fyuA (a gene codes for an outer-membrane protein; namely ferric yersiniabactin uptake) and biofilm was identified in 75% and 60% of the UTI urine and stool strains, respectively, and in 55% of the healthy individual stool strains, figure 7.

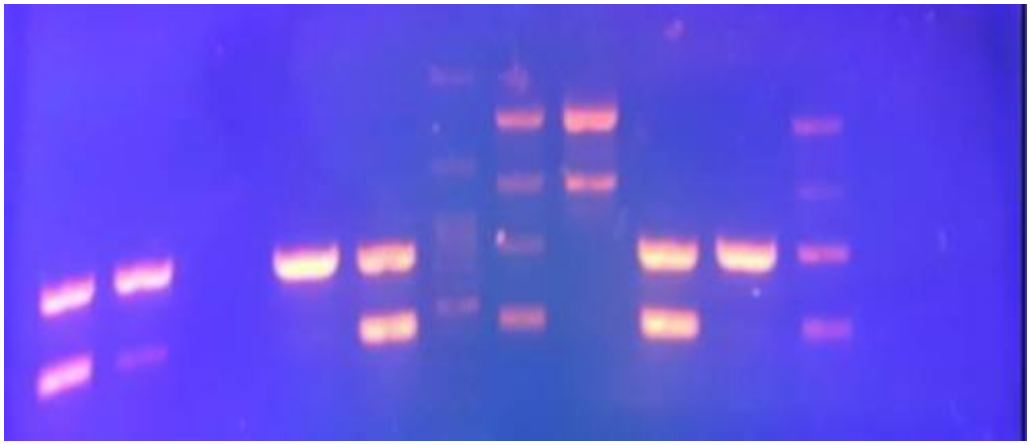

FIGURE 6. - Agarose gel stained with

5. Discussion

The human symptoms of urinary infection was a high incidence rate, and mostv of anaerobic microbial was plays a key part in their pathogenesis. A There are several virulence factors. regarded significant components of the UPEC, giving those strains with varied capacities like adhesion to host cells improved by certain proteins, such as adhesins, which play important roles in bacterial attachment to urinary epithelium. Some of these factors are related to nutrient limited sources such as environmental niche-based deficiency of iron that encourages UPEC strains to acquire certain genes encoding for iron uptaking such as fyuA gene [22]. According to the presence of those virulence factors, the UTI etiology is not well defined in understandable context. UPEC-associated O-serogroups were found at a high incidence of 60% for the O serogroup, [2]. The current study discovered that two (10%) of the isolates recovered from urine samples did not belong to a specific serogroup. This is in agreement with [2] who discovered that around (28%) of their strains in a certain serogroup were unidentifiable in their results. Issazadeh et al [10] have also discovered that 25.5 percent of their The isolates discovered were to unknown serogroups. Moreover, [15] The similar percentage of unknown strains, 25%, was reported. However, enterohemorrhagic E. coli (O157 H7) and enteroaggregative E. coli (O44 H7) were only found in two strains of UTI-based urine samples. This was also mentioned by [1] who discovered that strains of UPEC from UTIs but not from feces may cause UTIs in mice.

This bacterial technique is thought to be a defensive strategy for biofilm development in order to breach bodily borders and elude components of the immune system. The biofilm was detected in 60 percent of human illnesses [20]. The current study found that biofilm-based UPEC strains had a significant advantage over other strains, especially when it was discovered that the fyuA gene was associated to biofilm formation due to the frequent detection of this gene in those strains. This gene is increased in strains that inhabit environmental niches with iron deficit, and urine-system-related relatives are among such niches with restricted iron availability. [8][7].

6. CONCLUSION

The present work gives details information regarding the Escherichia coli-based biofilm., virulence gene, and antibiotic resistance properties from recurrent urinary tract infection patients and healthy individuals. we have looking for more study to identification of virulence factor which are due to caused infection for human.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest

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