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INVESTIGATING DRUG INTERACTIONS AND ANTIBACTERIAL ACTIVITY OF VITAMIN C ON PATHOGENIC FACTORS OF ESCHERICHIA COLI CLINICAL ISOLATES

Osamah. A Tuqi
Alkut- university college Iraq wasit

Aya Aziz Hussein
Alkut hospital for Gynecology and Pediatrics Iraq wasit

Tabark abdukarim Hussein
Alkut- university college Iraq wasit

REYADH R AL-RASHIDI
Alkut- university college Iraq wasit

Mustafa Khudair Abbas
Alkut hospital for Gynecology and Pediatrics Iraq wasit

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Abstract: Today, the phenomenon of multi-drug resistance has become a major cause of concern, and insufficient achievements have been made in the development of new antibiotics for the treatment of bacterial infections. Therefore, there is an unmet need for new auxiliary search. Vitamin C is one such promising supplement. The current study was conducted with the aim of explaining the antibacterial interaction effect of vitamin C in different concentrations (5-20 mg/ml) and drugs effective on Escherichia coli bacteria. In order to collect clinical samples during the months of September to November 2022, clinical samples were collected from 100 patients suspected of urinary tract infection who referred to medical centers. For identification, biochemical tests and gram staining and culture in IMViC and EMB environments were used to see metallic green polish. Then antimicrobial sensitivity test And the effect of antibiotic on biofilm formation, the effect of antibiotic and ascorbic acid on biofilm formation, biofilm inhibition rate was done for the single and combined effects of both antimicrobial substances. Finally, in order to investigate and identify the virulence gene of the isolated strains, DNA extraction was performed by PCR method. 45 samples of urinary infection culture were identified from the total of 100 samples collected, in 45 (45%) of them colonies with metallic polish were observed. 45% were men and 55% were women, and of these, a total of 45 positive E. coli cases were found in women and 17 positive cases were seen in men. Less than one year, teenagers, young and old were divided. 2 cases (2%) were in the age group of less than one year, 3 cases (3%) were teenagers, 63 cases (63%) were young and 32 cases (32%) were in the age group of middle-aged people. In a situation where the ability to form biofilm under vitamin C (ascorbic acid) treatment was not very effective and did not differ much from normal conditions, antibiotic treatment (under MBC gentamicin treatment) was somewhat successful in inhibiting biofilm formation, finally with the combination of antibiotic and Ascorbic acid was completely isolated in many resistant strains, the inability to form biofilm was evident, and this indicated the synergistic effects in the investigated composition. As the results showed, the ability to produce biofilm in the samples that had the target genes in this study was not much different from the other samples. Considering the increasing resistance to some antibiotics and wide changes in the effective spectrum of drugs and preventing the increase of drug-resistant cases, the necessity of conducting drug sensitivity tests seems very

necessary. The high prevalence of E. coli antibiotic resistance and the high percentage of resistance genes in patients suffering from infections caused by this bacterium can indicate the excessive use of antibiotics. Finally, the prevalence of antibiotic resistance is a useful marker for investigating resistance genes and can be useful in ecological investigations. Also, with clear inhibitory effects, the selected antibiotic in this study along with ascorbic acid, this vitamin can be used as a supplement in some infections caused by E. coli bacteria.

Keywords: Synergistic, Biofilm, E. Coli, Ascorbic Acid.

INTRODUCION

Dedicated to German physician Theodor Escherich (1885), proper intestinal and oral flora prevent bacterial infections in the digestive tract and produce trace amounts of vitamins K and B12. Neonatal gastrointestinal tracts populate within hours after birth. Facultative anaerobe, gram-negative short rod, commonly seen in normal human feces in large amounts (108/g). Lactose ferments and is mobile. The E. coli that causes UTIs comes from the patient's own gut flora, which includes both humans and animals (cattle). The mother's birth canal is the source of E. Coli that causes newborn meningitis; contaminated food or water is the source of E. Coli that causes traveler's diarrhea. Vitamin C is also known as ascorbic acid or ascorbate. Humans are one of the few organisms that cannot synthesize vitamins. Fruit bats, guinea pigs, primates, and certain birds are examples of species that cannot synthesize vitamin C. The last enzyme in the vitamin C production pathway, gulonolactone oxidase, is lacking, preventing the body from generating vitamin C. Vitamin C was isolated in 1928 and its structure determined in 1933. Scurvy, which is caused by a lack of vitamin C, has been prevalent for generations. Among the most famous stories are those of British sailors who regularly died at sea from scurvy Ascorbate has a reduction potential, which allows it to easily provide electrons or hydrogen ions to replace other antioxidants such as glutathione, vitamin E, and uric acid, as well as reduce a range of reactive oxygen species (ROS) and nitrogen species (RNS). Before oxidants harm the nucleus and cell lipids, they interact with them in the aqueous phase (blood or intracellular).There is some dispute over the relationship between vitamin C and cancer. Research has found a link between Vitamin C and stomach, esophagus, and oral malignancies. Furthermore, several clinical studies have shown that Vitamin C may improve the chances of survival for cancer patients. Vitamin C is known to have the potential to decrease and detoxify carcinogens, which might be one of its potential protective mechanisms.

LITERATURE REVIEW

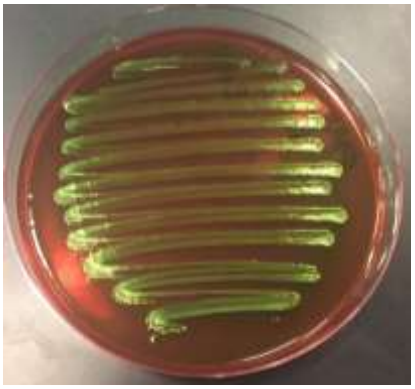
Verghese and colleagues conducted a 2017 study titled The effects of vitamin C and the antibiotic ciprofloxacin on uropathogenic bacteria E. coli using 50 isolates of E. coli from urine samples collected between August and September 2016. The samples were put in medium containing vitamin C (ascorbic acid and sodium ascorbate) at doses of 5 and 10 mg/ml, respectively, both alone and in conjunction with ciprofloxacin inoculation. lowered to 1 µg/ml. After an overnight incubation, absorbance was measured using spectrophotometry at 450 nm wavelength.The absorption values of bacterial solutions decreased as the vitamin C level rose.The combination of ciprofloxacin and vitamin C did not significantly reduce absorption (p-value=1). When on E. coli, Ascorbic acid and ciprofloxacin don't harmonize. No uropathogen was detected. However, ascorbic acid notably boosted restrained E. Coli when tested independently. Vitamin C, known for its antibacterial properties, is commonly used alongside antibiotics for UTIs, as noted by Kwiecińska-Piróg et al. in 2019. Among UTI-causing bacteria like Proteus, often treated with aminoglycosides or fluoroquinolones, Mirabilis stands out as the third most prevalent. Its tendency to form biofilms on urinary catheters poses challenges. This study aims to explore how ascorbic acid and antibiotics interact with P. mirabilis's biofilm formation. Spectrophotometric analysis revealed that combining ascorbic acid (0.4 mg × ml-1) with antibiotics affected biofilm production. Without ascorbic acid, P. mirabilis strains were more effectively inhibited from forming biofilms with lower doses of aminoglycosides. However, ascorbic acid supplementation reduced the inhibitory effects of fluoroquinolones and enhanced biofilm production by P. mirabilis strains. Supplementing aminoglycoside therapy with ascorbic acid may offer a solution for UTIs caused by P. mirabilis biofilms

METHOD

Sample Collection

For the purpose of collecting clinical samples, during the months of September to November 2022, 100 clinical samples of patients suspected of urinary tract infection, who refer to medical centers, were collected. During this period, 45 bacterial isolates were isolated. Then the isolates were passaged on blood agar medium and transferred to the microbiology department laboratory in special flasks (Figure 1).

Figure 1: *E. coli* bacteria cultured in EMB medium



Purification of bacteria

With the help of a sterile loop, several colonies were removed from the colonies on the blood agar medium and inoculated in the specific and differential eosin methylene blue (EMB) culture medium. E.coli bacteria, which strongly ferments lactose and produces a lot of acid, in this environment in the presence of methylene blue eosin, creates a metallic green color, which is to some extent the key to distinguish E.coli bacteria from other intestinal bacteria. It is (31). Biochemical tests performed to determine the collected strains, The following biochemical tests were used to identify the isolates: warm reaction, culture on McConkey's medium, catalase, oxidase, IMViC test (Indole, Methyl red), Voges-Proskauer test and consumption of citrate), fermentation of different sugars such as glucose, sucrose and lactose, movement and production of H2S and oxidative/fermentative reaction. Tests performed after the identification of bacteria , Tests performed after the identification of bacteria : Determination of antimicrobial sensitivity in isolated strains , Antimicrobial sensitivity test , Investigating the ability of biofilm formation under the influence of ascorbic acid , The effect of antibiotics on biofilm formation , The effect of antibiotics and ascorbic acid on biofilm formation , Biofilm inhibition rate.

RESULTS

Frequency of isolates in terms of tested samples Collection of samples, isolation and identification of isolates : Number of 45 samples from urinary infection culture Submissions were collected to medical diagnosis laboratories and medical centers and hospitals in the months of September to November. From a total of 100 collected samples, it was isolated that its contamination in terms of bacteria E.coli was checked in the microbiology laboratory, in 45 (45%) samples, colonies with metallic polish were observed according to figures 1. with confirmatory tests including the observation of gram-negative coccobacilli in gram staining, creating a purple colony on McConkey's medium and biochemical tests including positive catalase test, acid/acid reaction with gas production in TSI medium, positive motility due to the turbidity spread in the medium SIM, reddening in the vicinity of COVAX reagent and positive indole, positive MR reaction, negative VP reaction and negative citrate reaction in Simon citrate medium of the isolates as E.coli Confirmed

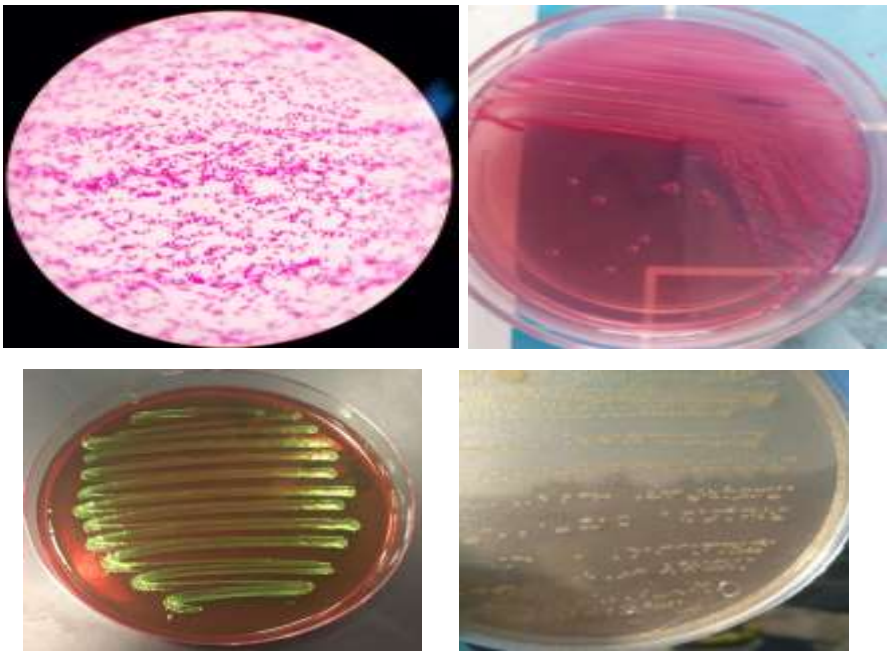


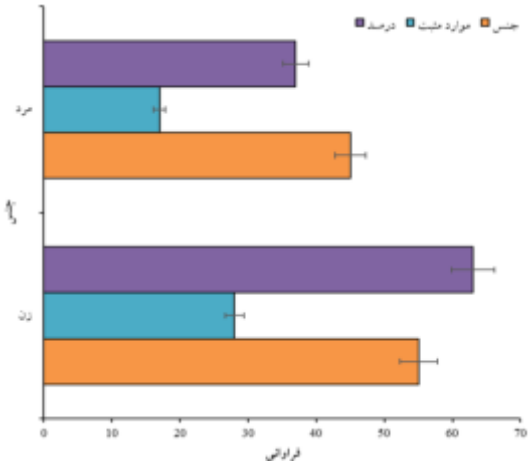
Figure 1: Colonies *E. coli* On and with green metallic polish on the surface of the environment EMB and Nutrient agar medium after 2 days of incubation

Descriptive Statistics

Frequency of patients by gender

In total, 100 cases of all patients who were sampled and studied, 45% were men and 55% were women, and out of these, 45 cases were positive , E. coli 28 positive samples were seen in women and 17 positive samples were seen in men .

Chart 1: Abundance of samples and positive cases for the presence of bacteria *E. coli* in the population under investigation



Patients based on age in 4 age groups (Age) included: children less than one year old, teenagers, young and old were divided. 2 cases (2%) were in the age group of less than one year, 3 cases (3%) were teenagers, 63 cases (63%) were young, and 32 cases (32%) were in the age group of middle-aged people (Chart 2).

PCR test results to identify target genes in E. coli strains isolated from clinical samples

- In order to choose the most suitable connection temperature, the corresponding temperature gradient (pictures 2 to 10) was done. According to the image of the bands obtained from PCR by forward and reverse primers, the optimal temperature of 68°C was chosen because the resolution of the band was better at this temperature (the desired sample number 23 was selected). In the following forms Virulence genes ibeA، hlyA، orC، fimA And fyuA has been specified.

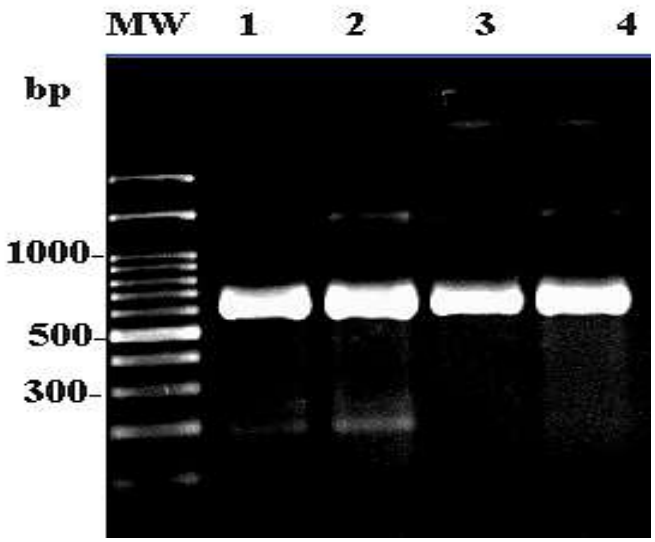


Figure 2: Temperatures chosen to perform a temperature gradient to select the binding temperature in a PCR reaction for the gene *ibeA*, respectively, the numbers from well 1 to 4 are 69-68-67-66 °C and on the left side is the molecular marker bp 1000.

ability to form biofilm in strains E. coli

- According to Table 3, also in molecular studies among the isolates E. coli isolated from urinary and gastrointestinal infections, almost more than half of them had at least one of the virulence genes in question

Table3: Frequency *E. coli* isolated from the collected samples along with the samples with at least one virulence gene and the ability to form biofilm under normal conditions

Separate number	have genes	Biofilm	Separate number	have genes	Biofilm
1	✓	Strong	24	×	medium
2	×	weak	25	×	medium
3	×	weak	26	×	weak
4	×	weak	27	×	weak
5	×	weak	28	✓	Strong
6	✓	Strong	29	✓	Strong
7	✓	Strong	30	✓	Strong
8	✓	Strong	31	✓	Strong
9	✓	Strong	32	×	weak
10	✓	Strong	33	×	weak
11	✓	Strong	34	✓	Strong
12	✓	medium	35	✓	Strong
13	✓	Strong	36	✓	Strong
14	✓	Strong	37	✓	Strong
15	✓	medium	38	✓	Strong
16	×	weak	39	✓	Strong
17	×	weak	40	✓	Strong

18	✓	medium	41	✓	medium
19	×	weak	42	✓	medium
20	×	weak	43	✓	Strong
21	×	weak	44	×	weak
22	✓	Strong	45	×	weak
23	✓	Strong	-		

DISCUSSION

In this study, bacterial resistance Escherichia coli uropathogen (UPEC) causing urinary tract infection compared to antibiotics was investigated. The strains isolated in this study show the highest antibiotic resistance to ampicillin, cotrimoxazole and ciprofloxacin. 72% of the isolates were resistant to ampicillin, 42% of the isolates were resistant to cotrimoxazole and 22% of them were resistant to ciprofloxacin. Similar outcomes were seen in a 2004 research carried out by Mathai and his associates (Mathai et al. 2004). The amikacin sensitivity of the Escherichia coli recovered in this study was greatest; in 2011, Khairabadi and colleagues reported that 98% of the Escherichia coli isolated from samples of urine was amikacin sensitive. Additionally, 95.3 percent of the isolates in a study by Milani et al. in Tabriz during 1998 and 2005 on the antibiotic susceptibility of bacteria isolated from UTI patients were the most resistant to ampicillin, and they shown the least resistance to ciprofloxacin (10.2%) and amikacin (6.6%). The resistance to ampicillin was greater in this study than in ours, and every isolate in our investigation demonstrated amikacin sensitivity. Over 47% of the isolates in this investigation were resistant to more than two antibiotics due to the multiple resistance (MDR) that some of the bacteria exhibited. In a study conducted by Eslami et al. in Iran in 2010, 85.5% of the isolates showed resistance to more than two antibiotics. Such antibiotic resistance among Escherichia coli strains causing urinary tract infections creates complex problems for experimental treatments of infections.The formation of biofilm causes bacteria to become resistant to antimicrobial agents. This biofilm resistance against harmful factors has many harmful aspects in different fields including industry. The first stage of biofilm formation is the attachment of bacterial cells to surfaces, in which many factors play a role, including the movement of bacteria, the hydrophobicity of their surface, the type of surface to be attached, etc. Rosenberg et al. reported that the high hydrophobicity of the surface of the cells causes bacteria to have a greater tendency to attach to the surfaces, which results in the formation of a biofilm and its destructive effects (Rosenberg, 1980). Many researchers pointed out the role and importance of high hydrophobicity for bacteria attachment and biofilm formation. In 2005, Cerca et al. confirmed the relationship between hydrophobicity and bacterial attachment (Cerca et al. 2005). In 2001, Frank showed that bacteria are selected to form biofilms on surfaces based on surface characteristics such as the presence of capsules, fimbriae and hydrophobicity of the cell surface (Frank. 2001). In this study, 60% of the isolates were strong, 16% medium and 20% weak in terms of biofilm formation ability. In 2011, Sevanan et al. used the tube method of biofilm formation and showed that 9.4% of the isolates were Strong Positive, 34.4% of the isolates were Positive, and 40.6% of the isolates were Weakly. Positive and 15.6% were negative. which showed more biofilm formation compared to the microtiter plate method.

CONCLUSION

This comprehensive study examines the complex dynamics of Escherichia coli (E. coli)-caused urinary tract infections (UTIs) and examines their resistance patterns to antibiotics, including ampicillin, cotrimoxazole, and ciprofloxacin. Through meticulous analysis, it is discovered that a large portion of the isolated E. coli strains show resistance to these antibiotics, highlighting the growing problem of antibiotic resistance in UTI treatment.

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