Antibiotic Resistance and Inflammatory Response in Urology Infections Caused by *E. coli*: A Molecular and Clinical Investigation

Amani Alhejely [1*]

Abstract

Background: Urinary tract infections (UTIs) are a common condition resulting from bacterial invasion of the urinary system, which includes the kidneys, ureters, bladder, and urethra. These infections are particularly prevalent among females due to anatomical differences and hormonal factors. UTIs can range from uncomplicated cases, where there are no structural abnormalities, to more severe infections that may ascend to the kidneys, causing pyelonephritis. Methods: This study aimed to isolate and diagnose Escherichia coli (*E. coli*) bacteria in patients with UTIs, identify specific virulence genes, and assess the bacteria's resistance to various antibiotics. A total of 100 clinical samples were collected from patients with UTIs at the General Hospital of Homadiyah and Ahmed Maher from October 2021 to December 2022. Bacterial identification was performed using biochemical tests and polymerase chain reaction (PCR) techniques, while antibiotic sensitivity was assessed using the Kirby-Bauer disk diffusion method. Additionally, inflammatory markers (TNF-α, IL-6, and IL-8) were measured using ELISA. Results: *E. coli* was isolated in 56% of the samples, with the highest prevalence in patients aged 31-40 years. The antibiotic sensitivity test revealed varying levels of resistance, with the highest sensitivity observed to third-generation cephalosporins, particularly ceftriaxone (32.2% resistance). PCR analysis identified the presence of several virulence genes, including the Hly gene in 96.6% of isolates and the iha gene in 10% of isolates. Elevated levels of inflammatory markers were observed in patients with UTIs, indicating a strong immune response to bacterial infection. Conclusion: *E. coli* is a significant pathogen in UTIs, exhibiting considerable antibiotic resistance, particularly to first-generation cephalosporins. The presence of specific virulence genes correlates with the severity of infection. These findings underscore the need for targeted antibiotic therapy and the development of strategies to manage antibiotic resistance in UTIs.

Keywords: Urology infections, *E. coli*, Antibiotic resistance, Inflammatory markers, PCR diagnostics

Significance | This study reveals the alarming antibiotic resistance of E. coli in urology infections, emphasizing the need for molecular diagnostics and targeted treatments to mitigate clinical risks.

*Correspondence. Amani Alhejely , Biology Department, Darb University College, Jazan University, Saudi Arabia
E-mail: research_inst@yahoo.com

Editor Md Shamsuddin Sultan Khan, And accepted by the Editorial Board July 20, 2024 (received for review Jun 04, 2024)

Introduction

Urinary tract infections (UTIs) are among the most prevalent bacterial infections, impacting millions of individuals globally. These infections arise when pathogenic bacteria enter the urinary tract, which includes the kidneys, ureters, bladder, and urethra. The urinary system's primary function is to filter and excrete metabolic waste and toxins from the bloodstream while maintaining fluid and electrolyte balance. Disruption or infection of this system can lead to significant health issues, including pyelonephritis, cystitis, and urethritis, among others (Kunin, 1994).

UTIs are particularly common and problematic in females, largely

Author Affiliation.
[1] Biology Department, Darb University College, Jazan University, Saudi Arabia

Please cite this article.
Amani Alhejely (2024). Antibiotic Resistance and Inflammatory Response in Urology Infections Caused by E. coli: A Molecular and Clinical Investigation, Journal of Angiotherapy, 8(7), 1-9, 9789

2207-8843/© 2024 ANGIOTHERAPY, a publication of Eman Research, USA.
This is an open access article under the CC BY-NC-ND license.
(http.//creativecommons.org/licenses/by-nc-nd/4.0/).
(https./publishing.emanresearch.org).

due to anatomical and physiological differences between genders. The female urethra is significantly shorter than that of males, measuring approximately 2.8 cm compared to over 10 cm in males. This shorter urethra facilitates easier bacterial entry into the urinary tract. Epidemiological studies suggest that between 40% and 50% of women will experience at least one UTI in their lifetime (Bollgren & Winberg, 1999). In contrast, UTIs are less common in males, though the rates have been reported to be approximately 39% (Raka et al., 2004). This disparity is partly attributed to hormonal differences and the presence of a longer urethra in males, which offers a more effective barrier against infection.

In children, the incidence of UTIs varies between genders as well. A study conducted in Australia revealed that 65% of pediatric UTI cases were in females, while 35% were in males (Cabral, 2010). Furthermore, another study estimated that the risk of UTIs before the age of 14 was 10.3% for girls and 3.3% for boys (Gunther et al., 2001). These findings underscore the gender-specific factors that contribute to the prevalence and management of UTIs.

Uncomplicated UTIs are those that occur in individuals with a normally functioning urinary tract and without structural abnormalities. These infections typically involve the bladder (cystitis) or the urethra (urethritis) and are often treatable with standard antibiotics (Lane & Takhar, 2011). However, UTIs can sometimes progress to more severe forms, including pyelonephritis, which affects the kidneys and can result in significant morbidity if left untreated (Hara et al., 2000).

A common complication of UTIs is vesicoureteral reflux (VUR), a condition where urine flows backward from the bladder into the ureters and sometimes the kidneys. This reflux can lead to recurrent infections and kidney damage if not managed properly (Bhat et al., 2011). The manifestations of UTIs vary but often include dysuria, increased urinary frequency, and lower abdominal pain (Chang & Shortliffe, 2006). Severe cases can result in systemic symptoms such as fever, nausea, and vomiting, indicating a more widespread infection (Dzidic et al., 2008).

The majority of UTIs are caused by Gram-negative bacteria from the Enterobacteriaceae family, with Escherichia coli (*E. coli*) being the most common pathogen. *E. coli* accounts for approximately 80% of UTI cases and is well-adapted to the urinary tract environment (Brooks et al., 2001). This bacterium can form biofilms and express various virulence factors, such as fimbriae, which enhance its ability to adhere to the urinary tract and evade host defenses (Wullt et al., 2000).

The body's response to a UTI involves various inflammatory markers and cytokines. For instance, interleukins (ILs) such as IL-6 and IL-8 play crucial roles in mediating the immune response to bacterial infections. Elevated levels of these cytokines can be indicative of an ongoing infection and contribute to the inflammatory process (Wullt et al., 2002). Cytokines and other inflammatory markers are critical in diagnosing and monitoring the progression of UTIs.

Treatment of UTIs generally involves antibiotics, but increasing resistance among uropathogens poses a significant challenge. *E. coli* strains resistant to common antibiotics, including beta-lactams, have been increasingly reported, complicating treatment strategies (Jacoby & Munoz-Price, 2005). Therefore, accurate identification of the causative bacteria and their antibiotic susceptibility profiles is essential for effective treatment (Fuda et al., 2004).

Recent research efforts have focused on understanding the mechanisms behind UTI pathogenesis and antibiotic resistance. Advances in molecular techniques, such as single and multiplex PCR, have improved the detection and characterization of bacterial pathogens (Al-Dupot et al., 1998). These techniques help identify specific genes responsible for virulence and resistance, providing valuable insights for developing targeted therapies (Collee et al., 1996).

In summary, UTIs are a significant health concern with complex etiology and varying presentations depending on the patient population. Understanding the underlying mechanisms, including bacterial virulence factors, host immune responses, and antibiotic resistance patterns, is crucial for effective management and prevention of these infections. Ongoing research and advancements in diagnostic and therapeutic approaches continue to enhance our ability to combat UTIs and mitigate their impact on public health.

2. Material and Methods

2.1 Preparation of Ready-Made Agricultural Media

All ready-made agricultural media were prepared according to the manufacturer's instructions. Each medium was sterilized at 121°C under 15 pounds of pressure for 15 minutes. For the Blood Agar Base, after sterilization, 7% human blood was added to the cooled medium. Similarly, a 31% urea solution was incorporated into the urea medium base following sterilization.

2.2 Preparation of Specific Media

The Motion Test Medium was created by dissolving 0.5 grams each of peptone and sodium chloride, and 0.25 grams of agar in purified water. The pH of the solution was adjusted to 7.0. The medium was then distributed into sterile glass tubes, sterilized at 121°C with 15 pounds of pressure for 15 minutes, and subsequently stored at 4°C until required.

The Methyl Red and Voges-Proskauer (MR-VP) Medium, as well as the Carbohydrate Fermentation Medium, were prepared following established protocols designed for assessing carbohydrate fermentation.

For dye solutions and reagents, the Wallet dye solution and physiological saline solution were prepared according to standard protocols. The physiological saline solution was specifically made using phosphate-buffered saline (PBS).

Sterilization of all heat-stable media, glassware, and most solutions not sensitive to heat was carried out at 168°C for 2 hours to ensure the complete removal of microbial contaminants.

2.3 Clinical Sample Collection

A total of 100 clinical samples were collected from urological cavities of patients at the General Hospital of Homadiyah and Ahmed Maher's education between October 2021 and December 2022. Samples included both blood and non-blood types. Midstream urine samples were collected in sterile tubes, discarding the initial drops to avoid contamination. These samples were plated using a planned technique and incubated at 37°C for 18-24 hours to identify bacterial growth. Additionally, 100 blood samples (5 mL per patient) were collected, with 20 extra samples taken from healthy individuals for control purposes. All blood samples were placed in sterile test tubes, centrifuged at 3,000 RPM for 15 minutes to separate the serum, and prepared for enzyme-linked immunosorbent assay (ELISA) testing.

2.4 Diagnosis of Isolated Bacteria

The appearance of bacteria was studied by examining samples directly from the culture media. Gram staining was used to assess bacterial cell wall characteristics, followed by observations of colony morphology, including color, shape, size, and edge characteristics on various media such as MacConkey and selective media. Various chemical tests were performed, including hydrogen sulfide production, urease activity, carbohydrate fermentation, and indole production. Hemolytic activity was tested using human blood agar. A 5% red blood cell suspension was added to the medium, and the presence of clear zones around colonies indicated hemolysis.

2.5 Investigation of E. coli Antibiotic Resistance

For antibiotic sensitivity testing, pure colonies from a 24-hour culture of *E. coli* in nutrient broth were used. The bacterial suspension was adjusted to the McFarland 0.5 standard. The bacteria were spread on Mueller-Hinton agar plates using a sterile cotton swab. Antibiotic disks were placed on the inoculated plates, which were then incubated at 37°C for 18-24 hours. Zones of inhibition around the disks were measured with a caliper and compared to standardized guidelines to determine antibiotic resistance.

2.6 ELISA for IL-6 Detection

Serum samples were allowed to reach room temperature after thawing. For the ELISA test, 100 µL of standard solutions and serum samples were added to wells of an ELISA plate. An additional 50 µL of enzyme conjugate was added to each well. The plate was covered and incubated at 25°C for 2 hours. After incubation, the wells were washed four times with washing solution. HRP-Streptavidin enzyme solution (100 µL) was added to each well, and the plate was incubated at room temperature for 30 minutes. Following this, the wells were washed four times. Substrate solution (100 µL) was added to each well, and the plate was incubated in the dark for 30 minutes at room temperature. Stop solution (100 µL) was then added to halt the reaction, changing the color from blue to yellow. The absorbance was read at 450 nm using a plate reader, and the results were analyzed using professional ELISA software.

2.7 Statistical Analysis

Data were described and analyzed using SPSS Version 16 and Microsoft Excel. Statistical significance and trends were determined based on the analyzed data.

3. Results

In this study, 56 samples of Escherichia coli (*E. coli*) were isolated from a total of 100 samples collected from patients with urinary tract infections. The samples were sourced from the General Hospital of Homadiyah and Ahmed Maher's educational facilities between October 2021 and December 2022.

Upon culturing on MacConkey agar, the developing colonies appeared as circular pink with bright surfaces, ranging in size from 1 to 2 millimeters. On blood agar, the colonies were small and gray with a beta-hemolytic pattern, showing a decaying zone around them. Microscopic examination of the isolates revealed that the bacterial cells were rod-shaped, Gram-negative, and exhibited a stunted, single- or double-ordered arrangement. They did not form spores.

Chemical testing of the isolates involved several diagnostic tests. The catalase test yielded a 100% positive result, indicating the presence of catalase enzyme in all isolates. Conversely, all isolates were negative for the oxidase test. The isolates were also tested on eosin methylene blue (EMB) agar, where they displayed a characteristic bright green metallic sheen, confirming their identity as *E. coli*. The Indole test showed that all isolates (100%) produced a red ring after the addition of Kovac's reagent, indicating the presence of indole. For the methyl red test, the isolates were negative, with no color change, whereas the urea hydrolysis test also returned negative results, as no color change to pink was observed. Of the 100 samples, 56 (56%) were identified as *E. coli*, while the remaining 44% contained other bacterial species. Further enzyme activity tests revealed that 85.7% of the *E. coli* isolates produced hemolysin, a significant virulence factor, while 28.3% produced a wallet enzyme.

Analysis of the demographic data showed that out of the 56 patients with urinary infections, 38 were female (67.86%) and 18 were male (32.14%). This indicates a higher prevalence of urinary infections among females. Age-wise, the highest incidence was found in the 31-40 year age group, with 17 cases (30.36%), whereas the 1-10 year age group had the fewest cases, with only one patient (1.79%).

Antibiotic sensitivity testing was performed using 16 standard antibiotics, including those from four generations of cephalosporins. The results revealed significant resistance among the *E. coli* isolates. The isolates were most sensitive to third-

generation cephalosporins, with resistance rates of 32.2% to Ceftriaxone, 35.7% to Ceftazidime, 37.5% to Cefixime, 35.7% to Cefdinir, and 30.4% to Ceftizoxime. The second-generation cephalosporins showed higher resistance rates, with Cefaclor at 59.0%, Cefonicid at 60.8%, Cefprozil at 55.0%, Cefoxitin at 64.2%, and Cefmetazole showing 62.5% resistance. First-generation cephalosporins also exhibited notable resistance, with rates of 78.6% to Cephalothin, 71.4% to Cefazolin, 69.7% to Cephalexin, and 66.1% to Cephadroxil. The fourth-generation cephalosporin Cefepime showed a resistance rate of 39.3%.

For the molecular characterization, DNA extraction was carried out from the *E. coli* isolates. Agarose gel electrophoresis of the DNA, stained with ethidium bromide and visualized under UV light, confirmed the presence of a single DNA band in all isolates. Polymerase chain reaction (PCR) techniques were employed to identify specific virulence genes. The PCR results showed that 96.6% of the isolates had the Hly gene, associated with hemolysin production, and 100% had the afa gene, which contributes to adherence. Conversely, only 30.0% of isolates contained the iha gene, which is responsible for capsule synthesis, and 10.0% had the tst gene, linked to toxic shock syndrome. Notably, no isolates were positive for the pap gene, which is involved in fimbrial adherence.

Overall, the study demonstrates a high prevalence of *E. coli* among urinary tract infection cases, with significant antibiotic resistance, particularly to second-generation cephalosporins. The molecular analysis confirms the presence of several key virulence factors, highlighting the pathogenic potential of the isolates.

4. Discussion

4.1 Insulation and Diagnosis of E. coli Bacteria:

Sample collection for this study was conducted with urology patients, focusing on diagnostic and preventive procedures. Initial diagnosis of bacterial isolates involved examining specific implantary and microscopic properties, as well as conducting diagnostic chemosynthetic tests. Blood agar was used to culture colonies, which were characterized as gray, soft, and slightly elevated, with a diameter ranging from 1 to 2 mm. These colonies exhibited beta-hemolysis. On MacConkey agar, colonies appeared bright pink, round, and approximately 1 to 2 mm in diameter, with a color change from pink to yellow due to lactose fermentation and acid production after 48 hours, aligning with findings from Dzidic, Suskovic, and Kos (2008) and Wullt et al. (2000). Microscopic analysis revealed that the bacterial cells were small, either singular or arranged bilaterally, and negative for Gram staining, consistent with Collee, Chambers, Peddie, and Mahanty (1996) and Brooks, Butel, and Morse (2001).

All isolates tested positive for the catalase test, crucial for distinguishing between aerobic and anaerobic bacteria, as indicated by the presence of air bubbles. Conversely, all isolates tested negative for the oxidase test, which is important for identifying bacterial species based on their ability to produce oxidase (MacFaddin, 2000). The use of eosin methylene blue agar yielded positive results for all isolates, with colonies showing green metallic sheen, and the indole test was positive for all isolates, which is used to differentiate *E. coli* from other enteric bacteria based on tryptophanase production (MacFaddin, 2000).

Sugar fermentation tests demonstrated that 95.3% of the isolates were positive, indicating their ability to ferment sugar, produce gas, and acid, consistent with findings from MacFaddin (2000). Urease tests were negative for all isolates, as the medium remained green, indicating the absence of urease production. All isolates also tested negative for hydrogen sulfide production, corroborating the results found by Dzidic et al. (2008).

4.2 Percentage of E. coli Bacteria in Isolations:

The results, detailed in Table 2, show that 56% of urology infections were caused by *E. coli*, which aligns with previous studies (Kunin, 1994; Raka et al., 2004). *E. coli*'s high adaptability to the human urological environment, due to its adhesive factors and ability to produce toxins, facilitates its invasion and infection of the urinary tract (Bollgren & Winberg, 1999; Gunther et al., 2001).

4.3 Gender and Age Relations with Urology:

Table 4 indicates that males were significantly more affected by urinary tract infections compared to females (67.86% vs. 32.14%), which is consistent with the findings of Lane and Takhar (2011). This may be due to anatomical differences, such as the shorter urinary tract in females and the protective effects of the prostate in males (Hooton, Steapleton, & Roberts, 2001). The results also show that urinary tract infections are more common in older adults, with age-related factors and chronic diseases contributing to increased susceptibility (Chang & Shortliffe, 2006; Hara, Brenner, & Miller, 2000).

Production of *E. coli* Bacteria for Hemolysin and Urease:

Table 3 presents data on the production of hemolysin and urease by *E. coli* isolates. Hemolysin production was observed in 85% of isolates, indicating its role in lysing red and white blood cells, which can contribute to bacterial virulence (Gunther et al., 2001). Urease production was detected in 28.3% of isolates, which is significant for its role in protecting bacteria from host defenses (Collee et al., 1996).

4.4 Antibiotic Sensitivity Test:

Antibiotic resistance remains a critical issue, with recent studies indicating a rise in resistance among *E. coli* strains, particularly to third-generation cephalosporins (Bhat, Katy, & Place, 2011). The current study found high resistance rates to various antibiotics, which aligns with findings from previous research (MacFaddin, 2000; Stukus, 1997). The resistance to Ceftriaxone (32.2%) and Cefixime (42.9%) reported here is consistent with the results of other studies (Hooton et al., 2001; Raka et al., 2004). The

Table 1. Numbers and percentages of isolated bacterial species in coronary samples.

Insulations	Number Insulations.	Number of E. coli seclusions	Other species
%	100	56	44

Table 2. Numbers and percentages of *E. coli.* Producers of the Himalayan enzyme

E. coli bacteria seclusions	Number of hemolycin-producing insulations.	Percentage (%)	Number of wallet-producing isolations	Percentage (%)
56	48	85.7	16	28.3

Table 3. Numbers and percentages of males and females infected with urinary tractors.

Type of Gender	The injured	
	%	Number
Male	32.14	18
Female	67.86	38
Total number	100	56

Table 4. Distribution of *E. coli* Disease by age group and percentage**.**

Relationship between sickness and age (year)	The injured	
	%	Number
1-10 (year)	1.79	1
11-20 (year)	7.14	4
21-30 (year)	14.33	9
31-40 (year)	31.35	17
41-50 (year)	19.88	11
51-60 (year)	11.65	6
61-70 (year)	12.52	7
Over 71 years old (year)	2.63	2

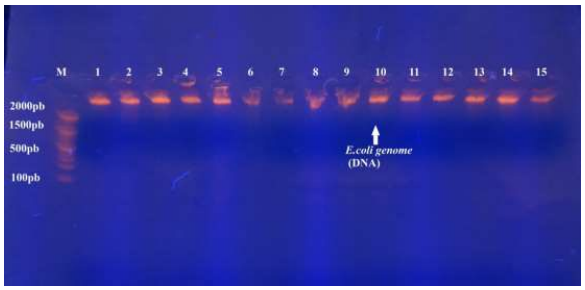

Figure 1. DNA extraction products by electro-deportation on agaroz gel (%1.5) and for an hour for coli. Isolations using Kit Mini DNA Genomic. Where M = DNA

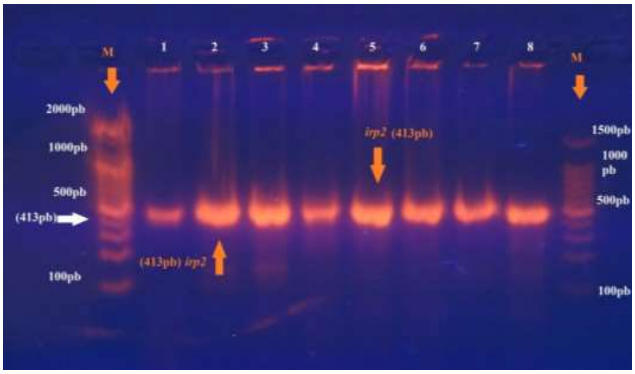

Figure 2. Gene magnification products 2irp for bacteria (*E. coli*) using PCR Single and PCR Single The stage is electrically on the Agaruz gel (%1.5) and voltage (100) for an hour. Since M = DNA bp100-2000Ladder, all the isolations showed a positive result for Jen 2irp, who is responsible for taking iron from the host's cell.

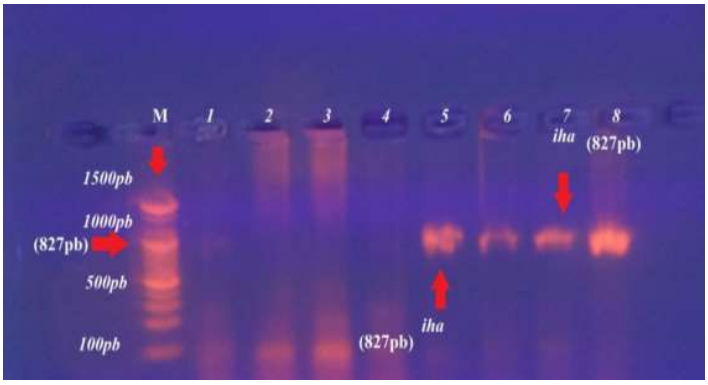

Figure 3. Gene iha magnification products for *E. coli* bacteria using PCR Single and the electrical phase I have to gel the Agaruz (1.5 percent) and voltage (100) for an hour. Since M = 100 (Ladder DNA bp 1500), all the insulations showed 4 seclusions as a positive result of the iha gene responsible **.**

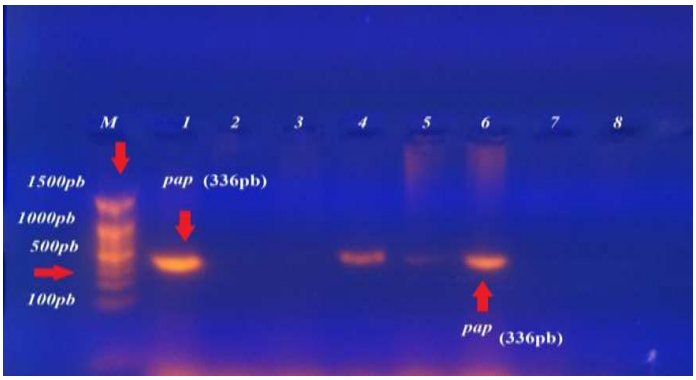

Figure 4. Gene pap products for *E. coli* bacteria using PCR Single and EP On the agaroz gel (1.5 percent) and voltage (100) for a quick run. Since M = bp100–1500 (ladder DNA), 11 solitudes were shown as a positive result of the pap gene responsible for the adhesive factors.

production of beta-lactamases and other resistance mechanisms contributes to this phenomenon, highlighting the need for ongoing surveillance and development of new antibiotics (Collee et al., 1996; MacFaddin, 2000).

Escherichia coli is the most common bacterial species responsible for urinary tract infections (UTIs). Females are more frequently affected by UTIs compared to males. Among different age groups, middle-aged and elderly individuals are the most vulnerable to UTIs, followed by children and adolescents.

Regarding treatment, third-generation cephalosporins are generally more effective against *E. coli* compared to other cephalosporin generations. This is due to their higher sensitivity and reduced resistance in treating UTIs.

In terms of genetic factors, the fhuA gene, which is involved in iron acquisition, is the most extensively studied gene related to UTIs. Following this, the hly gene, which encodes the production of hemolysin, is also significant in understanding the pathogenesis of UTIs.

PCR multiplex technology has demonstrated significant advantages in terms of speed and efficiency in generating results compared to traditional single PCR methods. Elevated levels of inflammatory markers such as interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor-alpha (TNF-α) are identified as key indicators of inflammation in patients with UTIs.

5. Conclusions

In conclusion, this study highlights the significant role of Escherichia coli (*E. coli*) in urinary tract infections (UTIs), revealing its prevalence and antibiotic resistance patterns. With *E. coli* identified in 56% of UTI cases, it underscores the bacterium's dominance in these infections. The high resistance rates observed, particularly to second- and third-generation cephalosporins, reflect a growing challenge in treatment efficacy. The molecular analysis confirmed the presence of key virulence factors, such as hemolysin and adherence factors, contributing to the pathogenicity of the isolates. These findings emphasize the urgent need for continuous monitoring of antibiotic resistance and the development of novel therapeutic strategies. Furthermore, the study demonstrates the necessity of tailored diagnostic and treatment approaches, considering the demographic factors that influence UTI prevalence. Addressing these issues is crucial for improving patient outcomes and combating the rising threat of antibiotic-resistant infections.

Author contributions

A.A. was responsible for the conceptualization, drafting, data analysis, and review of the manuscript.

Acknowledgment

Author was thankful to their department.

Competing financial interests

The authors have no conflict of interest.

References

Abdullah, A. M., Abdullah, R. M., & Salman, S. L. (2009). Use of chromogenic agar in detection of urinary tract pathogens and antimicrobial susceptibility. Journal of the Faculty of Medicine Baghdad, 51(1), 39-42. https://doi.org/10.32007/jfacmedbagdad.5111168

Abu Daia, J. M., Al-Aaly, M. A., & De Castro, R. (2000). Urinary tract infection in childhood. Saudi Medical Journal, 21(7), 711-714. https://pubmed.ncbi.nlm.nih.gov/11423881/

Agace, W. W., Hedges, S. R., Ceska, M., & Svanborg, C. (1993). IL-8 and the neutrophil response to mucosal gram-negative infection. Journal of Clinical Investigation, 92(3), 780-785. https://doi.org/10.1172/JCI116635

Akbar, D. H., Ahmed, M. M., & Algamdi, A. A. (2003). Cardiovascular risk factors in Saudi Arabian and non-Saudi Arabian diabetic patients in Saudi Arabia. Eastern Mediterranean Health Journal, 9(5-6), 884-892. https://pubmed.ncbi.nlm.nih.gov/16450518/

AL-Bero, B. R., Agrawal, K. C., & Agnihotri, V. (2000). Transferable drug resistance in Escherichia coli isolated from women with chronic UTI. Indian Journal of Medical Research, 71(1), 83–91.

Al-Dupot, L. B., Wullt, Z. X., Shen, D., & Svanborg, C. (1998). Cytokine repertoire of epithelial cells lining the human urinary tract. Journal of Urology, 159(6), 2185–2192. https://doi.org/10.1016/S0022-5347(01)63303-2

Al-Hadithi, H. A. (1996). Urinary tract infection in Ramadi city: A bacteriological study. Journal of Al-Anbar University, 1(1), 76–81. https://doi.org/10.37652/juaps.2007.15330

Alkasaby, N. M., Alsayed, M. A., Zaki, M. E., Bakr, A. O., & Rizk, M. A. (2022). Study of enteropathogenic Escherichia coli producing extended spectrum β-lactamase in children with acute gastroenteritis. Egyptian Journal of Medical Microbiology, 31(3), 83-90. https://doi.org/10.21608/EJMM.2022.248898

Bachur, R., & Caputo, G. L. (1995). Bacteremia and meningitis among infants with urinary tract infections. Pediatric Emergency Care, 11(4), 280–284. https://doi.org/10.1097/00006565-199510000-00004

Bert, F., & Lambert, Z. N. (1999). Current microbiological problems: Antibiotic resistance and therapeutic problems raised by *E. coli*. Press Medical, 28(1), 451. https://pubmed.ncbi.nlm.nih.gov/10093607/

Bhat, R. G., Katy, T. A., & Place, F. C. (2011). Pediatric urinary tract infections. Emergency Medicine Clinics of North America, 29(3), 637–653. https://doi.org/10.1016/j.emc.2011.04.004

Bollgren, I., & Winberg, J. (1999). The periurethral aerobic flora in girls highly susceptible to urinary infections. Acta Paediatrica Scandinavica, 65(1), 81-87. https://doi.org/10.1001/Actaps.1981.033102700090

Bradford, P. A. (2001). Extended-spectrum beta-lactamases in the 21st century: Characterization, epidemiology, and detection of this important resistance threat. Clinical Microbiology Reviews, 14(4), 933-951. https://doi.org/10.1128/CMR.14.4.933-951.2001

Brooks, G. F., Butel, J. S., & Morse, S. A. (2001). Jawetz, Melnick, and Adelberg's medical microbiology (22nd ed.). Lange Medical Books/McGraw-Hill.

Cabral, J. P. S. (2010). Water microbiology. Bacterial pathogens and water. International Journal of Environmental Research and Public Health, 7(10), 3657–3703. https://doi.org/10.3390/ijerph7103657

Chang, S. L., & Shortliffe, L. D. (2006). Pediatric urinary tract infections. Pediatric Clinics of North America, 53(3), 379–400. https://doi.org/10.1016/j.pcl.2006.02.011

Collee, G. J., Chambers, S. T., Peddie, B., & Mahanty, K. H. (1996). The association between colicinogenicity and pathogenesis among uropathogenic isolates of Escherichia coli. Microbial Pathogenesis, 20(3), 185-190. https://doi.org/10.1006/mpat.1996.0017

Delzell, J. E., Jr., & Lefevre, M. L. (2000). Urinary tract infections during pregnancy. American Family Physician. Retrieved from https://www.aafp.org/pubs/afp/issues/2000/0201/p713.html

Dontas, A. S., Papanayiotou, P., & Marketos, S. (1997). Bacteriuria in old age. Bulletin of the New York Academy of Medicine, 2(1), 305. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1805129/pdf/bullnyacadmed00111-0019.pdf

Dzidic, S., Suskovic, J., & Kos, B. (2008). Antibiotic resistance mechanisms in bacteria: Biochemical and genetic aspects. Acta Pharmaceutica, 46(1), 11-21. https://hrcak.srce.hr/file/34842

Engel, D., Dobrindt, U., Tittel, A., Peters, P., Maurer, J., Gütgemann, I., Kaissling, B., Kuziel, W., Jung, S., & Kurts, C. (2006). Tumor necrosis factor alpha- and inducible nitric oxide synthase-producing dendritic cells are rapidly recruited to the bladder in urinary tract infection but are dispensable for bacterial clearance. Infection and Immunity, 74(2). https://doi.org/10.1128/iai.00881-06

Fair, W. R. (1999). Observations on the origin of urinary tract infections. In P. E. Kincaid-Smith & K. F. Ferley (Eds.), Renal infections and renal scarring (pp. 98-123). Mercedes.

Farshad, S., Ranjbar, R., Japoni, A., Hosseini, M., Anvarinejad, M., & Mohammadzadegan, R. (2012). Microbial susceptibility, virulence factors, and plasmid profiles of uropathogenic Escherichia coli strains isolated from children in Jahrom, Iran. Journal of Archives of Iranian Medicine, 15(5), 312-316. https://pubmed.ncbi.nlm.nih.gov/22519382/

Fuda, C., Suvorov, M., Vakulenko, S. B., & Mobashery, S. (2004). The basis for resistance to beta-lactam antibiotics by penicillin-binding protein 2a of methicillin-resistant Staphylococcus aureus. Journal of Biological Chemistry, 279(40), 40802-40806. https://doi.org/10.1074/jbc.M403589200

Gunther, N. W., Virginia, L., David, E., & Harry, L. (2001). In vivo dynamics of type 1 fimbriae regulation in uropathogenic Escherichia coli during experimental urinary tract infection. Infection and Immunity, 69(5), 2838-2846. https://doi.org/10.1128/IAI.69.5.2838-2846.2001

Hara, C. M., Brenner, F. M., & Miller, J. M. (2000). Classification, identification, and clinical significance of Proteus, Providencia, and Morganella. Clinical Microbiology Reviews, 13(4), 534-546. https://doi.org/10.1128/cmr.13.4.534-546.2000

Hedström, M., & Gröndal, L. (1999). Urinary tract infection in patients with hip fractures. Journal of Family Practice, 30(5), 341-343. https://doi.org/10.1016/s0020-1383(99)00094-7

Ho, G. Y. F., Xue, X.-N., Burk, R. D., Kaplan, R. C., Cornell, E., & Cushman, M. (2005). Variability of serum levels of tumor necrosis factor-alpha, interleukin 6, and soluble interleukin 6 receptor over 2 years in young women. Cytokine, 30(1), 1-6. https://doi.org/10.1016/j.cyto.2004.08.008

Hooton, T., Steapleton, A., & Roberts, P. (2001). Perineal anatomy and urine-voiding characteristics of young women with and without recurrent urinary tract infections. Clinical Infectious Diseases, 29(1), 1600. https://doi.org/10.1086/313528

Hows, P. F., Jarvis, J. A., & Mitchell, C. K. (2003). Urinary tract infections. Primary Care: Clinics in Office Practice, 30(1), 41-61.

Jacoby, G. A., & Munoz-Price, L. S. (2005). The new beta-lactamases. New England Journal of Medicine, 352(4), 380-391. https://doi.org/10.1056/NEJMra041359

Jawetz, E., Brooks, G. F., Butel, J. S., & Morse, S. A. (1998). Jawetz Melnick and Adelberg's Medical Microbiology (21st ed.). Appleton and Lange.

Kunin, C. M. (1994). Urinary tract infections in females. Clinical Infectious Diseases, 18(1), 1-12. https://doi.org/10.1093/clinids/18.1.1

Lane, D. R., & Takhar, S. S. (2011). Diagnosis and management of urinary tract infection and pyelonephritis. Emergency Medicine Clinics of North America, 29(3), 539–552. https://doi.org/10.1016/j.emc.2011.04.001

Lee, K., Hong, S. G., Park, Y. J., Lee, H. S., Song, W., Jeong, J., Yong, D., & Chong, Y. (2005). Evaluation of phenotypic screening methods for detecting plasmid-mediated AmpC β-lactamases-producing isolates of Escherichia coli and Klebsiella pneumoniae. Diagnostic Microbiology and Infectious Disease, 53(4), 319-323. https://doi.org/10.1016/j.diagmicrobio.2005.07.004

MacFaddin, J. F. (2000). Biochemical tests for identification of medical bacteria (3rd ed.). Lippincott Williams & Wilkins.

Mahyar, A., Ayazi, P., Maleki, M. R., Daneshi-Kohan, M. M., Sarokhani, H. R., Hashemi, H. J., & Talebi-Bakhshayesh, M. (2013). Serum levels of interleukin-6 and interleukin-8 as diagnostic markers of acute pyelonephritis in children. Korean Journal of Pediatrics, 56(5), 218-223. https://doi.org/10.3345/kjp.2013.56.5.218

Naylor, G. H. (1998). A 16-month analysis of urinary tract infection in children. Journal of Medical Microbiology, 17(1), 31-36. https://doi.org/10.1099/00222615-17-1-31

Nicolle, L. E. (2009). Urinary tract infection in the elderly. Journal of Antimicrobial Chemotherapy, 33(1), 99. https://doi.org/10.1016/j.cger.2009.04.005

Prat, C., Domínguez, J., Andreo, F., & Hernández, M. (2006). Urinary antigen test for pneumococcal pneumonia. Chest, 130(3), 849-853. https://doi.org/10.1378/chest.130.3.849

Raka, L., Mulliqi-Osmani, G., Berisha, L., et al. (2004). Etiology and susceptibility of urinary tract isolates in Kosova. International Journal of Antimicrobial Agents, 23(1), 2-5. https://doi.org/10.1016/j.ijantimicag.2003.09.009

Riff, F. (1988). Current microbiological problems: Antibiotic resistance and therapeutic problems raised by *E. coli*. Press Med, 28(1), 451.

Roilides, E., Papachristou, F., Gioulekas, E., Tsaparidou, S., Karatzas, N., Sotiriou, J., & Tsiouris, J. (1999). Increased urine interleukin-6 concentrations correlate with pyelonephritic changes on 99mTc-dimercaptosuccinic acid scans in neonates with urinary tract infections. Journal of Infectious Diseases, 180(3), 904-907. https://doi.org/10.1086/314960

Segreti, J., Levin, S., & Marshal, A. (1996). Bacteriological and clinical applications of a new extended spectrum parenteral cephalosporin. American Journal of Medicine, 100(6), 45-55. https://doi.org/10.1016/s0002-9343(96)00107-6

Stamm, W. E., & Hooton, T. M. (1993). Management of urinary tract infection in adults. New England Journal of Medicine, 329(18), 1328-1334. https://doi.org/10.1056/NEJM199310283291808

Stukus, P. E. (1997). Investigating microbiology. Harcourt Brace and Company. https://doi.org/10.29079/vol13iss1art275

Tada, D. G., Gandhi, P. J., & Patel, K. N. (2012). A study on antibiotic-related resistance in UTI patients: A comparison between community-acquired and hospital-acquired *E. coli*. National Journal of Community Medicine, 3(2), 255-258. https://njcmindia.com/index.php/file/article/view/1691

Umegaki, H., Yamada, K., Naito, M., Kameyama, T., Iguchi, A., & Nabeshima, T. (1996). Protective effect of interleukin-6 against the death of PC12 cells caused by serum deprivation or by the addition of a calcium ionophore. Biochemical Pharmacology, 52(6), 911–916. https://doi.org/10.1016/0006-2952(96)00422-4

Vernon, S., Foo, C. K., & Coulthard, M. G. (1997). How general practitioners manage children with urinary tract infection: An audit in the former Northern Region. British Journal of General Practice, 47(418), 297-300. https://pubmed.ncbi.nlm.nih.gov/9219406/

Winberg, G. T., Funk, B. R., & Case, C. L. (1998). Microbiology: An introduction (6th ed.). The Benjamin Cummings Publishing Company.

Wullt, B., Bergsten, G., Connell, H., Röllano, P., Gebretsadik, N., Hull, R., & Svanborg, C. (2000). P-fimbriae enhance the early establishment of *E. coli* in the human urinary tract. Molecular Microbiology, 38(3), 456-464. https://doi.org/10.1046/j.1365-2958.2000.02165.x

Wullt, B., Bergsten, G., Samuelsson, M., & Svanborg, C. (2002). The role of P fimbriae for Escherichia coli establishment and mucosal inflammation in the human urinary tract. International Journal of Antimicrobial Agents, 19(6), 522–538. https://doi.org/10.1016/s0924-8579(02)00103-6