



Microbial Science Archives (ISSN: 2583-1666)

An International Research Journal

Journal homepage: www.microbial.sciencearchives.org

<https://doi.org/10.47587/MSA.2023.3309>



Research Article

Assessment of antibiotics sensitivity and resistance in *Escherichia Coli* isolated from patients diagnosed with urinary tract infections

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Received: Aug 14, 2023/ Revised: Sept 15, 2023/Accepted: Sept 17, 2023

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Abstract

Escherichia coli (*E. coli*), a ubiquitous bacterium in the human gut, is a joint causative agent of Urinary Tract Infections (UTIs). Despite its typical role in maintaining gut health, certain strains can invade the urinary tract, leading to infections. This investigation's principal objectives were to isolate these pathogenic *E. coli* strains from UTI patients, evaluate their susceptibility to an array of pre-selected antibiotics, and subsequently compare the efficacies of these antibiotics. The study amassed 100 urine specimens from various hospitals, incorporating private and educational institutions. These samples were procured from male and female patients displaying symptoms indicative of a bacterial UTI and validated through microscopic examination. Among these collected specimens, 42 exhibited positive *E. coli* growth, translating to a 42% infection rate. These were diagnosed with the assistance of the biomérieux vitek 2 system, and antibiotic sensitivities were inferred from the diagnostic analyzer's data. The results illuminated Meropenem as the most effective antibiotic against the examined bacteria, with a sensitivity profile of 78%. This was followed by Amikacin (74%), Gentamicin (69%), Tobramycin (57%), Cefepime (47%), Ceftazidime (31%), Ciprofloxacin (31%), Aztreonam (28%), Ticarcillin (9.5%), and Piperacillin (7%). By elucidating the sensitivity profiles of several antibiotics on *E. coli* induced UTIs, this research contributes valuable data to develop more precise and effective treatment strategies.

Keywords: Sensitivity, Resistance, Isolation, *Escherichia Coli*, UTI, *E. coli*

Introduction

Escherichia coli, or *E. coli*, is a Gram-negative, facultatively anaerobic bacterium that adopts a rod-like shape. *E. coli* is typically located in the lower intestine of warm-blooded animals (Allocati et al., 2013). While most *E. coli* strains are harmless and part of the regular gut microbiota, some can cause food poisoning and urinary tract infections (UTIs). Some strains can produce vitamin K2 or inhibit the colonization of the intestine by pathogenic bacteria, hence forming a symbiotic relationship with their human hosts (Gomes et al., 2016).

E. coli gets excreted into the environment via fecal matter and can survive outside the body for a limited time. This

characteristic makes it a viable indicator organism for evaluating fecal contamination in environmental samples (Cabral, 2010; Rodrigues & Cunha, 2017). Certain *E. coli* strains can also trigger severe disease in the intestinal tract and other areas within the host (Alteri & Mobley, 2012; Blaser, 2005). In terms of detection, *E. coli* possesses unique characteristics such as somatic (O) antigen 157 and flagella (H) antigen seven. It also exhibits delayed D-sorbitol fermentation and lacks the ability to produce β -glucuronidase (Gomes et al., 2016; Vidovic, 2008). Various *E. coli* infections, including those brought on by enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), and enterohaemorrhagic *E. coli* (EHEC), have various diagnostic methods (Kong et al., 1999; Zeighami et al., 2015). *E. coli* can grow at temperatures as high as 49 °C, flourish on a variety of

substrates, and engage in mixed acid fermentation in anaerobic environments (Van Elsas et al., 2011). It is categorized as a facultative anaerobe, which means that it may grow in the absence of oxygen by fermenting or engaging in anaerobic respiration when it is present (Clark, 1989; Shewaramani et al., 2017).

E. coli-related urinary tract infections (UTIs) may not usually show symptoms. However, when symptoms do occur, they can include a persistent urge to urinate, a burning sensation during urination, frequent urination, strong-smelling urine, and pelvic discomfort. Measures to prevent UTI include sufficient hydration, consumption of cranberry juice, maintaining hygiene after urinating and bowel movement, emptying the bladder soon after sexual activity, avoiding potential irritating feminine products, and altering birth control methods if required (Pullukcu et al., 2007; Tasbakan et al., 2012).

Typically, bacterial infections due to *E. coli* are treated with antibiotics. Nonetheless, antibiotic resistance is becoming an increasing problem due to the over-prescription of antibiotics in humans and their use as growth enhancers in animal feeds (Da Costa et al., 2013; Puvača & de Llanos Frutos, 2021; Qiao et al., 2018). There is growing concern about resistance to beta-lactam antibiotics as strains producing extended-spectrum beta-lactamases become more prevalent (Ghafourian et al., 2015; Rao et al., 2014). Phage therapy has emerged as an alternative to antibiotics. This therapy employs viruses that specifically target harmful bacteria and has been used to mitigate diarrhea caused by *E. coli*, while not currently available in the US for *E. coli* treatment, some dietary supplements contain phage strains that target *E. coli* (Oechslin, 2018; Sulakvelidze et al., 2001; Viertel et al., 2014).

Materials and Methods

Specimen Collection and Initial Examination

Material and Equipment

Urine cup and urine bag (for children), Centrifuge, Pipette, Slide, Cover slide, Microscope, Urine dipstick for macroscopic examination.

Collection

A total number of 100 urine samples were gathered into sterile receptacles for urine analysis. It is most beneficial to collect the morning's first urine due to its higher concentration. To reduce contamination, a "midstream clean-catch" approach was adopted, which involves cleaning the genital area prior to urination and collecting the sample during mid-urination. Alternatively, urine could be collected via a urinary catheter or suprapubic aspiration. In cases of infants and young children, the practice of attaching a bag to the genital area for urine collection was implemented with caution owing to an increased contamination risk.

Macroscopic Examination

Urine dipsticks or test strips were employed for swift evaluation of various urinary parameters and substances. The strip was submerged in the urine sample, and after a stipulated period, the color alterations on the reagent pads were assessed either visually or instrumentally. Tests included parameters like glucose, ketones, bilirubin, urobilinogen, blood, leukocyte esterase, protein, nitrite, pH, and specific gravity. Nitrite was documented as either negative or positive, whereas other elements were rated on a scale or noted based on the intensity of the color shift.

Microscopic Examination

Microscopic examination allowed for identifying and quantifying cells and elements such as urinary casts. This provided extensive information and potential indicators of specific diagnoses. Microscopy was performed on samples that showed abnormal results in preliminary testing or were taken from specific patient demographics, like infants. Abnormal color or clarity and positive dipstick outcomes for blood, leukocytes, nitrite, or protein were common triggers for microscopic examination. Centrifugation was performed to concentrate solid elements for better visibility. A droplet of the concentrated sample was placed under a coverslip and scrutinized using light microscopy at magnifications of 100x and 400x. Outcomes were documented according to the quantity in the microscope's field of view at low and high magnifications. Some elements were reported in a qualitative manner, while others were reported in a numerical form (Becker et al., 2016)

Culturing

Material and Equipment: Agar powder (e.g., nutrient agar), Distilled water, Blood sample, Lab thermometer, Glass stir rod, Heat-resistant hand protection, Boiling mixture, Sterile Petri dish. Beaker/flask.

Preparation of Agar Medium

According to the manufacturer's instructions, suspend the appropriate amount of agar powder (e.g., nutrient agar) in distilled water. Heat the mixture while stirring until all components are dissolved. Autoclave the dissolved mixture at the appropriate temperature and pressure for the specified time. Allow the agar medium to cool but not solidify, add any required supplements or additives to the cooled agar medium, if necessary, mix gently but thoroughly to ensure homogeneity, dispense the agar medium into sterile Petri dishes while in a liquid state.

Culturing of Samples

Seventy samples from patients with UTIs and bacterial findings with elevated pus count in the urine were cultured on

an appropriate agar medium, such as blood agar, as *E. coli* can grow easily on this medium (Becker et al., 2016)

Material and Equipment: Agar plates (e.g., blood agar plates), Sterile inoculating loop

Procedure

Tip over the container to remix the urine sample, using a sterile inoculating loop, obtain a small amount of urine and streak it evenly on the surface of the agar plate, Repeat the streaking process for each sample, using a new sterile inoculating loop for each sample, Incubate the agar plates at the appropriate temperature and for the specified duration suitable for *E. coli* growth, after incubation, observe and record any bacterial growth on the agar plates.

Bacterial Identification and Antibiotic Sensitivity Testing with VITEK 2 System (Prod'hom et al., 2013)

Required Materials and Equipment

1. Culture plate containing test organisms
2. Classification cards for Gram-negative (GN), Gram-positive (GP), Yeast (YST), and Bacilli (BCL)
3. Saline solution and a dispenser for saline
4. Test tubes
5. VITEK tube rack or VITEK tube compact cassette
6. Inoculating loop
7. Bunsen burner
8. Biosafety cabinet (BSC)
9. Densitometer (DensiCHECK plus)
10. Vortex mixer
11. Micropipette and tips
12. Reagent cards for organism identification (GN, GP, YST, BCL)
13. Antibiotic susceptibility testing (AST) cards for Gram-positive, Gram-negative, and yeast
14. VITEK 2 Compact machine

Testing Method

Assign transferred colony of organisms according to the suitable reagent cards (GN, GP, YST, BCL), Set up two test tubes for each organism marked with identification numbers, and Fill each tube with saline using the supplied dispenser, Develop the inoculum by adding an isolated colony to the first tube and mix using a vortex mixer, Determine the optical density of the inoculum, Transfer a specific volume of the inoculum from the first tube to the second tube following the instructions, Put the reagent card and AST card in their respective tubes, Load the cards into the VITEK tube compact cassette, Place the cassette in the filler module and then move it to the reader or incubator module of the VITEK 2 Compact machine, Allow 5-7 hours for the report (Prod'hom et al., 2013)

Interpreting Results

Identification of Organisms

1. The GN Reagent Card identifies Gram-negative bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and others.
2. The GP Reagent Card reveals Gram-positive cocci and non-spore-forming bacilli like *Staphylococcus aureus*, *Enterococcus faecalis*, *Streptococcus pyogenes*, and more.
3. The YST Reagent Card showcases yeast and yeast-like organisms including *Candida albicans*, *Candida krusei*, *Cryptococcus neoformans*, and others.
4. The BCL Reagent Card demonstrates Gram-positive spore-forming bacilli such as *Bacillus cereus*, *Clostridium difficile*, *Clostridium perfringens*, and others.

Antibiogram of Organisms

The panel indicates the minimum inhibitory concentration (MIC) of antibiotics for each isolate and classifies their susceptibility as susceptible, intermediate, or resistant.

Results and Discussion

Table 1. Comprehensive Analysis of Patient Sensitivity and Resistance to Various Antibiotics

N	Ticar	Piperacillin	Ceftazidime	Cefepime	Aztreonam
1	Res	Res	SS	SS	SS
2	SS	SS	SS	SS	SS
3	Res	Res	Res	SS	Res
4	Res	Res	Res	Res	Res
5	Res	Res	Res	SS	Res
6	Res	Res	Res	SS	Res
7	Res	Res	Res	SS	Res
8	Res	Res	Res	Res	Res
9	Res	Res	Res	SS	Res
10	Res	Res	Res	Res	SS

11	Res	Res	SS	SS	Res
12	Res	Res	Res	Res	S
13	Res	Res	SS	SS	Res
14	SS	Res	Res	Res	SS
15	Res	Res	Res	Res	Res
16	Res	Res	Res	Res	SS
17	Res	Res	SS	SS	Res
18	Res	Res	Res	Res	Res
19	Res	Res	Res	SS	SS
20	Res	Res	Res	Res	Res
21	Res	Res	SS	SS	Res
22	Res	Res	Res	SS	Res
23	Res	Res	Res	Res	Res
24	Res	Res	Res	Res	Res
25	Res	Res	Res	Res	SS
26	Res	SS	SS	SS	SS
27	Res	Res	SS	SS	Res
28	SS	Res	SS	SS	Res
29	Res	Res	Res	R	Res
30	Res	Res	Res	Res	Res
31	Res	Res	Res	SS	SS
32	Res	Res	SS	SS	I
33	Res	SS	Res	Res	SS
34	I	Res	SS	SS	Res
35	SS	Res	Res	Res	Res
36	Res	Res	Res	SS	Res
37	I	Res	Res	Res	Res
38	Res	Res	Res	Res	Res
39	Res	Res	Res	Res	Res
40	Res	Res	Res	Res	Res
41	Res	Res	Res	Res	Res
42	Res	Res	Res	Res	SS
	Meropenem	Amikacin	Gentamicin	Ciprofloxacin	Tobramycin
43	SS	SS	SS	Res	SS
44	SS	SS	SS	SS	SS
45	SS	SS	SS	SS	SS
46	SS	SS	SS	Res	SS
47	SS	I	R	Res	Res
48	SS	SS	R	Res	Res
49	Res	SS	SS	Res	SS
50	SS	Res	R	Res	Res
51	Res	SS	SS	Res	SS
52	SS	SS	SS	Res	SS
53	Res	SS	SS	SS	SS
54	Res	Res	Res	Res	Res
56	Res	SS	SS	SS	SS
57	Res	SS	SS	Res	SS
58	Res	SS	SS	Res	SS

59	SS	SS	SS	Res	Res
60	SS	SS	SS	Res	SS
61	SS	SS	SS	Res	SS
62	Res	SS	R	Res	Res
63	SS	SS	SS	Res	SS
64	SS	SS	SS	Res	SS
65	SS	SS	SS	Res	SS
66	SS	SS	SS	Res	Res
67	SS	I	SS	Res	Res
68	SS	I	SS	Res	Res
69	SS	I	SS	SS	SS
70	SS	SS	SS	SS	SS
71	SS	SS	Res	Res	Res
72	Res	SS	SS	SS	SS
73	SS	SS	R	Res	Res
74	SS	SS	SS	SS	SS
75	SS	SS	Res	Res	Res
76	SS	SS	Res	Res	Res
77	SS	SS	SS	SS	I
78	SS	SS	R	Res	SS
79	SS	SS	SS	SS	Res
80	SS	SS	Res	Res	SS
81	SS	Res	SS	SS	Res
82	SS	Res	I	Res	SS
83	SS	Res	Res	Res	Res
84	SS	Res	SS	SS	Res
85	SS	Res	SS	SS	SS
86					

Table 2. Quantitative Assessment of Patient Sensitivity and Resistance to Antibiotics: A Percentage-Based Study

Antibiotics	Sensitive (No. %)	Resistant (No. %)	Intermediate (No. %)
Ticar	4 (9.5%)	37 (88.1%)	1 (2.4%)
Piperacillin	3 (7.1%)	39 (92.9%)	0 (0%)
Ceftazidime	13 (31%)	29 (71.4%)	0 (0%)
Cefepime	20 (47.6%)	22 (52.4%)	0 (0%)
Aztreonam	12 (28.6%)	29 (69.0%)	1 (2.4%)
Meropenem	33 (78.6%)	9 (21.4%)	0 (0%)
Amikacin	31 (73.8%)	7 (16.6%)	4 (9.5%)
Gentamicin	29 (69.0%)	12 (28.6%)	1 (2.4%)
Ciprofloxacin	13 (31.0%)	29 (69.0%)	0 (0%)
Tobramycin	24 (57.2%)	17 (40.5%)	1 (2.4%)

Ticarcillin and Piperacillin, two antibiotics, exhibit a startlingly high percentage of resistance while having relatively low sensitivity, at 88.1% and 92.9%, respectively. This implies that these specific antibiotics may be less successful in treating patient infections, which may be caused

by various factors, such as usage, which increases resistance (Jacoby & Carreras, 1990; Padmini et al., 2017).

On the other hand, 78.6% of patients respond sensitively to the antibiotic Meropenem, which paints a different image. Given its high sensitivity rate, this medicine is highly likely to produce effective treatment results. The sensitivity percentages of Amikacin and Gentamicin, which are more significant at 73.8% and 69.0%, respectively, follow a similar pattern (Terbtothakun et al., 2021; Zhanel et al., 2012).

Aztreonam presents an intermediate finding, with patient sensitivity and resistance circling similar numbers (28.6% and 69.0%), indicating varied efficacy (Ramsey & MacGowan, 2016; Sonnevend et al., 2020).

The spread between sensitivity and resistance is more evenly distributed with ceftazidime, cefepime, and ciprofloxacin. This could indicate that these antibiotics have moderate effectiveness, but additional factors such as dosage and infection type could also come into play (Chen & Livermore, 1993; Mandal et al., 2012; Oteo et al., 2006).

Tobramycin has an exciting profile with sensitivity in just over half of the patients and resistance in 40.5%. The presence of some intermediate cases suggests a possibility of varied responses in different individuals (D'Arrigo et al., 2010). Overall, this analysis underlines the complex nature of patient responses to different antibiotics. It highlights the crucial role that personalized treatment can play, as patient sensitivity and resistance can significantly influence the effectiveness of antibiotic treatment.

Conclusion

In summary, this research highlights the significant role of *E. coli* in UTIs, with a prevalence of 42% identified across diverse patient samples. The use of the biomérieux vitek 2 system was instrumental in isolating the pathogenic strains and evaluating their antibiotic sensitivities. Among the tested antibiotics, Meropenem proved most effective, showing a 78% sensitivity profile. This study's findings contribute to the critical understanding of antibiotic sensitivity profiles in *E. coli* induced UTIs, potentially guiding more targeted treatment strategies. Further research is recommended to build upon these insights, enhancing patient outcomes and reducing healthcare burdens.

Conflict of Interest

The author hereby declares no conflict of interest.

Consent for publication

The author declares that the work has consent for publication.

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How to cite this article

Al-Sammarraie, A. M. Y. (2023). Assessment of Sensitivity and Resistance in the Isolation of *Escherichia Coli* from Patients Diagnosed with Urinary Tract Infections. *Microbial Science Archives*, Vol. 3(3), 134-140. <https://doi.org/10.47587/MSA.2023.3309>

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