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Research Article

Antimicrobial Resistance Pattern of *Escherichia Coli (E. coli)* and *Klebsiella Spp* Isolated from Patients with Urinary Tract Infection

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Abstract

Background: To better understand the frequency of *E. coli* and *Klebsiella spp*. among patients with urinary tract infections at the University of Benin Teaching Hospital, Benin City. *Objective:* This study aimed to analyze their patterns of antibiotic resistance. *Methods:* The Kirby-Bauer disk-diffusion method was employed to test the susceptibility of various microbes. *Results: E. coli* was the most prevalent pathogen in this study's 50 samples, with a prevalence of 64%, followed by *Klebsiella spp*. with a prevalence of 36%. *Escherichia coli* isolates were highly susceptible to imipenem (75%) and nitrofurantoin (65.6%), but showed high resistance to cefotaxime (100%), amoxicillin-clavulanate acid (100%), and nalidixic acid (96.9%). *Klebsiella spp* isolates were highly susceptible to Imipenem (50%) but showed 100% resistance to amoxicillin-clavulanate acid and cefotaxime, respectively. Therefore, these results should form the basis for preliminary decision-making about the best course of treatment for urinary tract infections (UTIs). *Conclusion:* This study demonstrated that the effectiveness of UTI therapy using available antibiotics is limited due to the increasing prevalence of antibiotic-resistant uropathogens.

Keywords

Escherichia Coli, *Klebsiella Spp*, Urinary Tract Infection (UTI)

1. Introduction

One of the most frequent extra-intestinal bacterial diseases is urinary tract infection (UTI). It now ranks among the most prevalent illnesses seen in clinical practice, affecting patients of all ages, from newborns to the elderly [1]. Each year, around 150 million people receive a UTI diagnosis worldwide [2]. The majority of infections are brought on by bacteria retrogradely ascending from the feces to the bladder and kidney via the urethra, particularly in females who have shorter, wider urethras and are more susceptible to microbial transmission [3].

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According to studies [4, 5], the anatomy of the female urethra and vagina makes it vulnerable to injury during sexual activity as well as bacterial massage into the urethra and bladder during pregnancy and childbirth. Most UTIs do not pose a threat to life and do not result in permanent harm. However, there is a chance of irreversible tissue damage when the kidneys are implicated, along with a higher chance of bacteremia [6]. Although many different organisms can cause UTIs, *Escherichia coli* is the main cause of infections. UTIs are almost universally known to affect people of all ages; however, some populations are more susceptible than others, including newborns, expectant mothers, and elderly patients. By acquiring possible virulence markers, *E. coli* strains may be better able to withstand and get past the host's immune system and cause a serious illness.

These virulence factors are typically found on the numerous islands associated with pathogenicity, which bacteria can easily spread through a variety of horizontal gene transfer processes. To further understand the pathogenicity of symptomatic or complex UTIs, it can be helpful to characterize virulence factors like adhesions, toxins, and iron uptake mechanisms. However, the rise of strains that are resistant to antibiotics has raised severe issues with public health, increasing mortality and morbidity [7]. The main cause of the rising rate of multi-drug resistant (MDR) strains, frequently linked to the rising trend of bacteria that produce extended-spectrum beta-lactamases (ESBLs), is the excessive and inappropriate use of antibiotics. [6, 8].

Klebsiella pneumoniae causes bloodstream and respiratory infections in UTIs [8]. It belongs to the species group called ESKAPE, which is defined by the ability of its members to resist the effects of antimicrobial drugs. *Enterococcus faecium, Staphylococcus aureus, K. pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Enterobacter species* are among the six highly pathogenic and antibiotic-resistant bacterial pathogens included in this acronym. Furthermore, due to the worldwide issue of antimicrobial resistance, the World Health Organization (WHO) recognizes *K. pneumoniae* as one of the species of high priority. It encourages the study and development of new antibiotics [9].

Infectious diseases, wars, and famines are among the most important elements threatening humanity's survival worldwide, with emerging countries bearing the brunt of the threat. [10]. Despite enormous improvements in our understanding of microorganisms and their control, epidemics caused by drug-resistant microbes and the introduction of new disease-causing microorganisms continue to be major public health problems in developed countries. [10]. Furthermore, due to the rise of multidrug-resistant bacteria, treatment options for some illnesses have become limited [10].

1.1. Statement of Problem

Antibiotic-resistant bacteria are thought to be responsible for 33,000 fatalities in Europe and 700,000 worldwide per year. In the worst-case scenario, bacterial infections will be the leading cause of mortality worldwide by 2050, killing more than 1.8 million people annually. Our ability to treat common infectious diseases is in jeopardy due to the growth of bacterial resistance, which can lead to prolonged sickness, disability, and death. As a result, antimicrobial resistance raises the price of healthcare due to longer hospital stays, which are anticipated to add 2.5 million days of hospitalization per year across the European Union, costing an estimated 900 million Euros. The rising occurrence and spread of antibiotic resistance among various microorganisms (bacteria, fungi, viruses, and parasites) is thus one of the most significant health issues. As mortality and morbidity rates rise, germs become more resistant to antibiotics in community and hospital settings. Diet, age, and stress, in addition to immunological function, affect a host's vulnerability to infection. As a result, opportunistic infections can infect immunocompromised patients but not healthy people.

This creates significant obstacles to the efficient operation of medical and healthcare systems and entails significant financial expenses for society at large. Determining the prevalence and antibiotic resistance profile of *Escherichia coli* and *Klebsiella spp* isolated from urinary tract infections in patients at the University of Benin Teaching Hospital, Benin City (UBTH), was the goal of the current investigation.

This aim is to investigate the antibiotic resistance pattern of *Escherichia coli* and *Klebsiella spp* isolated from urinary tract infection in patients at the University of Benin Teaching Hospital (UBTH), Benin City.

1.2. Specifically, the Study Objectives Are

1) Isolation of *Escherichia coli* and *Klebsiella spp.* from patients with urinary tract infections at the University of Benin Teaching Hospital, Benin City (UBTH).
2) Identification of *Escherichia coli* and *Klebsiella spp.* isolated from a urinary tract infection in a patient at the University of Benin Teaching Hospital, Benin City (UBTH).
3) Determination of the antibiotic resistance pattern of the isolates collected from the University of Benin Teaching Hospital, Benin City (UBTH).

2. Methodology

This study was carried out at the Dora Akunyili College of Pharmacy, Igbinedion University, Okada, Edo State, Nigeria, from November 2022 to August 2023.

2.1. Sample Collection

A total of 50 clinical samples of patients with urinary tract infections were collected from the Department of Microbiology laboratory, University of Benin Teaching Hospital, between November 2022 to April 2023.

2.1.1. Culturing and Identification of Clinical Isolates

All isolates previously isolated from the hospital were sub-cultured into a differential medium, MacConkey and Eosin methylene blue agar; thereafter, the plates were incubated at 37 ℃ for 24 hours. Pure colonies were then picked onto nutrient agar slants and stored for further use.

2.1.2. Biochemical Tests and Gram Staining

(i). Gram Staining

A thin smear of the organism was prepared using a glass slide, the smear was flooded with a crystal violet reagent, and the flooded glass slides with crystal violet were allowed to stand for one minute and then rinsed gently under tap water. The smear was flooded with Gram's iodine (mordant) and allowed to stand for one minute, and then the smear was rinsed again gently under tap water. The smear was decolorized with 95% ethanol and rinsed again gently under tap water.

The smear was flooded gently for one minute with a safranin reagent and then rinsed under tap water gently, and the smear was air-dried. The smear was observed first under the low power (10x) objective, and then under the oil immersion (100x) objective using a compound light microscope, and then the result was taken.

2.1.3. Biochemical Tests

(i) Test for Indole Activity

A tryptophan broth used for growing bacteria before testing for indole production was prepared, and a syringe was used to transfer 4 ml of the broth into each test tube. The test tubes were sterilized in the autoclave for 20 minutes and then removed. The Bunsen burner was turned on for sterilization, and the test tubes were taken to the sterilized zone. The inoculating loop was heated over the flame and used to inoculate the sterilized test tubes with growth from the overnight culture. The test tubes were then placed in the incubator and incubated at 37 ℃ for 24 hours. After incubation, 0.5 ml of Kovac's reagent was added to each broth culture, and the presence or absence of a ring was observed to determine the result.

(ii). Test for Citrate Utilization

A citrate agar was used to prepare the agar slant, which was sterilized in the autoclave for 15 minutes. The agar slant was brought out from the autoclave after 15 minutes, then the Bunsen burner was turned on for heat sterilization, and the inoculating loop was heated over the flame. The slant was streaked back and forth using a sterile inoculating loop with a light inoculum picked from the center of the isolated colony.

The agar slants were placed in the incubator and incubated at 37 ℃ for 4-7 days. A colour change from green to blue indicates a positive result.

(iii). Oxidase Test

Take a filter paper and soak it with tetramethyl-p-phenylenediamine dihydrochloride. Pick your test colony with a wooden or platinum loop and smear on the filter paper, observing the inoculated area for a color change to deep blue or purple within 10 – 30 seconds.

(iv). Urease Test

Urease Agar was prepared as instructed by the manufacturer, i.e., 2.1 grams dissolved in 95 ml of distilled water and sterilized. The urease salt was also prepared as directed (i.e., lg in 10 ml of distilled water), and 5 ml of the solution was added to the urease base agar. This was distributed as 5 ml per test tube and sterilized. The test tube was placed in a slant position for cooling, and the isolates were aseptically introduced.

(v). Catalase Test

Certain bacteria possess a defense mechanism that can minimize the harm done by the two compounds. These resistant bacteria use two enzymes to catalyze the conversion of hydrogen peroxide into diatomic oxygen and water. One of these enzymes is catalyzed and its presence can be detected. The catalase test, which involves adding hydrogen peroxide to a cultured sample, if the bacteria produce catalase, there will convert the hydrogen peroxide and oxygen gas will be evolved. The evolution of gas causes bubbles to form, indicative of a positive test. One drop of hydrogen peroxide solution was placed on a slide, a small portion of the colony was spotted onto the center of a cover slip, and the cover slip was inverted and placed on the drop of hydrogen peroxide solution. They were vigorously bubbling within 10 seconds.

Positive result: vigorous bubbling indicates the presence of catalase.

2.1.4. Antibacterial Susceptibility Test

The Kirby-Bauer susceptibility testing technique [11] was adopted. The antimicrobial testing was determined by a modified agar diffusion method.

(i). Preparation of Mueller Hinton Agar Plates

The Mueller Hinton Agar which is considered to be the best for the susceptibility testing of non-fastidious bacteria because it gives satisfactory growth of most non-fastidious pathogens, was prepared using a beaker and sterilized in the autoclave for 20 minutes. The Mueller Hinton Agar was brought out from the autoclave and allowed to cool for some minutes. The petri dish which is used to culture the bacteria was labelled accordingly. A syringe was used to transfer 20 ml of the Mueller Hinton Agar into each petri dish. The petri dish was allowed to set for a few hours.

(ii). Preparation of an Overnight Culture

The nutrient broth was prepared and boiled with stirring until it had melted and distributed throughout the medium. The test tubes were labeled alphabetically according to the isolates taken from the microbiological laboratory UBTH and also a test tube was labeled for the standard *E. coli*. A syringe was used to transfer about 5 ml of the nutrient broth into the test tubes and the top of the test tubes was plugged using a cotton wool to allow the passage of air but prevent the passage of micro-organisms. The test tubes were sterilized in the autoclave for 20 minutes to prevent the contamination of the test tubes.

The distilled water was measured using a measuring cylinder and transferred into a beaker. A syringe was used to transfer 9 ml of the distilled water into the test tube which was labeled accordingly, the top of the test tubes was plugged using cotton wool to prevent the passage of micro-organisms and sterilized in an autoclave for 20 minutes.

The Bunsen burner was turned on, with an inoculating loop heated over the flame isolates were transferred into test tubes containing the nutrient broth which was incubated at 37 ℃ for 24 hours.

(iii). Serial Dilution

The test tube containing the isolates was brought out from the incubator after 24 hours. The Bunsen burner was turned on, and the test tubes were placed in the sterilized zone with the test tubes containing the sterilized water.

A syringe was used to collect 1 ml of each isolate inside the test tubes, and was transferred into the test tubes containing the sterilized water. The mouth of the test tubes was flamed before and after collection and plugged with cotton wool to prevent the entry of micro-organisms.

This procedure was carried out for the 50 isolates, which were labeled accordingly.

The labeled Mueller Hinton Agar plate was brought to the sterilized zone. Using a syringe, 0.1 mL of the isolate was transferred into each petri dish, and the isolate was spread evenly over the surface of the agar using a sterile glass spreader. This procedure was carried out for each labeled Mueller-Hinton Agar Plate.

(iv). Introduction of Antibiotic Disc

Multiple discs containing Antibiotics, which include Ceftriaxone/Sulbactam, Cefuroxime (30 µg), Gentamicin (30 g), Ofloxacin (5 µg), Amoxicillin/clavulanic acid (30 µg), Nitrofurantoin (300 µg), Cefotaxime (30 µg), Levofloxacin (30 µg), Nalidixic Acid, Cefixime, Imipenem/ Cilastatin (10 µg), and Meropenem (10 µg). were used to carry out the Antimicrobial Susceptibility Test. The Bunsen burner was turned on for sterilization, and the Mueller-Hinton agar plates were brought to the sterilized zone. Each disc was carefully removed from the container, which was positioned in the middle of the petri dish, using a pair of forceps that had been heated over a flame. This process was followed for each petri dish with a label. For 24 hours, the plates were in an incubator set at 37°C. The inhibition zones were measured and interpreted by CLSI standards.

2.2. Statistical Analysis of Data

Statistical analysis of the data was performed using GraphPad Prism Software version 10.2.0.392. Results were presented in a graph as percentages.

3. Results

Table 1. *Clinical isolates according to the age, and gender of patients.*

Characteristics	N	%
Gender		
Male	19	38
Female	31	62
Age		
18-30	20	40
31-40	11	22
41-50	9	18
>60	10	20

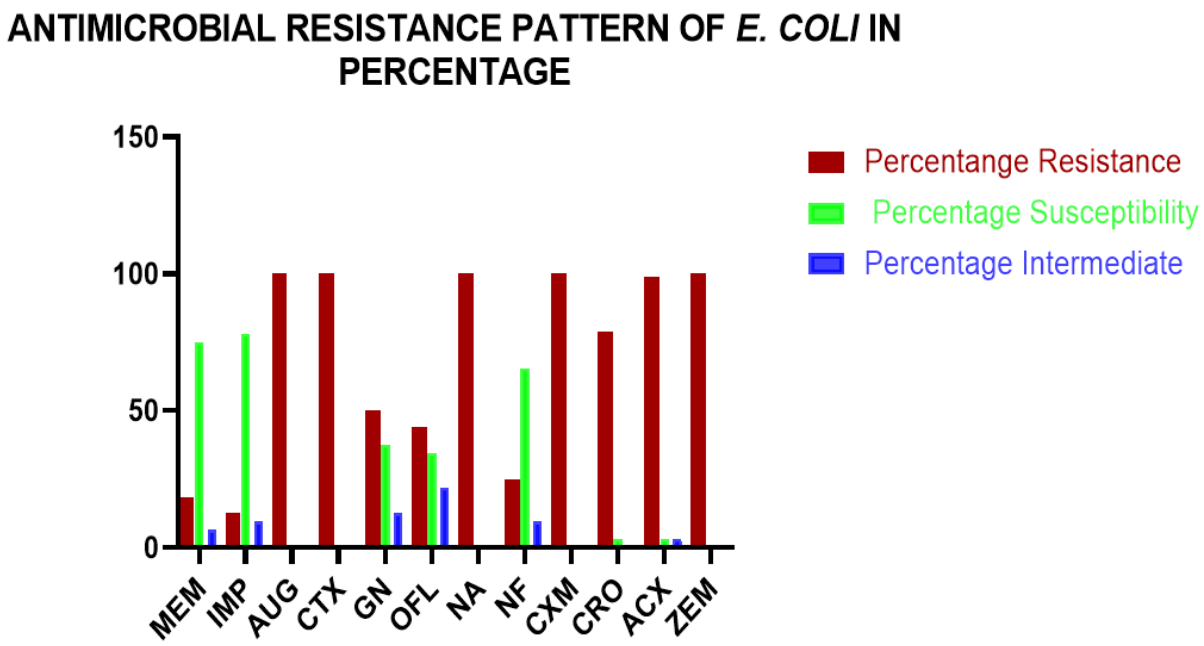

Figure 1. *Graph showing Percentage resistance of Escherichia Coli for intermediate, and susceptibility to various antibiotics.*

Key: S= Susceptible, I= Intermediate, R =Resistance, Zone of inhibition are measured in millimeters. CRO= Ceftriaxone/Sulbactam, GEN= Gentamicin, OFL=Ofloxacin, AUG=Amoxicillin/clavnalic acid, NF=Nitrofurantoin, CTX =Cefotaxime, LBC =Levofloxacin, NA= Nalidixic Acid, ZEM= Cefixime, IMP= Imipenem/ Cilastatin, MEM= Meropenem.

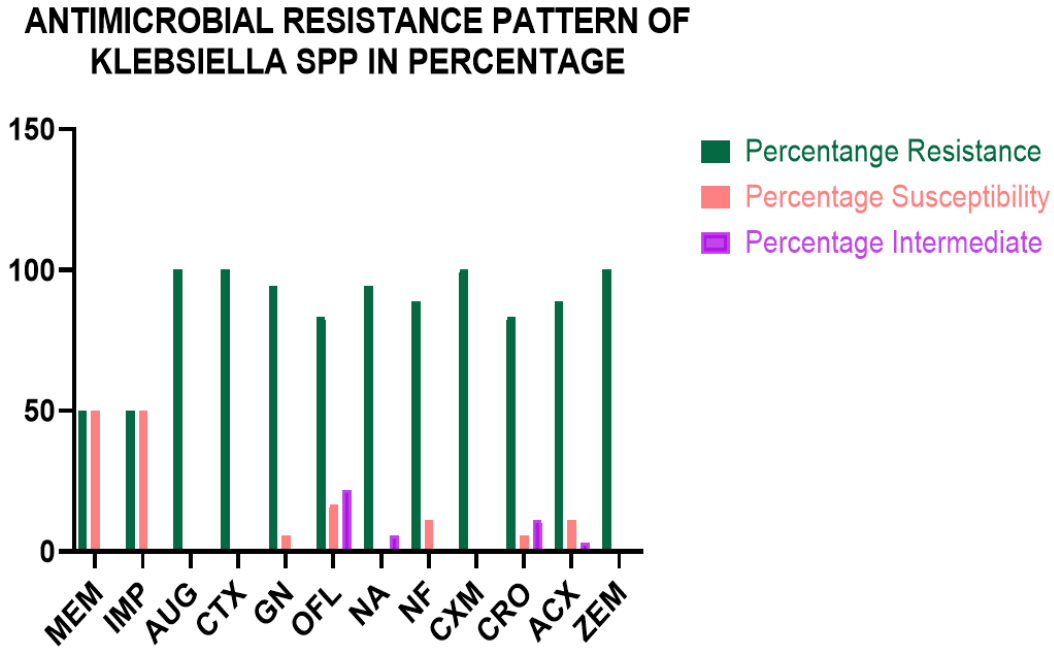

Figure 2. *Graph showing Percentage resistance of Klebsiella Spp for intermediate and susceptibility to various antibiotics.*

Key: S= Susceptible, I= Intermediate, R =Resistance, Zone of inhibition are measured in millimeters. CRO= Ceftriaxone/Sulbactam, GEN= Gentamicin, OFL=Ofloxacin, AUG=Amoxicillin/clavnalic acid, NF=Nitrofurantoin, CTX =Cefotaxime, LBC =Levofloxacin, NA= Nalidixic Acid, ZEM= Cefixime, IMP= Imipenem/ Cilastatin, MEM= Meropenem.

Table 2. Biochemical test result for the samples.

TEST	RESULT E. coli *Klebsiella spp*
Colonies on MacConkey agar	pink colonies pink colonies
Colonies on EMB agar	Green sheen pink colonies
Gram staining	-Rod -Rod
Oxidase	-
Indole test	+ -
Citrate test	+ +
Urease test	- +
Catalase test	+ +
KEY: + = Positive = Negative	

4. Discussion

Escherichia coli and Klebsiella species use different virulence factors to colonize and spread in the human body and cause a variety of illnesses. The scientific community is extremely concerned about this bacteria's rising antibiotic resistance in recent years.

According to this study, women (62% of patients at the University of Benin Teaching Hospital) were more likely to have UTIs. These findings align with those of other studies, which showed that 62.6–86.9% of UTI cases involved females. [12, 13]. The higher prevalence of UTIs in women can be due to various risk factors for this type of infection, as well as the close proximity of the urethra, anus, and vagina in females [14]. Gram-negative bacteria are the main cause of UTIs, with E. coli being the most common, accounting for 70–95% of cases [15]. Other Gram-negative bacteria, such as Klebsiella spp., Enterobacter spp., and Proteus spp., are also causes of UTIs [12]. Enterococcus spp. and Staphylococcus spp. are examples of potential Gram-positive human uropathogens [16]. In this study, E. coli was the most common isolate, found in 64% of cases. Similar results were reported by [12, 17], who identified E. coli in 56.7% and 58.9% of patients, respectively. K. pneumoniae, the second most frequently isolated bacterium, was found in 36% of cases, consistent with findings from other studies [12, 13, 18]. In this study, K. pneumoniae was significantly more often isolated from male patients.

K. pneumoniae-related UTIs are probably more likely to be caused by host factors than by the virulence of this opportunistic pathogen, such as impaired urine flow caused by conditions like vesico-ureteral reflux, strictures at the uretero-vesical junction, urinary tract malformations, neurogenic bladder, or benign prostate hyperplasia [19]. While *K. pneumoniae* was much more common in older people (40–60 and over 60), *E. coli* was significantly more frequently isolated in the age group of 0–18. This is consistent with the findings of [20]. Due to the immune system's diminished effectiveness and, in the case of elderly patients, the urinary tract's compromised function, these two patient groups—young patients and older patients—are more vulnerable to urinary tract infections. The presence of any studied genes encoding virulence factors in *K. pneumoniae* was not associated with the emergence of upper UTIs, according to a [19] study. According to these authors, *K. pneumoniae* UTIs are influenced by the patient's health and are linked to urinary system dysfunction, which is more frequently seen in elderly individuals. Human UTIs that are challenging to treat have increased as a result of the global expansion of MDR bacteria [16].

In many regions of the world, ampicillin is one of the antibiotics that are most frequently used in empirical treatment [12]. In this study, ampicillin resistance was found in (88.9%) K. pneumoniae isolates and 93.8%) E. coli isolates. Numerous regions of the world have reported high rates of uropathogens' ampicillin resistance [12, 15, 20]. This is why using ampicillin as the sole drug in empirical therapy for UTI is neither acceptable nor advised. For simple cystitis or as a first-line treatment for moderate pyelonephritis, amoxicillin-clavulanic acid was suggested [21]. In this investigation, the amoxicillin-clavulanic acid combination was completely ineffective against E. coli and Klebsiella spp. Cephalosporins 2nd G (cefuroxime-100%) and 3rd G (cefotaxime-100%) resistance was observed in this investigation in isolates of E. coli and Klebsiella spp. Cefuroxime and cefotaxime are not recommended for the empirical treatment of UTIs in this area due to the high K. pneumoniae resistance to these antibiotics. The antibiotics carbapenems (ertapenem, imipenem, and meropenem) are suggested for the treatment of acute, simple pyelonephritis, complex UTIs, and urosepsis [23]. According to [22], carbapenems are also utilized to treat infections caused by ESBL-producing bacteria. Carbapenems were sensitive to 50% of Klebsiella and 75% of E. coli. Treating UTIs has become more

difficult as carbapenem-resistant uropathogens have emerged. Worldwide reports of clinical isolates with increased carbapenem resistance due to MBL production have increased recently [22]. In this investigation, a high percentage of K. pneumoniae (94.4%) and E. coli (84.4%) displayed levofloxacin resistance.

5. Conclusion

Although *K. pneumoniae* was also often identified from UTI cases, our study demonstrated that *E. coli* is the primary uropathogen. UTIs are more common in women and people between the ages of 18 and 30. This study demonstrated that the rising prevalence of uropathogens that are resistant to the existing antibiotics limits the options for treating UTIs with them. Penicillin and cephalosporin should not be used frequently to treat UTIs because these organisms are resistant to them. The combination of carbapenem and nitrofurantoin was quite efficient, and its agent's use may be increased.

Abbreviations

S	Susceptible
I	Intermediate
R	Resistance
CRO	Ceftriaxone/Sulbactam
GEN	Gentamicin
OFL	Ofloxacin
AUG	Amoxicillin/clavnalic Acid
NF	Nitrofurantoin
CTX	Cefotaxime
LBC	Levofloxacin
NA	Nalidixic Acid
ZEM	Cefixime
IMP	Imipenem/ Cilastatin
MEM	Meropenem

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Author Contributions

Ogunsina Olabode Isaiah is the sole author. The author read and approved the final manuscript.

Conflicts of Interest

The author declares no conflicts of interest.

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