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Antimicrobial Activity of Four Different Commercially Available Antibiotics against Selected Bacteria Isolated from Urine Samples of UTI Patients

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Abstract

More than thirty percent of the global population suffers from urinary tract infections. While many infections are currently treated empirically, appropriate standards for the prudent use of antibiotics should be concentrated on preventing the emergence of bacterial resistance and the elevated risk of infections that are challenging to treat. The article includes and discusses pertinent information about risk factors, severity levels, and the proper microbiological interpretation of pathogens. Research was done to compare the in vitro antibacterial activity of commercial antibiotics that are frequently used to treat urinary tract infections (UTIs) to clinical isolates that were obtained from urine samples of patients who were suffering from UTIs. A total of 5 isolates were identified as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa*, following different morphological, physiological and biochemical tests. Antibiotic sensitivity of four commercial antibiotics viz., cefpodoxime, cefuroxime, amoxicillin, and ciprofloxacin was screened by disc diffusion assay. Among the four antibiotic discs tested 40% of isolates were resistant to cefuroxime followed by 20% to cefpodoxime, amoxicillin and ciprofloxacin. 40% of the isolates were found to be resistant to multiple antibiotics and termed as multi-drug resistant (MDR) isolates. Cefpodoxime shows smallest zone of inhibition against *Staphylococcus epidermidis* and *Staphylococcus aureus* of about 7 mm and 8 mm, respectively. Hence, further research should be carried out to identify the active biomolecules or Phyto-molecules from the plants and determine the effects of them *in vitro* as well as *in vivo*.

Keywords: *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, multi-drug resistant, cefpodoxime, cefuroxime, amoxicillin, ciprofloxacin, urinary tract infections, antibacterial activity, colony morphology.

Introduction:

A Urinary Tract Infection (UTI) is a common ailment that can produce symptoms like painful and frequent urination, bloody or hazy urine, and pelvic pain. An UTI can be caused by an infection in any portion of the urinary system, including the kidneys, ureters, bladder, and urethra **[1]**.

Uropathogenic *Escherichia coli* is the primary cause of urinary tract infections (UTIs), accounting for over 85% of recurrent cystitis and at least 35% of recurrent pyelonephritis. While non-pathogenic *Escherichia coli* lacks virulence properties, uropathogenic *Escherichia coli* possesses them. For many years, antibiotics and other chemotherapeutic agents have been used to treat *E. coli*-associated UTIs. However, uropathogenic *Escherichia coli* is becoming more resistant to these antibiotics as well as other widely available antimicrobials **[2]**.

Recent reports have indicated that the clinical administration of antibiotics, such as nitrofurantoin, ceftizoxime, ciprofloxacin, pipemidic acid, nitrofurantoin, cefamendol, cefotaxime, chloramphenicol, kanamycin, cefuroxime, nalidixic acid, augmentin, tobramycin, and ampicillin, against the pathogenic bacteria has been gradually prohibited due to the emergence of multi drug resistant (MDR) bacterial strains. As a result, searching for new alternative medicines to control MDR pathogens has become an essential aspect of drug development research in the last few years **[3]**.

In actuality, more than 70% of the antibacterial drugs authorized between 1981 and 2005 were beta-lactams, macrolides, and quinolones. Semi-synthetic adaptations of the first naturally occurring substance discovered more than 50 years ago make up the majority of new antibacterial medications produced by pharmaceutical companies **[4]**.

Complex tannins, which are components of living organisms, are the main source of naturally occurring anti-microbials found in both plants and microbes. Tannase, also known as tannin acyl hydrolase, is an extracellular enzyme that has the ability to hydrolyze the ester bonds in tannins to break them down to produce glucose and GA **[5]**.

Their efficacy against UMs was also compared to that of other commercially available antibiotics, such as sulfamethoxazole (SXT), levofloxacin (LEV), ciprofloxacin (CIP), amikacin (AK), and nitrofurantoin (F), in order to ascertain whether the Tannase (TN) and Gallic Acid (GA) could replace the frequently used antibiotic for treating UTIs **[4, 5]**.

Materials and Methods:

1. Collection of Clinical Samples

Human clinical samples were obtained from Bhubaneswar private clinics where the bacteria were isolated from urinary tract infection (UTI) patients. Urine samples were preserved and used for additional bacterial isolation and identification.

2. Isolation of Pure Culture

Using a standard microbiological method, five bacteria were isolated as pure cultures **[6]**. A milliliter of urine sample was suspended in nine milliliters of sterile water, and the sample was serially diluted in sterile water up to a 10^{-10} dilution. 100 µl of each dilution was taken and evenly spread over the surface of nutrient agar (NA) medium **[7]**, which was supplemented with 50 µg/ml of cycloheximide to inhibit the growth of yeast and fungi **[8]**. Plates were then incubated at 32°C for five days.

The white colonies were sub-cultured and then pure cultured after five days of incubation. The purified bacterial isolates were then stored at 4°C in nutrition broth (NB) media **[9]**.

3. Morphological Characterization

Under a high-power magnifying lens, bacterial colonies were examined, and the morphology of the colonies was recorded in relation to the seven characters: form, margin, elevation, size, colour, texture, and opacity **[10]**.

4. Gram Staining

A thin smear was prepared by spreading the broth culture on a glass slide followed by heat fixing. The smear was flooded with crystal violet (primary stain) for 45 seconds and washed off with water. The smear was then covered with Gram's iodine (mordant) for 45 seconds, decolorized with alcohol, and washed with water. Finally, the smear was stained with safranin (counter stain) for 1 minute. After washing and air drying, the slides were viewed at 45X under microscope **[10]**.

5. Biochemical Characterization

Following initial investigations, the isolates were chosen for biochemical analyses. Biochemical tests generally used are starch hydrolysis, urea hydrolysis, fermentation of different carbohydrates, motility test, indole test, methyl red test, Voges-Proskauer test, citrate utilization test, and catalase test, oxidase test **[11]**.

6. Antibiotic Sensitivity Test

The antibiotic sensitivity pattern of the bacterial isolates was determined against four commercially available antibiotic discs by disc diffusion method. Each bacterial isolate was cultured into nutrient broth and incubated at 37°C for 18 hours. Mueller-Hinton agar was selected for the disc diffusion assay and prepared by mixing beef extract (2.00g), casein (17.50g), starch (1.50g), and agar (17.00g) in 1000 ml distilled water **[12]**.

Using an L-spreader, the broth culture was spread aseptically onto the plate. Four commercially available antibiotic discs: cefpodoxime (30 µg/disc), cefuroxime (20 µg/disc), amoxicillin (15 µg/disc), and ciprofloxacin (30 µg/disc) were then placed on the surface of the medium with sterile forceps and gently pressed to ensure proper contact with the medium. The plates were then incubated at 37°C for a full day. Following this incubation, the organism was classified as resistant if it showed no zone of inhibition and as sensitive if there was complete zone of inhibition. The diameter of the discs and the diameter of the zone of inhibitions were measured using a measuring scale.

Results and Discussion:

The colony morphologies and biochemical features of the bacterial isolates are displayed in Tables 1 and 2, respectively, and were determined by examining various attributes as described in the materials and methods. Total of five bacterial pure cultures were isolated, from which three are gram negative bacteria: *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa* and two are gram positive bacteria: *Staphylococcus aureus* and *Staphylococcus epidermidis*.

ISOLATES	SHAPE	SIZE	ELEVATION	MARGIN	COLOUR	OPACITY	TEXTURE
C1	circular	small	convex	entire	grayish white	opaque	rough, mucoid
C2	circular	medium	convex	entire	golden yellow	opaque	smooth, mucoid
C3	circular	large	convex	entire	off white	translucent	smooth, shiny
C4	circular	medium	raised	entire	gray	opaque	smooth, shiny
C5	irregular	large	flat	lobate	off white	opaque	rough, mucoid

[Table-1: Colony Morphology of Five Bacterial Isolates from Clinical Samples]

ISOLATES	C1	C2	C3	C4	C5
GRAM'S STAIN	negative / rod	positive / cocci	negative / rod	positive / cocci	negative / rod
STARCH HYDROLYSIS	negative	positive	negative	negative	negative
UREA HYDROLYSIS	negative	positive	negative	positive	negative
GLUCOSE FERMENTATION	positive	positive	positive	positive	negative
MOTILITY	positive	negative	positive	negative	positive
INDOLE	positive	negative	negative	negative	negative
METHYL RED	positive	positive	positive	negative	negative
VOGES-PROSKAUER	negative	positive	negative	positive	negative
CITRATE UTILIZATION	negative	positive	negative	negative	positive
CATALASE	positive	positive	positive	positive	positive
OXIDASE	negative	negative	negative	negative	positive

[Table-2: Biochemical Characterization of Bacterial Isolates]

An antibiotic susceptibility test was carried out on 5 bacterial isolates against 4 commercially available antibiotics are shown in Table 3. Among the all isolates, *Escherichia coli* is more susceptible to amoxicillin followed by cefuroxime having zone of inhibition about 37 mm and 32.5 mm, respectively. However, *Salmonella typhi* is 100% resistant to cefpodoxime and amoxicillin; *Staphylococcus epidermidis* is resistant to cefuroxime and ciprofloxacin; and *Pseudomonas aeruginosa* is resistant to cefuroxime. Cefpodoxime shows smallest zone of inhibition against *Staphylococcus epidermidis* and *Staphylococcus aureus* of about 7 mm and 8 mm, respectively.

ISOLATES	CEFPODOXIME	CEFUROXIME	AMOXICILLIN	CIPROFLOXACIN
C1	21	32.5	37	13
C2	8	31.5	32	23
C3	-	17	-	9
C4	7	-	17	-
C5	12	-	15.5	18

[Table-3: Antibacterial Activity of Commercially Available Antibiotics Against Five Bacterial Isolates]

The development of drug resistance coupled with low patient compliance, medication side effects, and the increased expense of therapeutic combinations points to the urgent need for a treatment plan that has the same or greater positive benefits of antibiotics but better adverse effects **[13]**.

The primary concern regarding this significant health issue is that the emergence of antibiotic resistance

limits the therapeutic choices now accessible to treat infectious infections, indicating the necessity of developing new antibiotic compounds **[14]**.

Since the discovery of these antibiotics and their use as chemotherapeutic agents, the medical community has believed that this will eventually lead to the eradication of infectious diseases; however, the emergence of resistant strains of E. coli and many other bacteria worldwide has become a major therapeutic problem in recent times. Antibiotics are the mainstay of treatment for microbial infections **[15]**.

Aside from the fact that natural products have been used in traditional medicine for thousands of years, long before antibiotics and other modern drugs were introduced, multi-drug resistant strains of E. Coli are also being isolated from community-acquired infections **[16]**. In this study, five bacterial samples were tested for antibiotic sensitivity using four different commercially available antibiotics (Cefpodoxime, Cefuroxime, Amoxicillin, and Ciprofloxacin).

Conclusion:

The worldwide emergence of multidrug-resistant (MDR), E. coli has become a potential threat for patient suffering from UTI. In the present study, five multi-drug resistant bacteria were isolated from Urinary Tract Infection patient, where some of them exhibited as sensitive to some commercial antibiotics. Escherichia coli is more susceptible to amoxicillin followed by cefuroxime having zone of inhibition about 37 mm and 32.5 mm, respectively. So, amoxicillin and cefuroxime could be used in future days to cure E. coli associated UTI of patient as 100% antibacterial activity obtained by these two commercial antibiotics.

Salmonella typhi is 100% resistant to cefpodoxime and amoxicillin; Staphylococcus epidermidis is resistant to cefuroxime and ciprofloxacin; and Pseudomonas aeruginosa is resistant to cefuroxime. Cefpodoxime shows smallest zone of inhibition against Staphylococcus epidermidis and Staphylococcus aureus of about 7 mm and 8 mm, respectively. According to the result, we can conclude that the bacteria are resistant to commercially available antibiotics. Hence, further research should be carried out to identify the active biomolecules or Phyto-molecules from the plants and determine the effects of them in vitro as well as in vivo.

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