Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE

DOI: 10.53555/rr33w096

ANTIMICROBIAL SUSCEPTIBILITY PATTERNS OF ESCHERICHIA COLI ISOLATED FROM PATIENTS IN CALCUTTA SCHOOL OF TROPICAL MEDICINE

Amrita Roy[1*], Zubia Zain[2], Prof. Dr. Banya Chakraborty[3], Dr. Arup Kumar Mitra[4]

[1*]M.Sc. Microbiology St. Xavier's College, Kolkata (Autonomous) amritaroy66666@gmail.com
[2]M.Sc. Microbiology St. Xavier's College, Kolkata (Autonomous)
[3]Professor Dept. of Microbiology Calcutta School of Tropical Medicine
[4]Associate Professor St. Xavier's College, Kolkata (Autonomous)

***Corresponding Author:** Amrita Roy
[*]M.Sc. Microbiology St. Xavier's College, Kolkata (Autonomous) amritaroy66666@gmail.com

Abstract

This study aimed to determine the prevalence and antimicrobial susceptibility patterns of Escherichia coli (E. coli) isolated from clinical samples, primarily urine, at the Calcutta School of Tropical Medicine. Increasing rates of antimicrobial resistance in E. coli, particularly the emergence of Extended Spectrum Beta-Lactamases (ESBLs), pose a growing worldwide concern in treating infections like Urinary Tract Infections (UTIs), which E. coli is the leading cause of.

OBJECTIVE: The aim of this study is to determine the prevalence and antimicrobial susceptibility of E. coli from clinical samples.

Methodology

A retrospective review was conducted on results from urine cultures, and data on E. coli isolates and their antimicrobial susceptibility were collected. Isolates were identified using MacConkey agar culture (showing pink-red colonies), Gram staining, and a panel of biochemical tests (Citrate, Urease, SIM, and TSI). Antimicrobial susceptibility was evaluated using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar.

Results and Conclusion

Of the 487 urine samples analyzed between June and July 2022, 12.3% yielded E. coli isolates, with the highest isolation rate observed in urine specimens. The study found a significantly high overall resistance of E. coli to certain groups of antibiotics, with resistance rates for Ampicillin being the highest (>60%) and for Cephalosporins being high (around <50% >). Ampicillin is no longer recommended for the empirical treatment of UTIs due to this high resistance. High resistance was also observed for Co-trimoxazole (55%) and Ciprofloxacin (53.44%).

Conversely, the isolates showed high susceptibility to certain antibiotics. Fosfomycin (resistance rate <5%) and Amikacin (resistance rate 5.17%) were found to be highly active.

The emergence of resistance among uropathogenic E. coli to penicillin, cephalosporin, quinolone, fluoroquinolones, and sulfonamides limits their use as first-line UTI treatments. The study concludes that fosfomycin and nitrofurantoin could be considered appropriate antimicrobials for empirical therapy of UTIs due to their high susceptibility. Regular and continuous monitoring of antibiotic susceptibility is vital to guide and optimize empirical treatment.

Introduction

Community-acquired urinary tract infection is considered as one of the most common reasons for consultation in everyday practice ,it represents a major source of antibiotic consumption. Escherichia coli is the leading cause of urinary tract, ear, wound and other infections in humans. Increasing rates of antimicrobial resistance among E. coli is a growing concern worldwide.It has been observed that *Escherichia coli* (E. coli) is the main pathogen incriminated and ESBLs being leading as a concern matter of gaining resistance against antibiotics.The aim of this study is to evaluate Antimicrobial susceptibility patterns of e.coli in Calcutta School of Tropical medicine.

As we know, Escherichia coli is a common inhabitant of the human and animal gut, but can also be found in water, soil and vegetation which are common sources. It is the leading pathogen causing urinary tract infections and is among the most common pathogens causing bloodstream infections, wounds, otitis media and other complications in humans as well as animals. E. coli is also the most common cause of food and water-borne human diarrhea worldwide and in developing countries, causing many deaths in children under the age of five years.

Gram-negative pathogens are particularly worrisome because they are becoming resistant to nearly all the antibiotic drug options available, creating situations reminiscent of the pre-antibiotic era. The most serious gram-negative infections occur in health care settings and are most commonly caused by Enterobacteriaceae (mostly *Klebsiella pneumoniae* and *Escherichia coli*), *Pseudomonas aeruginosa*, and Acinetobacter.

Urinary tract infection (UTI) is one of the most common infectious diseases so far.Studies show that it is the second most common bacterial infection managed in primary care, accounting for approximately millions visits to health care providers each year. UTI affects mostly women, with an estimated two in every three women experiencing at least one episode of UTI during a lifetime.To treat UTI, an empirical antibiotic treatment is frequently initiated, since antibiotic susceptibility necessitates a minimum of 48 h for testing. However, this strategy of treatment leads to the emergence of resistance to several first-line antimicrobial agents, multidrug resistance and extended spectrum beta-lactamases (ESBLs),which are raising major concern worldwide. To control the increasing prevalence of antibiotic resistance, many scientists recommend that resistance rates against antibacterial drugs should not exceed 10–20% for starting empirical treatment.At 20% for uncomplicated cystitis and 10% for acute pyelonephritis, male UTI, cystitis in pregnancy, and other cystitis presentations at risk of complication. For that reason and in order to choose the appropriate antimicrobial empirical treatment, knowledge of region specific antimicrobial susceptibility patterns that is based on up-to-date epidemiological data is vital.The most effective way for bacteria to counteract antibiotics has been by producing β-lactamases, enzymes that inactivate the drugs by hydrolyzing the β-lactam ring.

Based on the sequence analysis, β-lactamases and the PBPs are believed to diverge from a common ancestor. All PBPs possess β-lactam catalyzing capability to a smaller extent. For instance, PBP5 was demonstrated to have the highest β-lactamase activity. At pH 7.0 and 30°C, the half-life of penicilloyl-PBP5 was averaged at 10 min. In addition, the monofunctional penicillin-binding DD-peptidases and penicillin-hydrolyzing serine beta-lactamases retain the same tertiary folding, three-motif amino acid (Lys-Thr-Gly) sequence signature, serine-assisted catalytic mechanism and active-site topology.

Because of the diversity of enzymatic characteristics of the many β-lactamases discovered, multiple attempts have been made to categorize them since the late 1960s. These Classifications involve two major approaches: the first and older one is based on the biochemical and functional characteristics of the enzyme, whereas the second approach is based on the molecular structure of the enzyme. In the former classification scheme, several criteria are used, including the spectrum of antimicrobial

substrate profile, enzyme inhibition profile, hydrolysis rate (Vmax), binding affinity (Km), isoelectric focusing (pI), protein molecular weight, and amino acid composition.

The molecular classification of β-lactamases is based on the nucleotide and amino acid sequences in these enzymes. To date, four classes are recognized (A-D), correlating with the functional classification. Classes A, C, and D act by a serine based mechanism, whereas class B or metallo β-lactamases need zinc for their action.

Extended spectrum beta-lactamases (ESBLs) are defined as enzymes produced by certain bacteria that are able to hydrolyze extended spectrum cephalosporin. They are therefore effective against beta-lactam antibiotics such as ceftazidime, ceftriaxone, cefotaxime and oxyimino-monobactam. The objective of this study is to provide a better understanding of ESBL and the epidemiology of ESBL producing organisms which are among those responsible for antibiotic resistant strains. Globally, ESBLs are considered to be problematic, particularly in hospitalized patients. There is an increasing frequency of ESBL in different parts of the world. The high risk patients are those contaminated with ESBL producer strains as it renders treatment to be ineffective in these patients. Thus, there is an immediate need to identify ESBL and formulate strategic policy initiatives to reduce their prevalence.It is estimated that more than 90% of ampicillin resistance among E.coli is related to the presence of TEM-1.TEM-1 is able to hydrolyze penicillin and first generation cephalosporins.

ESBLs are found in Gram-negative bacteria, especially in enterobacteriaceae and Pseudomonas aeruginosa. The most important beta-lactamase that is prevalent is TEM-1. It is estimated that more than 90% of ampicillin resistance among E.coli is related to the presence of TEM-1 . TEM-1 is able to hydrolyze penicillin and first generation cephalosporins. The first derivative of TEM-1 is TEM-2, with a single replacement of amino acids . The difference between beta- lactamase enzymes is the substitution of amino acids that produces different phenotype of enzymes.The substitutions are more common among TEM, SHV and OXA enzymes in defined amino acids positions. The combination of altered amino acids produces different phenotypes of beta-lactamase enzymes with varying ability to hydrolyze 3rd generation cephalosporin and increases the level of resistance to beta-lactamase inhibitors.

According to the Bush, Jacoby and Medeiros scheme,
beta-lactamases are divided into four groups:

A) Group I (Ambler Class C) beta-lactamases
B) Group 2 (Ambler Class A) enzymes
C) Group 3 (Ambler Class B) enzymes
D) Group 4 beta-lactamases

Methodology

Study Design:

A retrospective review is done on results of cultures of urine that have been performed. The sex and age of patients, as well as E. coli isolates and antimicrobial susceptibility data are collected from the registration records using a standard data collection form.

1.Specimen collection :

A)Pre-Urine Collection:

1. The patient ID labels are laid on specimen collection cup so that when the urine cups are upright, the barcode looks like a ladder.

B)Urine Collection:

Instruction are previously given to each patient to do the following for urine collection:

1. Hands are to be washed with soap and water beforehand.

2. not to remove the cap from the urine cup until ready to collect.
3. Refrain from touching the inside of cup or cap at any time
4. Collect 50 - 60 mL of urine in the cup.
5. Recaping the urine cup.

In order to maintain sample integrity of the specimen we must be collected in a sterile and dust-free environment. After collection , we immediately secure the cap on the cup and transfer the sample to at least -20° C cold storage freezer.

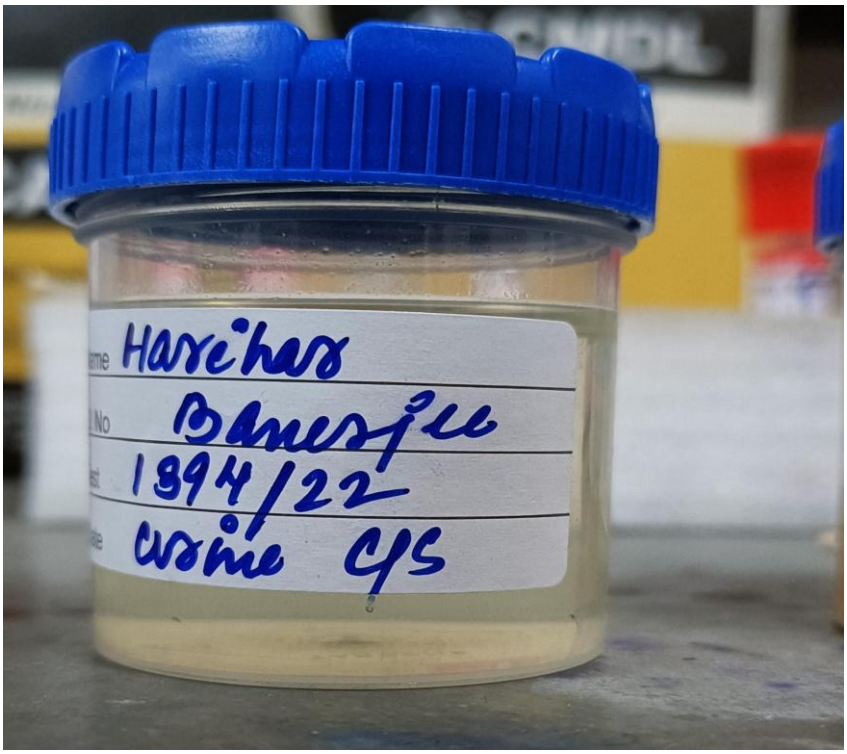


Fig: Urine sample

C) Wet Mount

This method is performed to determine the culture of a living organism, where the sample is placed over a slide and then a coverslip is placed over this specimen. After that, the slide is observed under a microscope to confirm if the specimen is motile or nonmotile, also the presence of pus cell in the sample. It also helps to determine the structure and shape of the organisms .

Procedure

1. Before going ahead , we have to ensure that the urine sample container , which we received has been tightly closed and devoid of any possible contamination.
2. Take a glease free slide and pipette 1 to 2 drops of urine onto the slide.
3. Carefully place the coverslip onto the slide with centre having the urine sample

Fig: A single drop of urine is taken with a help of sterile Strawer
Fig: Placing the coverslip onto the slide

Culture and Identification:

1. Tip over the container to re-mix the urine sample.
2. Removing the cap and dipping the end of a sterile 1-µL inoculating loop (white) into the urine and removing it vertically making sure that there is no urine up the loop.
3. Tip and spread the inoculum over the surface of a standard MacConkey agar plate (60 × 15 mm) prepared according to the instructions of the manufacturing company.
• First of all we have to make a single streak across the centre. Then, spread the inoculum evenly distributed in a Diagnostics cross-zigzag arrangement to the primary streak, as shown in picture.
4. Re-dip the end of the same 1-µL loop into the urine and removing it vertically making sure that there is no urine up the loop.
5. Tip and spread the inoculum over the surface of MacConkey agar plate (60 × 15 mm). Spread as described above. Prepare the MacConkey agar plates.
6.Incubate the plates aerobically at 35–37 ◦C for at 18–24 h.
7.The following day, count the number of colonies on the surface of MacConkey medium. Each colony growing on the agar plate represents one colony forming unit (cfu)/µL
(according to the size of the loop), which is equal to 1000 cfu/mL.

- As the standard operating procedures show, clean-catch midstream morning urine specimens are collected using sterile wide mouth glass containers.
- The urine container are kept at low temperature immediately after the collection, this step makes us to devoid of any possible contamination and maintaining the bacterial growth.
- Specimens are inoculated onto respective agar plates. The plates were incubated at 37o C aerobically and examined after 24 and 48 hours.

Fig:Urine sample are plated onto culture media Gram staining

Preparation of a slide smear

1. Inoculation loop is used to transfer a drop of suspended culture to the microscope slide.
2. If a Petri dish or a slant culture tube has the colony, a drop or a few loopful of water is added to facilitate a minimal amount of colony transfer to the examination slide.
3. A minimal amount of culture is required. If culture can be detected visually on an inoculation loop, it indicates the collection of too much culture.
4. Culture is spread with an inoculation loop to an even thin film over a circle of 15mm in diameter. A typical slide can contain up to 4 small smears if examining more than one culture.
5. The slide can be either air-dried or dried with the help of heat over a gentle flame. The slide should be moved circularly over the flame to prevent overheating or forming of ring patterns in the slide. The heat helps the cell adhesion to the glass slide and prevents the significant loss of culture during rinsing.

Gram staining:

- Crystal violet stain is added over the fixed culture.
- After 10 to 60 seconds, the stain is poured off, and the excess stain is rinsed with water. The goal is to wash off the stain without losing the fixed culture.
- Iodine solution is used to cover the smear for 10 to 60 seconds. This step is known as "fixing the dye." Iodine solution is poured off, and the slide is rinsed with running water. Excess water from the surface is shaken off.
- A few drops of decolorizer are added to the slide. Decolorizers are often the mixed solvent of ethanol and acetone. This step is known as "solvent treatment." The slide is rinsed with water in 5 seconds. To prevent excess decolorization in the gram-positive cells, stop adding decolorizer as soon as the solvent is not colored as it flows over the slide.
- The smear is counterstained with basic fuchsin solution for 40 to 60 seconds. The fuchsin solution is washed off with water, and excess water is blotted with the bibulous paper. The slide can also be air-dried after shaking off excess water.

Microscopic examination of slide

- The slides are taken to undergo an examination under a microscope under oil immersion.
- Then they are to be examined using the X100 oil immersion objective.

MacConkey Agar

MacConkey Agar Is the earliest selective and differential medium for cultivation of coliform organisms. Subsequently MacConkey Agar and Broth have been recommended for use in microbiological examination of foodstuffs and for direct plating / inoculation of water samples for coliform counts. Pancreatic digest of gelatin and peptones (meat and casein) provide the essential nutrients, vitamins and nitrogenous factors required for growth of microorganisms.

Lactose monohydrate is the fermentable source of carbohydrate. The selective action of this medium is attributed to crystal violet and bile salts, which are inhibitory to most species of gram-positive bacteria.

Sodium chloride maintains the osmotic balance in the medium.After enrichment of Escherichia coli in MacConkey Broth, it is then sub cultured on MacConkey Agar. Gram-negative bacteria usually grow well on the medium and are differentiated by their ability to ferment lactose. Lactose fermenting strains grow as red or pink and may be surrounded by a zone of acid precipitated bile.

Ingredients	Gms / Litre
Peptones (meat and casein)	3.000
Pancreatic digest of gelatin.	17.000
Lactose monohydrate	10.000
Bile salts	1.500
Sodium chloride	5.000

Crystal violet	0.001
Neutral red	0.030
Agar	13.500

pH after sterilization(at 25°C) 7.1±0.2

Gelling

Firm comparable with 1.35% Agar gel.

Organisms -Escherichia coli
Inoculum -50 -100
Growth Recovery - 25 -100 >=50 %
Color of colony -pink-red with bile precipitate
Temperature -30-35 °C
Incubation period- 18 -72 hrs

Principle:

MacConkey agar is a selective and differentiating agar that only grows gram-negative bacterial species; it can further differentiate the gram-negative organisms based on their lactose metabolism. The fermentation of lactose produces organic acids, particularly lactic acid, which decreases the pH of the agar. MacConkey agar contains the essential nutrients required for microorganism growth. Additional key components include crystal violet dye, bile salts, lactose, and neutral red (a pH indicator). The lactose in the agar is a source of fermentation. Lactose-fermenting microorganisms will produce organic acids, particularly lactic acid, which will lower the pH. Neutral red is a pH indicator that turns from off-white to bright red/pink as the pH drops below 6.8.Lactose fermenting species will grow pink colonies. Lactose fermentation will produce acidic byproducts that lower the pH, and this turns the pH indicator to pink.

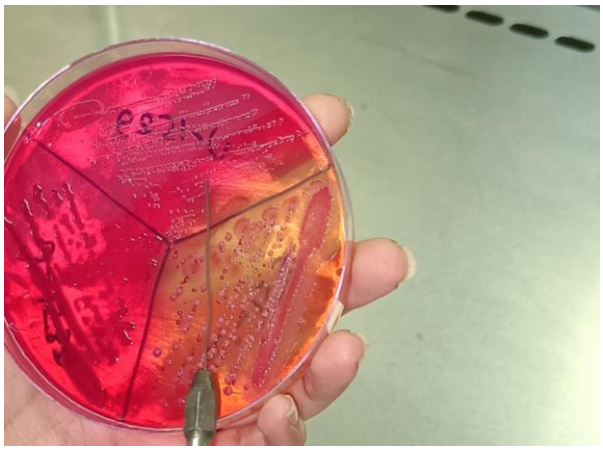


Fig: visible pink colonies are *Escherichia coli.*

Biochemical test

BIOCHEMICAL TESTS FOR ESCHERICHIA COLI (E.COLI)

Biochemical test are performed for the identification of bacterial species, based on differences in biochemical activities. Bacterial physiology differs from one bacterium to another.The differences in carbohydrate metabolism, protein metabolism, fat metabolism, production of certain enzymes, ability to utilize a particular compound etc. help them to be identified.

In our lab the following mentioned tests are performed for biochemical test for Escherichia coli.

- Citrate Test
- Sim / Indole test
- Urease Test
- And TSI (Triple Sugar Iron) tesr

Citrate Utilization Test

Citrate agar is used to test the ability of an organism to utilize citrate as a source of energy.The agar medium contains citrate as the sole carbon source and inorganic ammonium salts as the sole source of nitrogen. The growth of the organism is indicative of the utilization of citrate as it is an intermediate metabolite in the Krebs cycle. The enzyme citrase breaks down citrate into oxaloacetate and acetate, where oxaloacetate is further broken down to form pyruvate and carbon dioxide.The release of carbon dioxide induces the metabolism of ammonium salts, causing the formation of ammonia or sodium carbonate, both of which increase the alkalinity of the medium.The shift in pH turns the bromthymol blue indicator in the medium from green to blue above pH 7.6.

Procedure of Citrate Utilization Tes

1. In a beaker , 24.38 grams of lab prepared media is taken and 1000ml of distilled water is added.
2. This beaker is then send for autoclave for sterilization of about 15lbs pressure at 121°C (15 mins) .
3. Once the autoclave is complete, the tubes are taken out and cooled at a slanted position to a temperature of about 40-45°C. The position should be maintained in order to obtain butts of 1.5 – 2.0 cm depth.

Utilization test

1. A well-isolated colony is taken from an 18-24 hour culture with a sterile inoculating needle.
2. The citrate agar tubes are inoculated by streaking the surface of the slant. The slant should be streaked back and forth with the loop or the inoculating stick.
3. The test tubes should be examined daily for 4 days before discarding the result as a negative.
4. The change in color, if present, is observed

Results:

1. A positive test is demonstrated by growth with a color change from green to intense blue along the slant.
2. A negative test is demonstrated by no growth and no color change, and the color of the slant remains green.

Fig: Gives negative for E.coli

Urease Test

Urea is the product of decarboxylation of amino acids. Hydrolysis of urea produces ammonia and CO2. The formation of ammonia alkalinizes the medium, and the pH shift is detected by the color change of phenol red from light orange at pH 6.8 to magenta (pink) at pH 8.1. Rapid urease-positive organisms turn the entire medium pink within 24 hours.Weakly positive organisms may take several days, and negative organisms produce no color change or yellow as a result of acid production.

Preparation

1. Dissolve the ingredients in 100 ml of distilled water, suspend the agar in 900 ml of distilled water, boil to dissolve completely.
2. Autoclave at 121 degree C and 15 psi for 15 minutes.
3. Cool the agar to 50 to 55 degree C.
4. Distribute 4 to 5 ml per sterile tube and slant the tubes during cooling until solidified.

Procedure of Urease Test

1. Streak the surface of a urea agar slant with a portion of a well-isolated colony or inoculate slant with 1 to 2 drops from an overnight brain-heart infusion broth culture.
2. Examine the development of a pink color for 24hrs.

Result:

1. Positive Reaction: Development of an intense magenta to bright pink color in 15 min to 24 h.
2. Negative Reaction: No color change

Fig: Negative Result for *Escherichia coli*

Sulphur Reduction Test

SIM medium (Sulphide Indole Motility medium) which is a combination differential medium that tests three different parameters, Sulfur Reduction, Indole Production and Motility.The medium having the constituents ferrous ammonium sulfate and sodium thiosulfate, which together serve as indicators for the production of hydrogen sulfide (H2S). Hydrogen sulfide production detects when ferrous sulfide, a black precipitate, is produced as a result of ferrous ammonium sulfate reacting with hydrogen sulfide gas. Casein peptone of this medium is rich in tryptophan. Organisms having the enzyme tryptophanase degrade tryptophan to indole. Indole detection is achieved after the addition of Kovac's reagent following incubation of the inoculated medium. Indole combines with p-dimethylaminobenzaldehyde and produces a red band at the top of the medium. A negative indole test produces no color change after the addition of Kovac's reagent i.e. Yellow color of Kovac's reagent. A lower concentration of agar added to the medium provides a semi-solid structure allowing for the detection of bacterial motility.Motile organisms diffuse from the stab line and produce turbidity or cloudiness throughout the medium. The growth of non-motile bacteria is restricted along the stab line and leaves the surrounding medium clear. Another constituent, animal tissue of this medium which provides amino acids and nutrients necessary for bacterial growth

Procedure of SIM Test

1. Take pure colonies from an 18-24-hour old culture on a solid medium.
2. Inoculate the SIM Medium by stabbing the center of the medium to a depth of half an inch.
3. Incubate the inoculated medium aerobically at 37°C for 18-24 hours.
4. Observe for hydrogen sulfide production and motility of test organism.
5. Only apply Kovac's reagent (three drops) after reading the result of H2S and motility reaction to the surface of the medium.
6. Observe for the development of a pink to red color.

Result interpretation of SIM Test

1. Positive H2Stest: blackening of the medium
2. A negative H2S test: absence of blackening
3. Positive motility test: a diffuse zone of growth flaring from the line of inoculation
4. Negative motility test: restricted growth along the stab line
5. Indole positive test: a pink to red color ring is formed at the top of the medium after the addition of Kovac's reagent
6. Indole negative test: A yellow color denotes a negative indole test after the addition of Kovac's reagent

Fig: Motility - positive, **Indole**- Positive, **H2S:**negative, **Growth**- Positive

Triple Sugar Iron (TSI) Agar:

Triple Sugar Iron Agar contains three carbohydrates: glucose (0.1%), sucrose (1%) and lactose (1%) + (beef extract, yeast, and peptones etc) + phenol red as an indicator of pH. During preparation, the tubes containing molten agar are tilted. This inclination makes it possible to have an aerobic metabolism in the slope and anaerobic in the pellet.Glucose is utilized first by a fermentative organism and the entire medium becomes acidic (yellow) in 8 to 12 hours. The butt remains acidic even after an 18 to 24 hours incubation period because of the presence of organic acids resulting from the fermentation of glucose under anaerobic conditions in the butt of the tube. The slant, however, reverts to the alkaline (red) state because of oxidation of the fermentation products under aerobic conditions on the slant. This change is a result of the formation of CO2 and H2O and the oxidation of peptones in the medium to alkaline amines.The formation of CO2 and hydrogen gas (H2) is indicated by the presence of bubbles or cracks in the agar or by the separation of the agar from the sides or bottom of the tube.

Preparation of TSI Agar

1. Combine the ingredients, and adjust the pH to 7.3
2. Boil to dissolve the agar
3. Dispense it into tubes
4. Sterilize by autoclaving at 121°C for 15 minutes
5. Cool in a slanted position to give a 2.5 cm butt and a 3.8 cm slant.

Procedure for TSI Agar Test

1. With a sterilized straight inoculation needle touch the top of a well-isolated colony
2. Inoculate TSI agar by first stabbing through the center of the medium to the bottom of the tube and then streaking on the surface of the agar slant**.**
3. incubate the tube at 35°C in ambient air for 18 to 24 hours.

Result:

Red/ Yellow	K/ A	Glucose fermentation only, peptone catabolized.
Yellow/Yellow	A/A	Glucose and lactose /or sucrose fermentation.
Red/Red	K/K	No fermentation, Peptone catabolized under aerobic and/or anaerobic conditions
Yellow/Yellow with bubbles	A/A,G	Glucose and lactose and/or sucrose fermentation, Gas produced.
Red/Yellow with bubbles	K/A,G	Glucose fermentation only, Gas produced.
Yellow/Yellow with bubbles and black precipitate	A/A,G,H 2S	Glucose and lactose and/or sucrose fermentation, Gas produced, H2S produced.

Fig: A/ A *Escherichia coli*

Antimicrobial susceptibility tests :

According to the standard operational procedures,antimicrobial susceptibility, all tests are done on Mueller Hinton agar using the Kirby Bauer disk diffusion method. The Kirby Bauer test is a selected method whereby the discs are placed onto the surface ofMH media. During the incubation period,the antibiotic diffuses outward the discs into agar.This will create a concentration gradient in the agar which depends on the solubility of the chemical and its molecular size. The absence of growth of the organism around the antibiotic discs indicates that the respective microorganism is susceptible to that particular antibiotic and the presence of the growth around the antibiotic disc indicates the organism is resistant to that antibiotic. The area around no growth is known as Zone of inhibition, which is a uniform circular shape.MH agar is considered the best medium to use for routine susceptibility testing of nonfastidious bacteria for the following reasons:

- It shows acceptable batch-to-batch reproducibility for susceptibility testing
- It supports satisfactory growth of most non fastidious pathogens
- A large body of data and experience has been collected concerning susceptibility tests performed with this medium

Beef, Infusion from 300.0 g
Casamino acid, technical 17.5 g
Starch 1.5 g
Agar 17.0 g

Table 1: Antimicrobial agent - widely used for urine sample

Ampicillin	10 microgram
Ciprofloxacin	5 microgram
Trimethoprim Sulfamethoxazole	1.25 microgram
Cefotaxime	30 microgram
Cefepime	30 microgram
Imipenem	10 microgram
Colistin	MIC - microdilution method (1mcg/2mcg/ 4mcg)
Nitrofurantoin	300 microgram

Table -2: Antimicrobial agent with symbol, disc content and diameter zone

Antimicrobial agent	Symbol	Disc content	Diameter of Zone of inhibition (mm)
Amikacin	AK	30mcg	16 -23
Amoxyclave	AMC	30 mcg	24-30
Ampicillin	AMP	10 mcg	20-30
Cefepime	CPM	30mcg	32-40
Cefotaxime	CTX	30 mcg	29-35
Ciprofloxacin	CIP	5 mcg	30-40
Ceftazidime	CAZ	10mcg	25-32
Co- Trimoxazole	COT	25 mcg	23-29
Ertapenem	ETP	10mcg	29-36
Fosfomycin	FO	200mcg	25-33
Gentamicin	GEN	20 mcg	19-26
Imipenem	IPM	10 ncg	26-32
Nitrofurantoin	NIT	300mcg	20-25
Piperacillin / Tazobactam	PIT	100mcg	24-27
Meropenem	MRP	10 mcg	28-35

Preparation of Mueller-Hinton plate

1.Suspend the qgar in 1 liter of purified water. Mix thoroughly. Heat with frequent agitation and boil for 1 minute to completely dissolve the components. Autoclave at 121°C for 15 minutes. Dispense as desired. Allow to solidify at room temperature, then store at 4 to 8°C. Mueller-Hinton agar is stable for approximately 70 days from the date of preparation. Each lab should verify the quality and functionality of each batch of prepared media by testing known strains of organisms against each antimicrobial compound being used as the 70-day expiration date approaches.

2.One thing to be noted that ,the plates have been instructed to be poured to a depth of 4 mm (approximately 25 ml of liquid agar for 100-mm plates and 60 ml of liquid agar for 150-mm plates, but in any case to a measured depth of 4 mm). Plates that are too shallow will produce false susceptible results as the antimicrobial compound will diffuse further than it should, creating larger zones of inhibition Conversely, plates poured to a depth >4 mm will result in false resistant results.

3.pH of the MH agar should fall between 7.2 and 7.4 at room temperature after solidification and should be tested when the media is first prepared. If the pH is <7.2 certain drugs will appear to lose potency , while other agents may appear to have excessive activity (as say tetracycline). If the pH is >7.4, the opposite results may occur.

4.Important thing to be noted is that MH agar should be tested with known strains of organism at least weekly in order to verify that the media and disks are working as expected.

Preparation of inoculum for MH agar:

1. Using a sterile inoculating loop or needle, touch four or five isolated colonies of the organism to be tested.
2. Suspend the organism in 2 ml of sterile saline.
3. Use this suspension within 15 minutes of preparation.

Fig: Using inoculating loop a loop full of colony is taken to resuspend within test tube containing saline solution

Fig: OD is measured for confirming the turbidity of solution.

Inoculation of the MH plate

1. Dip a sterile swab into the inoculum tube.
2. Rotate the swab against the side of the tube (above the fluid level) using firm pressure, to remove excess fluid. The swab should not be dripping wet to be kept in mind.
3. Inoculating the dried surface of a MH agar plate by streaking the swab three times over the entire agar surface; rotate the plate approximately 60 degrees each time to ensure an even distribution of the inoculum is done.
4. Rim the plate with the swab to pick up any excess liquid.
5. Discard the swab into an appropriate container.
6. Leaving the lid slightly ajar, allow the plate to sit at room temperature at least 3 to 5 minutes, but no more than 15 minutes, for the surface of the agar plate to dry before proceeding to the next step.

Fig: With a help of sterile cotton swab , the inoculum is uniformly streak against the MH agar media

Placement of the antibiotic disks

1. After streaking with swap is done, Lift the dispenser off the plate and use forceps sterilized by either cleaning them with an alcohol pad or flaming them with isopropyl alcohol, touch each disk on the plate to ensure complete contact with the agar surface.
2. Not move a disk once it has contacted the agar surface even if the disk is not in the proper location, because some of the drug begins to diffuse immediately upon contact with the agar.
3. Sterilizing the forceps by cleaning them with a sterile alcohol pad and allowing them to air dry or immersing the forceps in alcohol then igniting.
4. Using the forceps carefully remove one disk from the cartridge.
5. Gently press the disk with the forceps to ensure complete contact with the agar surface.
6. Replace the lid to minimize exposure of the agar surface to room air
7. Continue to place one disk at a time onto the agar surface until all disks have been placed.
8. Once all disks are in place, replace the lid, invert the plates, and place them in a 35°C air incubator for 16 to 18 hours.

Fig1: Antibiotic disk are taken with a sterile forcep.
Fig2 : Antibiotic disk are placed carefully onto the agar media

Disk placement

It has to be noted that, Disks should not be placed closer than 24 mm (center to center) on the MH agar plate. Ordinarily, no more than 12 disks should be placed on a 150-mm plate or more than 5 disks on a 100-mm plate.

Avoid placing disks close to the edge of the plate as the zones will not be fully round and can be difficult to measure.

Measuring zone sizes

1. Following incubation, measure the zone sizes to the nearest millimeter using a ruler or caliper; include the diameter of the disk in the measurement.

2. It is instructed that, when measuring zone diameters, always round up to the next millimeter.
3. All measurements are made with the unaided eye while viewing the back of the petri dish.
4. Recorded the zone size on the recording sheet.
5. Growth up to the edge of the disk can be reported as a zone of 0 mm, which resembles resistant organisms.
6. Conclusion : The colonies which shows 0 mm are either mutant organisms that are more resistant to the drug being tested, or the culture was not pure and they are a different organism. If it is

determined by repeat testing that the phenomenon repeats itself, the organisms must be considered resistant to that drug.

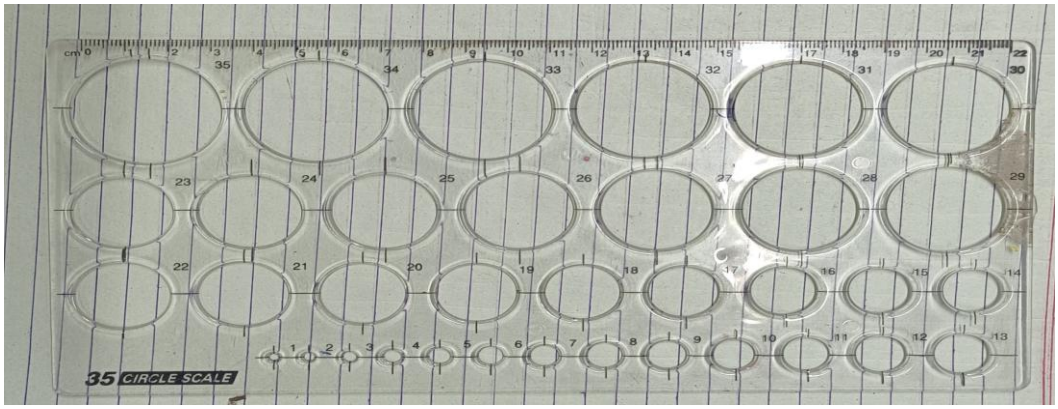


Fig: Measuring Scale for Zone inhibition

Analysis:

Between Jun and July 2022, a total of 487 urine samples
were analyzed for isolation and identification of bacteria and antimicrobial susceptibility testing. E. coli was isolated from 487 (12.3%) samples. Of these positive cases, the isolation rate of E. coli was the highest in urine samples. followed by 160 (4.7%) in miscellaneous sample (sputum, discharge of body etc). The overall susceptibility patterns of E. coli isolates from various clinical sources is calculated. Significantly high resistance rates to (as per as National AMR surveillance network AST pannel)

Ampicillin AMP 36 (66%),
amoxiclave AMC 19 (33%)
Piperacillin Taz- 12(20%)
Cefotaxime (CTX) -; 11(18%)
Ertapenem (ETP) - 1 (1.6%)
Meropenem- (MRP) -7(11.8%)
Amikacin -3 (5%)
Fosfomycin -2 (2.33) were detected.

Discussion

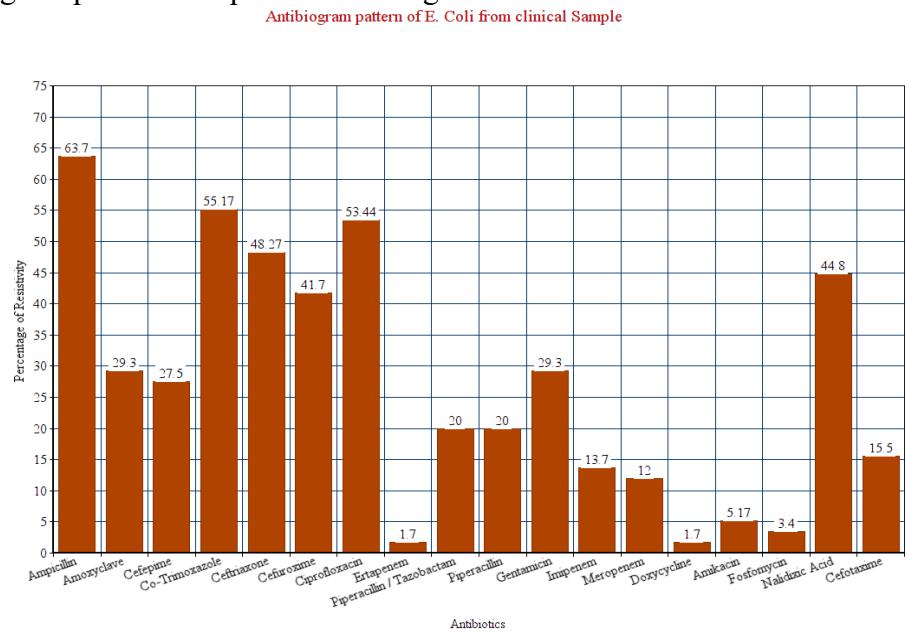
The isolation rate of E. coli in the present study was 12.3% and it was commonly isolated from urine samples (16.7%). However females are the most prone, as has been proven in this study.In this study, the overall resistance of E. coli to antimicrobials was high to some particular group of antibiotics. Penicillin and cephalosporin are much more resistant to E coli than other family of antibiotics.The most common antibiotic resistance rate is observed for ampicillin and cephalosporin. The highest resistance in E. coli is reported to Ampicillin, showing that ampicillin is the least active antimicrobial agent against E. coli; with resistance rates ranging > 60% to ampicillin and around <50% > to cephalosporin.These high levels of E. coli resistance to ampicillin may be a consequence of frequent and inappropriate use of this antibiotic in empirical therapy.That's why, ampicillin is no longer recommended for empirical treatment of UTIs

The antibiotics like Cefepime with 27.5% , ceftriaxone 48.27, cefuroxime -41.7 and cefotaxime - 15.5 have resistance among cephalosporin. And ceftriaxone having higher resistance rate amongst all. Antibiotics of carbapenem class like imipenem, Meropenem and ertapenem , imipenem and Meropenem are more likely similar in Resistivity than ertapenem.

The Drug class Quinoline where an Antibiotic is given named Nalidixic Acid which has quite higher resistance rate of 44.8% comparatively greater to some penicillin and to some extent of

cephalosporin. It is given to treat UTI and other genital infections.Studies have also shown that the The Group I producer beta-lactamases are resistant to beta lactam/beta-lactamase inhibitor combinations, penicillins, cephamycins, as well as 1st, 2nd and 3rd generation cephalosporins. They are sensitive to cefepime and carbapenems.
Gentamicin and amikacin are proven to be active against uropathogenic E. coli. Resistance Rate ranged between 29% and 5.17 respectively. Though Amikacin is considered to be much more susceptible than gentamicin.
Co-trimoxazole is a combination of trimethoprim and sulfamethoxazole and is in a class of medications called sulfonamides. It works by stopping the growth of bacteria. It has a higher resistance rate sulfonamides. It has a range of 55% which is quite arising concern against UTI.
Antibiotic Fosfomycin which is isolated from *Streptomyces fradiae* which has quite below 5% resistance rate.It is quite sensitive to bacteria infecting UTI.

Fluoroquinolones have been largely prescribed in the treatment of UTI, particularly in the empirical treatment of uncomplicated acute cystitis in women.Resistance to fluoroquinolones has become a growing concern. 41% of E.coli are resistant to fluoroquinolones.Increased bacterial resistance is the consequence of increased consumption without proper consultation from a physician.Though it has been found that, Quinolone resistance is more prevalent in strains that produce ESBL (E.coli) although, the mechanism of co-resistance is not cleared yet.
High sensitivity to Piperacillin and tazobactam , fosfomycin, doxycycline, co-trimoxazole , Meropenem, imipenem, amikacin, have been recorded and they were found to be the most effective antimicrobials against UTI.
Here a Antibiogram pattern is depicted in this figure



Conclusion

The emergence of antimicrobial resistance among uropathogenic E.coli to penicillin, cephalosporin, Quinolone , fluoroquinolones and sulfonamides, the rise of ESBL-producing organisms limited the use of these drugs as first-line treatment of UTIs. However, due to high susceptibility, fosfomycin and nitrofurantoin could be considered as appropriate antimicrobials for empirical therapy of UTIs. Nevertheless, regular and continuous monitoring of antibiotic susceptibility of the most common uropathogen, E. coli, is necessary in order to optimize empirical treatment and control antimicrobial resistance.

Acknowledgments

I specially thank to Prof (Dr.) Banya Chakraborty ma'am, Dr. Anindita Rakshit Ma'am and Dr. Malabika Biswas ma'am for this immense support in this study. It would had incomplete without them. And also for provoking gaining new knowledge and letting us experiencing as well as exploring the entire work phenomenon of microbiology lab. I specially thank to lab technician who helped to cope up with all the doubts and difficulties faced into the work area.

References

1. Wagenlehner FM, Naber KG, Weidner W. Rational antibiotic therapy of urinary tract infections. Med Monatsschr Pharm. 2008; 31: 385-90.
2. Antimicrobial susceptibility patterns of E. coli from clinical sources in northeast Ethiopia *Kibret M1 ,Abera B2
3. Kahlmeter G. An international survey of the antimicrobial susceptibility of pathogens from uncomplicated urinary tract infections: the ECO-SENS Project. J Antimicrobial Chemother. 2003; 51:69–76.
4. Aibinu, I. E., Ohaegbulam, V. C., Adenipekun, E. A., Ogunsola, F. T., Odugbemi, T. O., and Mee, B. J. (2003) Extended-spectrum beta-lactamase enzymes in clinical isolates of Enterobacter species from Lagos, Nigeria. J Clin Microbiol, 41 (5), 2197-200.