IJPSR (2020), Volume 11, Issue 2 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 19 April 2019; received in revised form, 24 August 2019; accepted, 01 September 2019; published 01 February 2020

ANTIBIOTIC SUSCEPTIBILITY PATTERN OF ESBL PRODUCING BACTERIA FROM URINARY TRACT INFECTION (UTI)

Juhi Hattewar *, Akshma Koul and Abhishek Mathur

NCS Green Earth Pvt. Ltd., M-7, MIDC Hingna Industrial Area, Nagpur - 440016, Maharashtra, India.

Keywords:

ESBL producing bacteria, Urinary tract infection, Antibiotic susceptibility, Antibiotic resistance

Correspondence to Author:
Juhi Hattewar

Junior Scientist (R&D),
NCS Green Earth Pvt. Ltd., M-7,
MIDC Hingna Industrial Area,
Nagpur - 440016, Maharashtra, India.

E-mail: hattewarjuhi@gmail.com

ABSTRACT: Antibiotics inhibit bacterial cell wall synthesis, protein synthesis or nucleic acid replication. The antibiotic must have access to and bind to its bacterial target site. Whether antibiotic resistance is intrinsic or acquired, the genetic determinants of resistance encode specific biochemical resistance mechanism that may include enzymatic inactivation of the drug, alteration to the structure of the antibiotic target site and changes that prevent access of an adequate concentration of the antimicrobial agents to the active site. Klebsiella organisms are often resistant to multiple antibiotics. Current evidence implicates plasmids as the primary source of the resistance genes. The susceptibility pattern of different antibiotics to ESBL producing *Escherichia coli* and Klebsiella spp. isolated from urine samples and they found that most of the infection caused by *E. coli* (61.29%) found to be most common bacteria in urinary tract infection followed by Klebsiella spp. (45.16%). Isolates were highly resistance to gentamicin followed by tetracycline, ampicillin, and amikacin. gentamicin showed resistance against *E. coli* and Klebsiella spp were 89.47% and 85.71% respectively. While tetracyclin and ampicillin showed 80% and 78% resistance to *E. coli* and Klebsiella spp. The isolates were highly susceptible and least susceptible to ciprofloxacin and norfloxacin against *E. coli* and Klebsiella spp. These antibiotics are considered as appropriate antimicrobials for empirical treatment of urinary tract infections. Most of the ESBL A producing isolates were multidrug-resistant. During present study, when urine sample inoculated on selective media like EMB, CLED, MSA, PIA and CIA, the growth of isolates on the medium were obtained as *E. coli, Klebsiella pneumonia, S. aureus,* and *Pseudomonas aeruginosa*.

INTRODUCTION: Antibiotics susceptibility tests including, ESBL screening and confirmation, were carried out by disc diffusion technique using Clinical Laboratory Standard Institute (CLSI) criteria. Ten different types of bacteria genera were observed from nine different clinical samples.

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DOI: 10.13040/IJPSR.0975-8232.11(2).799-10

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.11(2).799-10

E. coli was the most frequently isolated bacteria (30.5%) followed by *Staphylococcus aureus* (21.3%). ESBL producers showed high-level resistance against the quinolones, aminoglycoside, and cotrimoxazole but were sensitive to carbapenems and levofloxacin.

Non-ESBL organisms showed increased resistance to amoxicillin-clavulanate, ceftazidime, cotrimoxazole, tetracycline and amoxicillin. The prevalence of ESBL producers was 12.8%. *E. coli, Klebsiella pneumonia, and Pseudomonas aeruginosa* showed ESBL production of 17.3, 14.9 and 10.0%, respectively [1].

Antimicrobial susceptibility pattern and ESBL prevalence in *Klebsiella pneumoniae* from urinary tract infections. The study included 92 *Klebsiella pneumoniae*, isolated from the urine of patients suffering from UTIs. Antibiotic susceptibility testing and ESBL detection were carried out according to Clinical and Laboratory Standards Institute (CLSI) criteria. Eighteen antibiotics were tested in this study. A total of 54 (58.7%) isolates were found to be ESBL producers [2]. The detection of ESBL producing *E. coli* isolated from clinical samples.

This study was to determine the prevalence of ESBL isolation and identification of ESBL producing *E. coli* isolation and its confirmation via different confirmative tests such as ESBLs screening test. Double disc synergy test (DDST) and double-disc diffusion test (Phenotypic confirmatory test). In antibiotic susceptibility test the antibiogram revealed that 4 (6.15%) isolates were resistance to amikacin, (4.61%) were resistance to amoxyclav, 36 (55.38%) were resistant to ampicillin, gentamycin, 8 (12.30%) were resistant to levofloxacin, 5 (7.69%) were resistance to nalidixic acid, 25 (38.46%) were resistant to norfloxacin, 10 (15.38%) isolates were resistant to ofloxacin, 8 (12.30%) isolates were resistant to piperacillin. The ESBL percentage was found to be more in female urine samples [3].

In the present investigation, antibiotic susceptibility pattern of ESBL producing bacteria from urinary tract infection.

MATERIALS AND METHODS:

Collection of Microorganism: The urine sample is collected from the pathological laboratory Nagpur.

Isolation and Identification of Bacteria:

- Urine samples are stricked on different selective media's.
- Plates were incubated at 37 ºC for 24 h.
- After 24 h plates were observed for colonies.

Morphological Characteristics:

- **Gram Character Examination:** For the study of gram character a well-isolated colony was selected by picking the colony with inoculating loop smear was prepared on clean slide. A staining technique was performed as per standard procedure. Shape, size, gram character was then observed.
- **Motility:** Bacterial motility is an important part of making final identification. Motility of the given isolates was observed by using hanging drop method.
- **Biochemical Characteristics:** Various bio-chemical tests unique to each organism were performed by inoculating small portion of the well isolated colony into series of media. This include following tests.
- **IMViC Reactions:** IMViC reactions are a set of four useful reactions that are commonly employed in the identification of members of family *Enterobacteriaceae*. The four reactions are Indole test, methyl red test, Voges proskauer test, and citrate utilization test.

Indole Test: Bacterium to be tested is inoculated in peptone water, which contains amino acid tryptophan and incubated overnight at 37 ºC. Following incubation few drops of Kovac's reagent are added. Kovac's reagent consists of para-dimethyl amino benzaldehyde, isoamyl alcohol, and con. HCl. Ehrlich's reagent is more sensitive in detecting indole production in anaerobes and non-fermenters.

Formation of a red or pink colored ring at the top is taken as positive. Example: *Escherichia coli*: positive; *Klebsiella pneumoniae*: negative.

Methyl Red Test:

Procedure: The bacterium to be tested is inoculated into glucose phosphate broth, which contains glucose and a phosphate buffer and incubated at 37 ºC for 48 h. Over the 48 h the mixed-acid producing organism must produce sufficient acid to overcome the phosphate buffer and remain acid.

The pH of the medium is tested by the addition of 5 drops of MR reagent. Development of red color is taken as positive. MR negative organism produces a yellow color. Example: *Escherichia coli*: positive; *Klebsiella pneumoniae*: negative.

Voges Proskauer (VP) Test:
Procedure: Bacterium to be tested is inoculated into glucose phosphate broth and incubated for at least 48 h. 0.6 ml of alpha-naphthol is added to the test broth and shaken. 0.2 ml of 40% KOH is added to the broth and shaken. The tube is allowed to stand for 15 min. The appearance of the red color is taken as a positive test.

The negative tubes must be held for one hour since maximum color development occurs within one hour after addition of reagents. Examples: *Escherichia coli*: negative; *Klebsiella pneumoniae*: positive.

Citrate Utilization Test:
Procedure: Bacterial colonies are picked up from a straight wire and inoculated into the slope of Simmon's citrate agar and incubated overnight at 37 ºC. If the organism has examples: *Escherichia coli*: negative; *Klebsiella pneumoniae*: positive.

Triple Sugar Iron Test: The peptone mixture and the Beef and Yeast extracts provide the nutrients essential for growth. Sodium chloride maintains the osmotic balance of the medium. The Bacteriological agar is the solidifying agent.

Procedure:

1. Sterilize the inoculating needle in the blue flame of the bunsen burner till red hot and then allowed to cool.
2. From the rack, take the Trypticase soy broth tube containing the 24-48 h culture, remove the cap and flame the neck of the tube.
3. Using aseptic technique, take the culture of the organism from the TSB (tryptic soy broth) tube with the needle.
4. Again flame the neck of the tube and replace the tube in the test tube rack.
5. Take a sterile TSI slant tube from the rack, remove the cap and flame the neck of the tube.
6. Stab the needle containing the pure culture into the medium, up to the butt of the TSI tube, and then streak the needle back and forth along the surface of the slant.
7. Again flame the neck of the TSI tube, cap it and place it in the test tube rack.
8. Incubate at 37 ºC for 18 to 24 h.

Catalase Test:

1. Take 3% of H_2O_2 in a clean test tube.
2. Take loopful of culture and insert it in the test tube.
3. Formation of bubbles within 10 sec indicates a positive test.

Urease Test:

1. Prepare Christensen's urea agar slants. Streak the slant with the inoculum.
2. Incubate the test tubes at 37 ºC for 24 h.
3. After incubation observes the tubes. A pink color indicates positive test reaction.

Oxidase Test:

1. Soak a small piece of filter paper in 1% Kovacs oxidase reagent and let dry.
2. Use a loop and pick a well-isolated colony from a fresh (18- to 24-h culture) bacterial plate and rub onto treated filter paper.
3. Observe for color changes.

Antibiotic Susceptibility Test: Once a culture is established, there are two possible ways to get an antibiogram:

- A semi-quantitative way based on diffusion (Kirby-Bauer method); small discs containing different antibiotics, or impregnated paper discs, are dropped in different zones of the culture on an agar plate, which is a nutrient-rich environment in which bacteria can grow. The antibiotic will diffuse in the area surrounding each tablet, and a disc of bacterial lysis will become visible. Since, the concentration of the antibiotic was the highest at the center, and the lowest at the edge of this zone, the diameter is suggestive for the Minimum Inhibitory Concentration, or MIC, (conversion of the diameter in millimeter to

the MIC, in µg/ml, is based on known linear regression curves).

- A quantitative way based on dilution: a dilution series of antibiotics is established (this is a series of reaction vials with progressively lower concentrations of antibiotic substance). The last vial in which no bacteria grow contains the antibiotic at the Minimal Inhibiting Concentration.

Kirby Diffusion Method: The agar diffusion test (Kirby–Bauer antibiotic testing, KB testing, or disc diffusion antibiotic sensitivity testing) is a test of the antibiotic sensitivity of bacteria. It uses antibiotic-impregnated wafers to test the extent to which bacteria are affected by those antibiotics. In this test, wafers containing antibiotics are placed on an agar plate where bacteria have been placed, and the plate is left to incubate. If an antibiotic stops the bacteria from growing or kills the bacteria, there will be an area around the wafer where the bacteria have not grown enough to be visible. This is called a zone of inhibition.

The size of this zone depends on how effective the antibiotic is at stopping the growth of the bacterium. A stronger antibiotic will create a larger zone because a lower concentration of the antibiotic is enough to stop growth. The bacteria in question are swabbed uniformly across a culture plate. A filter-paper disk, impregnated with the compound to be tested, is then placed on the surface of the agar. The compound diffuses from the filter paper into the agar. The concentration of the compound will be highest next to the disk and will decrease as distance from the disk increases. If the compound is effective against bacteria at a certain concentration, no colonies will grow where the concentration in the agar is greater than or equal to the effective concentration. This is the zone of inhibition. These along with the rate of antibiotic diffusion are used to estimate the bacteria's sensitivity to that particular antibiotic. In general, larger zones correlate with smaller minimum inhibitory concentration (MIC) of antibiotics for that bacterium. Inhibition produced by the test is compared with that produced by known concentration of a reference compound. This information can be used to choose appropriate antibiotics to combat a particular infection [4-8].

Procedure: All aspects of the Kirby-Bauer procedure are standardized to ensure consistent and accurate results. Because of this, a laboratory must adhere to these standards. The media used in Kirby-Bauer testing must be Mueller-Hinton agar at only 4 mm deep, poured into either 100 mm or 150 mm Petri dishes. The pH level of the agar must be between 7.2 and 7.4. A close-up looks at the results of an agar diffusion test. Inoculation is made with a broth culture diluted to match a 0.5 McFarland turbidity standard, which is roughly equivalent to 150 million cells per ml [9-13].

Incubation Procedure:

1. Using an aseptic technique, place a sterile swab into the broth culture of a specific organism and then gently remove the excess liquid by gently pressing or rotating the swab against the inside of the tube.

2. Using the swab, streak the Mueller-Hinton agar plate to form a bacterial lawn.

   - To obtain uniform growth, streak the plate with the swab in one direction, rotate the plate 90° and streak the plate again in that direction.

   - Repeat this rotation 3 times.

   - Allow the plate to dry for approximately 5 minutes.

1. Use an Antibiotic Disc Dispenser to dispense discs containing specific antibiotics onto the plate.

2. Using a flame-sterilized forceps, gently press each disc to the agar to ensure that the disc is attached to the agar.

3. Plates should be incubated overnight at an incubation temperature of 37 °C (98.6 °F) [14-16].

E–Strip Method: The E - test principle was developed to standardize the disc diffusion method and to improve its reproducibility and reliability for clinical susceptibility predictions. The inhibition zone sizes from disc test results were compared to Minimum Inhibitory Concentration (MIC) values based on the reference agar dilution procedure.

The correlation between zone sizes and MIC values was then assessed using regression analysis and regression lines were used for extrapolating zone interpretive limits that corresponded to the MIC breakpoint values that defined susceptible, intermediate and resistant categorical results [17-28].

RESULTS:

Isolation and Characterization of Microbes from Clinical Specimens: After inoculation on EMB agar plates were observed for growth forms green metallic sheen. Similarly on MSA agar plates were observed for growth observed as golden yellow, smooth colonies along with the production of acid which turns the media color yellow. On PIA plates showed bluish-green, pigmented colonies were observed. On Mac Conkey agar bluish color colonies observed. The results are shown in **Table 1-13**; **Fig 1-14**.

Characterization of Isolates: The clinical isolates were characterized on the basis of microscopic and biochemical test various test performed were shown in tables.

Microscopic and Biochemical Characterization of Clinical Isolates: Total no. of samples (n) = 8.

TABLE 1: MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF URINE SAMPLE 1

S. no.	Bacterial Isolates	Sugar Fermentation				IMVIC				Identified Bacteria
		lactose	Glucose	Mannitol	Sucrose	Indole	MR	VP	C	
1	US -1	Acid +gas	Acid +gas	Acid +gas	Acid +gas	+ve	+ve	-ve	-ve	*E. coli*
2	US -1	Acid +gas	Acid +gas	Acid +gas	Acid +gas	- ve	-ve	+ ve	-ve	*Klebsiella pneumonia*
3	US -1	Acid +gas	Acid +gas	Acid +gas	Acid +gas	-ve	-ve	-ve	+ve	*S. aureus*
4	US -1	Acid +gas	Acid +gas	Acid +gas	Acid +gas	+ ve	-ve	+ve	+ve	*Pseudomonas aeruginosa*

On the basis of the above results, it can be said that the clinical isolates were found to be *Escherichia coli, Klebsiella pneumonia, S. aureus,* and *Pseudomonas aeruginosa.*

TABLE 2: MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF URINE SAMPLE 2

S. no.	Bacterial Isolates	Sugar Fermentation				IMVIC				Identified Bacteria
		lactose	Glucose	Mannitol	Sucrose	Indole	MR	VP	C	
1	US -2	Acid +gas	Acid +gas	Acid +gas	Acid +gas	- ve	-ve	+ ve	-ve	*Klebsiella Pneumonia*
2	US -2	Acid +gas	Acid +gas	Acid +gas	Acid +gas	-ve	-ve	-ve	+ve	*S. aureus*
3	US -2	Acid +gas	Acid +gas	Acid +gas	Acid +gas	-ve	-ve	-ve	+ve	*S. aureus*
4	US -2	Acid +gas	Acid +gas	Acid +gas	Acid +gas	+ ve	-ve	+ve	+ve	*Pseudomonas aeruginosa*

On the basis of above result, it can be said that, the clinical isolates were found to be *Klebsiella pneumonia, S. aureus and Pseudomonas aeruginosa.*

TABLE 3: MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF URINE SAMPLE 3

S. no.	Bacterial Isolates	Sugar Fermentation				IMVIC				Identified Bacteria
		lactose	Glucose	Mannitol	Sucrose	Indole	MR	VP	C	
1	US -1	Acid +gas	Acid +gas	Acid +gas	Acid +gas	+ve	+ve	-ve	-ve	*E. coli*

On the basis of above result, it can be said that the clinical isolate was found to be *Escherichia coli.*

TABLE 4: MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF URINE SAMPLE 4

S. no.	Bacterial Isolates	Sugar Fermentation				IMVIC				Identified Bacteria
		lactose	Glucose	Mannitol	Sucrose	Indole	MR	VP	C	
1	US -2	Acid +gas	Acid +gas	Acid +gas	Acid +gas	-ve	-ve	-ve	+ve	*S. aureus*
2	US -2	Acid +gas	Acid +gas	Acid +gas	Acid +gas	+ ve	-ve	+ve	+ve	*Pseudomonas aeruginosa*

On the basis of above result, it can be said that the clinical isolates were found to be *S. aureus and Pseudomonas aeruginosa.*

TABLE 5: MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF URINE SAMPLE 5

S. no.	Bacterial Isolates	Sugar Fermentation				IMVIC				Identified Bacteria
		lactose	Glucose	Mannitol	Sucrose	Indole	MR	VP	C	
1	US -1	Acid +gas	Acid +gas	Acid +gas	Acid +gas	+ve	+ve	-ve	-ve	*E. coli*
2	US -1	Acid +gas	Acid +gas	Acid +gas	Acid +gas	- ve	-ve	+ ve	-ve	*Klebsiella pneumoniae*
3	US -1	Acid +gas	Acid +gas	Acid +gas	Acid +gas	-ve	-ve	-ve	+ve	*S.aureus*
4	US -1	Acid +gas	Acid +gas	Acid +gas	Acid +gas	+ ve	-ve	+ve	+ve	*Pseudomonas aeruginosa*

On the basis of the above results, it can be said that the clinical isolates were found to be *Escherichia coli, Klebsiella pneumonia, S. aureus,* and *Pseudomonas aeruginosa.*

TABLE 6: MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF URINE SAMPLE 6

S. no.	Bacterial Isolates	Sugar Fermentation				IMVIC				Identified Bacteria
		lactose	Glucose	Mannitol	Sucrose	Indole	MR	VP	C	
1	US -1	Acid +gas	Acid +gas	Acid +gas	Acid +gas	+ve	-ve	+ve	+ve	*Pseudomonas aeruginosa*

On the basis of the above results, it can be said that the clinical isolates were found to be *Pseudomonas aeruginosa*.

TABLE 7: MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF URINE SAMPLE 7

S. no.	Bacterial Isolates	Sugar Fermentation				IMVIC				Identified Bacteria
		lactose	Glucose	Mannitol	Sucrose	Indole	MR	VP	C	
1	US -1	Acid +gas	Acid +gas	Acid +gas	Acid +gas	-ve	-ve	-ve	+ve	*S. aureus*

On the basis of the above results, it can be said that the clinical isolates were found to be *S. aureus*.

TABLE 8: MORPHOLOGICAL AND BIOCHEMICAL CHARACTERISTICS OF URINE SAMPLE 8

S. no.	Bacterial Isolates	Sugar Fermentation				IMVIC				Identified Bacteria
		lactose	Glucose	Mannitol	Sucrose	Indole	MR	VP	C	
1	US -1	Acid +gas	Acid +gas	Acid +gas	Acid +gas	+ve	+ve	-ve	-ve	*E. coli*
2	US -1	Acid +gas	Acid +gas	Acid +gas	Acid +gas	+ve	+ve	-ve	-ve	*E. coli*
3	US -1	Acid +gas	Acid +gas	Acid +gas	Acid +gas	- ve	-ve	+ ve	-ve	*Klebsiella pneumoniae*

On the basis of the above results, it can be said that the clinical isolates were found to be *Escherichia coli* and *Klebsiella pneumonia.*

TABLE 9: COLLECTION OF ISOLATES FROM URINE SAMPLES

S. no.	Clinical samples	Total no. of isolates collected	*E. coli*	*Klebsiella pneumonia*	*S. aureus*	*Pseudomonas aeruginosa*
1	US-1	4	1	1	1	1
2	US-2	4	-	1	2	1
3	US-3	1	1	-	-	-
4	US-4	2	-	-	1	1
5	US-5	4	1	1	1	1
6	US-6	1	-	-	-	1
7	US-7	1	-	-	1	-
8	US-8	3	2	1	-	-

TABLE 10: ANTIBIOTIC SUSCEPTIBILITY OF *E. COLI*

Samples	Antibiotics	Concentration of antibiotics	Diameter of zones (mm)	Interpretation
US-1	Norfloxacine	10 mcg	17 mm	Resistant
US-1	Amikacin	30 mcg	17 mm	Sensitive
US-2	Gentamycin	10 mcg	13 mm	Resistant
US-2	Erythromycin	15 mcg	NZ	Resistant
US-3	Ceprofloxacin	10 mcg	NZ	Resistant
US-3	Amoxicillin	30 mcg	NZ	Resistant
US-4	Morifloxcine	5 mcg	28 mm	Sensitive
US-4	Ofloxacine	5 mcg	28 mm	Sensitive
US-5	Tetracycline	30 mcg	NZ	Resistant
US-5	Streptomycine	10 mcg	NZ	Resistant
US-6	Methicilline	5 mcg	NZ	Resistant
US-6	Clindamycine	20 mcg	NZ	Resistant
US-7	Penicillin	10 units	NZ	Resistant

US-7	Rifamicine	5 mcg	16 mm	Resistant
US-8	Lomefloxacine	10 mcg	17 mm	Resistant
US-8	Cotrimoxazole	25 mcg	23 mm	Sensitive
US-8	Tobramicine	10 mcg	15 mm	Resistant

*Note: Control = antibiotics without Tris, S = Sensitive, NZ = NO zone of inhibition, I = Intermediate, R = Resistant

TABLE 11: ANTIBIOTIC SUSCEPTIBILITY OF *KLEBSIELLA PNEUMONIAE*

Samples	Antibiotics	Concentration of antibiotics	Diameter of zones (mm)	Interpretation
US-1	Norfloxacine	10 mcg	22 mm	Sensitive
US-1	Amikacin	30 mcg	18 mm	Sensitive
US-2	Gentamycin	10 mcg	16 mm	Resistant
US-2	Erythromycin	15 mcg	NZ	Resistant
US-3	Tobramicine	10 mcg	13 mm	Resistant
US-3	Amoxicillin	30 mcg	NZ	Resistant
US-4	Morifloxcine	5 mcg	18 mm	Resistant
US-4	Ofloxacine	5 mcg	21 mm	Sensitive
US-5	Teicoplanin	30 mcg	19 mm	Sensitive
US-5	Tetracycline	10 mcg	13 mm	Resistant
US-6	Methicillin	5 mcg	NZ	Resistant
US-6	Linezoid	30 mcg	NZ	Resistant
US-7	Penicillin	10 units	NZ	Resistant
US-7	Amphicillin	30mcg	16 mm	Intermediate
US-8	Lomefloxacine	10 mcg	16 mm	Resistant
US-8	Tobramicine	10 mcg	17 mm	Resistant

*Note: Control = antibiotics without Tris, S = Sensitive, NZ = NO zone of inhibition, I = Intermediate, R = Resistant

TABLE 12: ANTIBIOTIC SUSCEPTIBILITY OF *STAPHYLOCOCCUS AUREUS*

Samples	Antibiotics	Concentration of antibiotics	Diameter of zones (mm)	Interpretation
US-1	Vancomycine	10 mcg	17 mm	Sensitive
US-1	Amikacin	30 mcg	15 mm	Sensitive
US-1	Gentamycin	10 mcg	15 mm	Resistant
US-2	Erythromycin	15 mcg	31 mm	Sensitive
US-2	Norfloxacine	10 mcg	21 mm	Sensitive
US-3	Amphicilline	30 mcg	26 mm	Sensitive
US-3	Morifloxcine	5 mcg	18 mm	Resistant
US-4	Ofloxacine	5 mcg	21 mm	Sensitive
US-4	Tetracycline	30 mcg	NZ	Resistant
US-5	Streptomycine	10 mcg	NZ	Resistant
US-5	Methicilline	5 mcg	NZ	Resistant
US-6	Clindamycine	20 mcg	NZ	Resistant
US-6	Penicillin	10 units	NZ	Resistant
US-7	Rifampicin	5 mcg	16 mm	Sensitive
US-7	Lomefloxacine	10 mcg	21 mm	Resistant
US-8	Cotrimoxazole	25 mcg	NZ	Resistant
US-8	Levofloxacine	10 mcg	15 mm	Resistant

*Note: Control = antibiotics without Tris, S = Sensitive, NZ = NO zone of inhibition, I = Intermediate, R = Resistant

TABLE 13: ANTIBIOTIC SUSCEPTIBILITY OF *PSEUDOMONAS AERUGINOSA*

Samples	Antibiotics	Concentration of antibiotics	Diameter of zones (mm)	Interpretation
US-1	Norfloxacine	10 mcg	17 mm	Intermediate
US-1	Amikacin	30 mcg	20 mm	Sensitive
US-2	Gentamycin	10 mcg	20 mm	Sensitive
US-2	Erythromycin	15 mcg	NZ	Resistant
US-3	Ceprofloxacin	10 mcg	18 mm	Sensitive
US-3	Meropenem	10 mcg	15 mm	Intermediate
US-4	Morifloxcine	5 mcg	28 mm	Sensitive
US-4	Cefepime	30 mcg	NZ	Resistant
US-5	Tetracycline	30 mcg	13 mm	Resistant
US-5	Streptomycine	10 mcg	NZ	Resistant
US-6	Methicilline	5 mcg	13 mm	Intermediate
US-6	Clindamycine	2 mcg	NZ	Resistant
US-7	Penicillin	10 units	NZ	Resistant
US-7	Rifamicine	5 mcg	16 mm	Resistant
US-7	Lomefloxacine	10 mcg	16 mm	Resistant
US-8	Cotrimoxazole	25 mcg	NZ	Resistant
US-8	Tobramicine	10 mcg	32 mm	Sensitive

*Note: Control = antibiotics without Tris, S = Sensitive, NZ = NO zone of inhibition, I = Intermediate, R = Resistant

FIG. 1: COLLECTION OF URINE SAMPLES

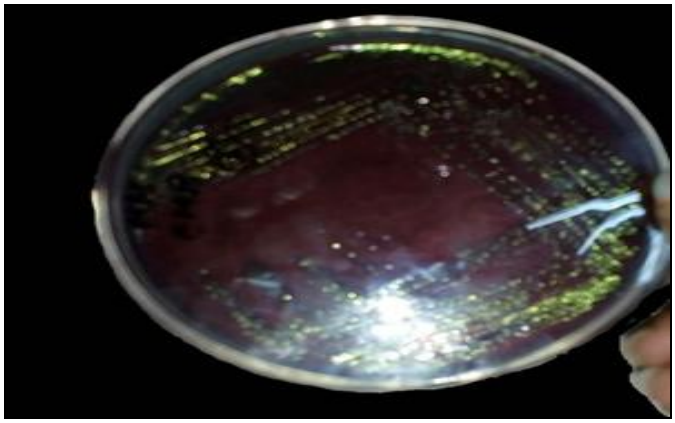


FIG. 2: GROWTH OF ISOLATES ON EMB

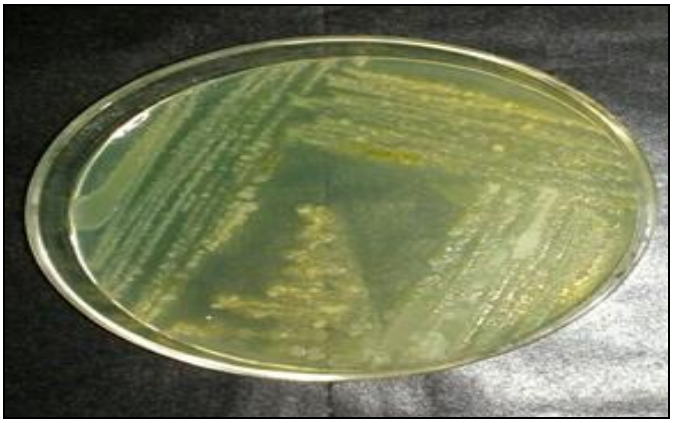


FIG. 3: GROWTH OF ISOLATES ON CLED

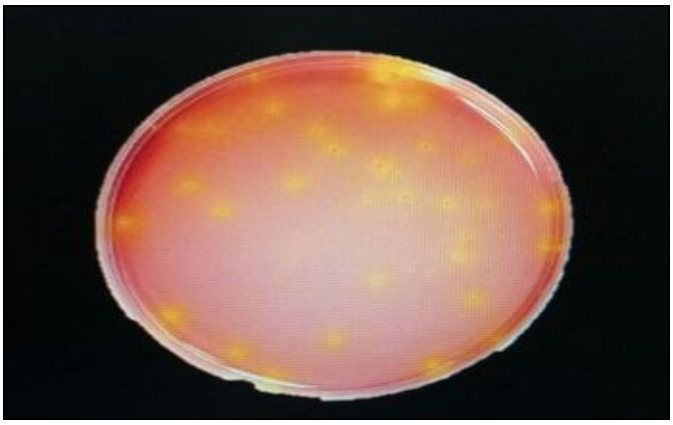


FIG. 4: GROWTH OF ISOLATES ON MSA

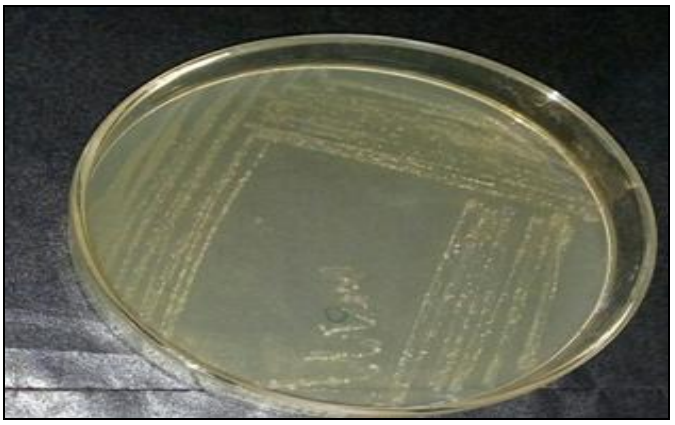


FIG. 5: GROWTH OF ISOLATES ON PIA

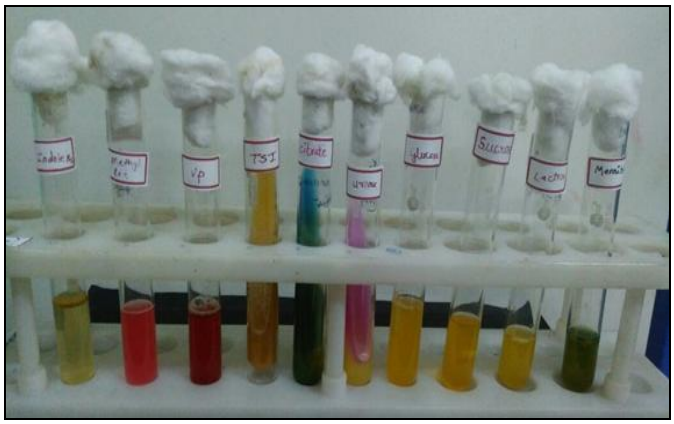


FIG. 6: RESULTS OF SUGAR FERMENTATION AND IMVIC FOR *E. COLI*

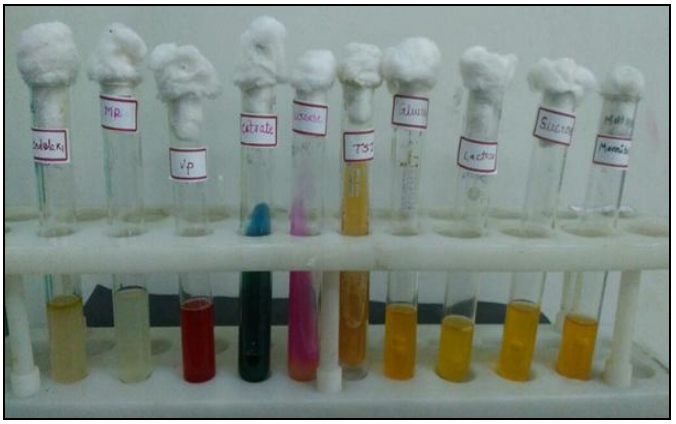


FIG. 7: RESULTS OF SUGAR FERMENTATION AND IMVIC TESTS FOR *KLEBSIELLA PNEUMONIAE*

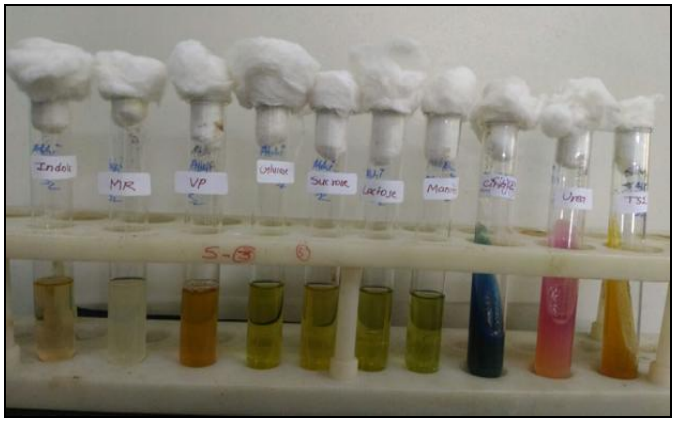


FIG. 8: RESULTS OF SUGAR FERMENTATION AND IMVIC TEST FOR *S. AUREUS*

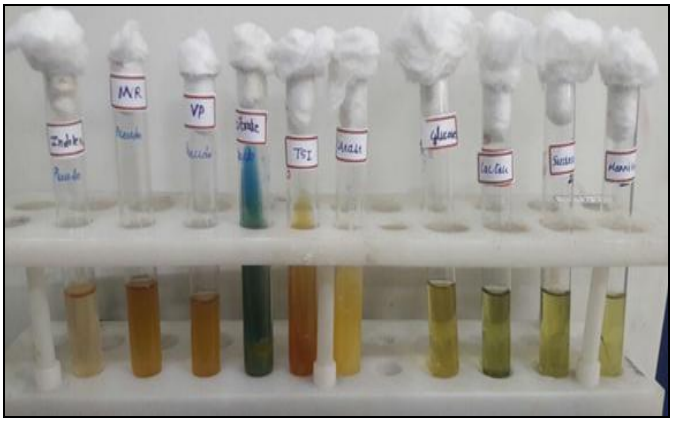
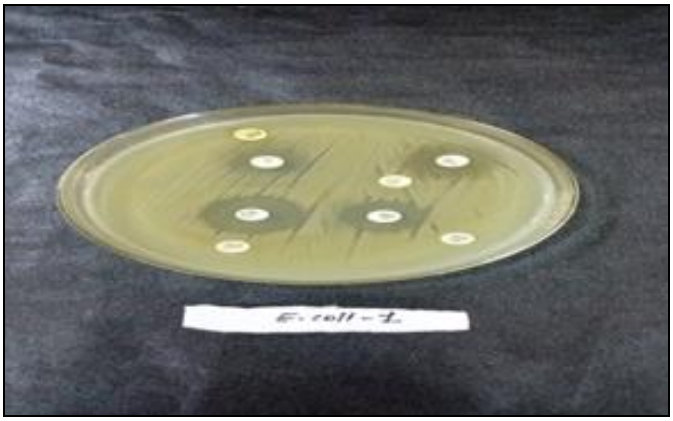


FIG. 9: RESULTS OF SUGAR FERMENTATION AND IMVIC TEST FOR *PSEUDOMONAS AERUGINOSA*



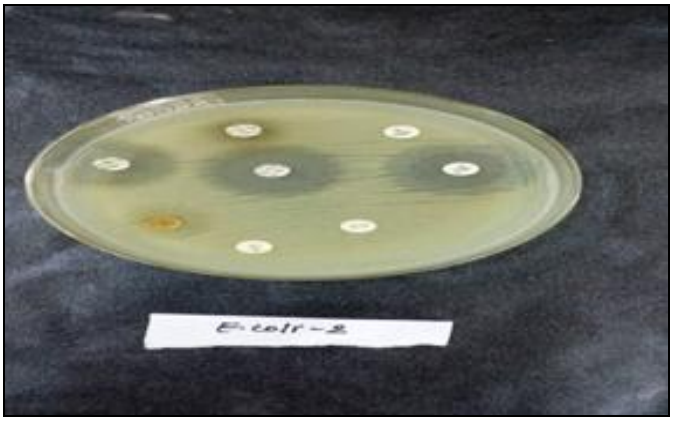
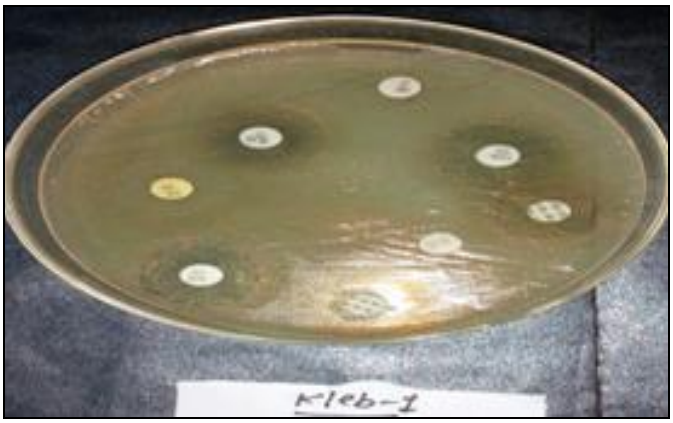


FIG. 10: ANTIBIOTIC SUSCEPTIBILITY TEST AGAINST *E. COLI*



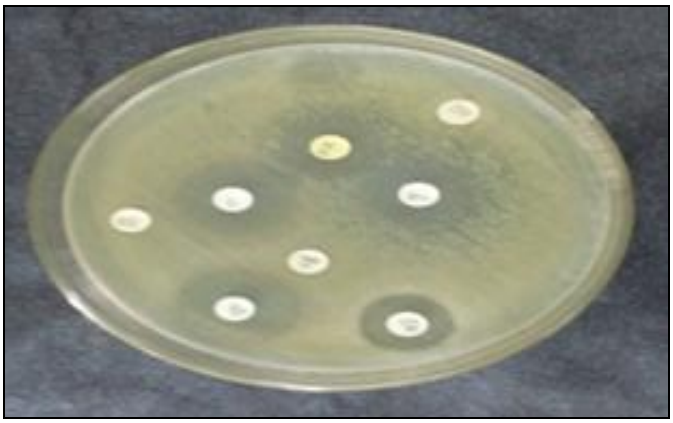


FIG. 11: ANTIBIOTIC SUSCEPTIBILITY TEST AGAINST *KLEBSIELLA PNEUMONIAE*

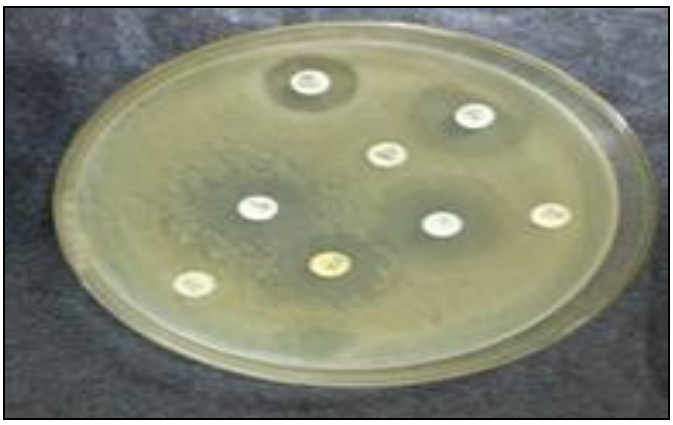


FIG. 12: ANTIBIOTIC SUSCEPTIBILITY TEST AGAINST *S. AUREUS*

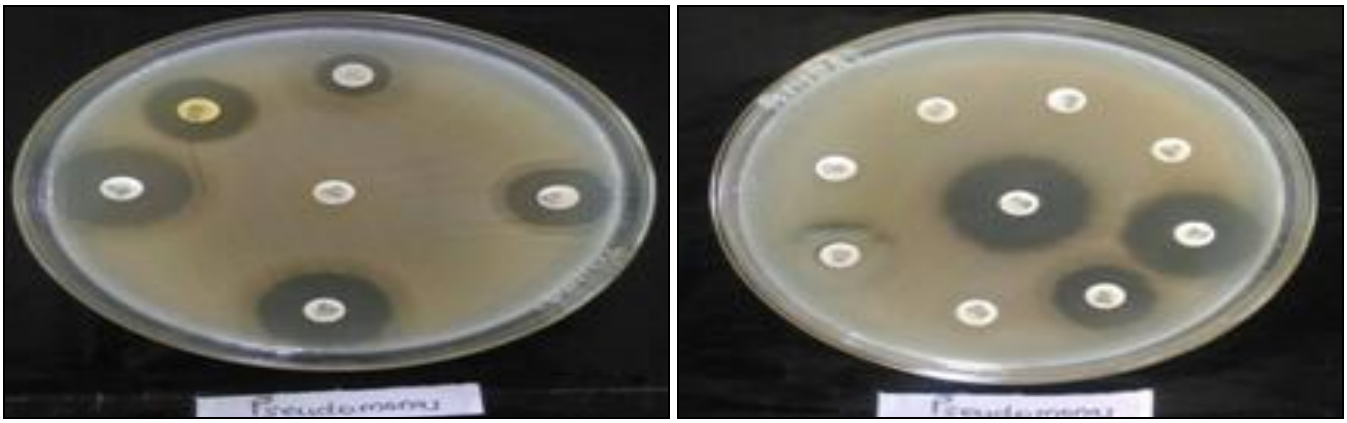


FIG. 13: ANTIBIOTIC SUSCEPTIBILITY TEST AGAINST *PSEUDOMONAS AERUGINOSA*

FIG. 14: RESULTS OF MIC TEST BY E- STRIP METHOD

DISCUSSION AND CONCLUSION: During the study, the urine sample was inoculated on selective media like EMB, CLED, MSA, PIA, CIA medium followed with biochemical tests. The results showed the isolation of *E. coli*, *K. pneumoniae*, *S. aureus*, and *Pseudomonas aeruginosa*.

The results of antibiotic susceptibility showed that norfloxacin, gentamycin, rifamycin, lomeflo-xacine and tobramycin, penicillin, clindamycin, methicillin, streptomycin, tetracycline, amoxi-cilline, ciprofloxacin and erythromycin are resistant to *E. coli* while amikacin, moxifloxacin, ofloxacin and cotrimoxazole are found to be sensitive against the same. It was observed that norfloxacin, oflo-xacin, and teicoplanin are the sensitive antibiotics for klebsiella while gentamycin, tobramycine, moxifloxacin, tetracycline, lomefloxacin-cine, and tobramycine are resistant antibiotics. Ampicillin shows intermediate state and methicillin, linozoids, penicillin, amoxicillin, erythromycin showed resistance against Klebsiella. Norfloxacine, amikacin, ofloxacin, vancomycin, erythromycin, ampicillin and rifamycin are sensitive antibiotics. Gentamycin, moxifloxacin, lomefloxacin, levo-floxacin, penicillin, clinda-mycine, methicillin, streptomycin, tetracycline were found resistant against *Staphylococcus aureus* while amikacin, gentamycin, ciprofloxacin and tobramycin were found to be sensitive antibiotics against the same. Tetracycline, rifamycin, lome-floxacine, erythr-omycin, cefepime, streptomycin, penicillin, clindamycin were found to be resistant against *P. aeruginosa*. Antibiotics like norfloxacin, meropenem, and methicillin were found to be intermediate antibiotics.

ESBL producing organisms, being commonest nosocomial pathogens, and thus it is much essential to detect and treat them as early as possible The study thus suggests that the present study will provide the basis for routine screening of antibiotics against ESBL producing bacteria.

The study will provide the basis for optimizing the different lines of antibiotics against specific bacterial cultures. Thus, the results will help in exploring the specific treatment against such pathogens borne infections.

ACKNOWLEDGEMENT: The authors acknowledge the research staff and colleagues of R&D project on the concerned topic.

CONFLICTS OF INTEREST: The authors declare no conflicts of interest.

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How to cite this article:
Hattewar J, Koul A and Mathur A: Antibiotic susceptibility pattern of ESBL producing bacteria from urinary tract infection (UTI). Int J Pharm Sci & Res 2020; 11(2): 799-10. doi: 10.13040/IJPSR.0975-8232.11(2).799-10.