

To study susceptibility pattern of ESBL producers in urinary isolates of *E. coli*

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Abstract- Extended spectrum beta-lactamase (ESBL) Producing *E. coli* has tremendously increased worldwide and it is one of the most common causes of morbidity and mortality associated with hospital-acquired infections, this could be attributed to association of multi drug resistance in ESBL producing isolate. The present study was aimed to determine the susceptibility pattern of ESBL producers by *E. coli* isolated from urine sample. Current knowledge of prevalence of ESBL production by commonly isolated organisms such as *E. coli* is necessary action to prevent the spread. Therefore, the present study was conducted with an objective to find out the prevalence of ESBL producing *E. coli* and to formulate effective antibiotic strategy.

Isolates of *E. coli* was isolated from urine sample collected from GMC Akola. Isolated bacteria were subjected to antibiotic sensitivity test by which the resistance of *E. coli* towards general antibiotics can be seen.

Then the *E. coli* was tested against ESBL specific antibiotics. This was done by double disc diffusion method and combination disc method.

Keywords: ESBL, *E. coli*, ESBL producing *E. coli*, DDS, Combination disc method, Multi-drug resistance.

INTRODUCTION:

Multidrug resistant bacteria are now a days a major cause of concern to the field of medicine. Their resistance can be shown by bacteria. In the case of ESBL producing microbes, the enzyme named **Extended Spectrum β Lactamases** is responsible to immunise the microbe from various antibiotics which are in common use. The prevalence of ESBL producing microbes has increased throughout the world and is a major cause of treatment failure.

Extended spectrum β lactamase is an enzyme produced by **many Gram negative organisms**. ESBL will provide resistant towards a broad range of antibiotics, due to which the organism can survive and continue to grow. ESBL producing organisms exhibit co-resistance to many other classes of antibiotics, resulting in limitation of therapeutic options. They were first reported in Germany in 1983.

ESBLs have been reported in all parts of the world. They are derivatives of classic β lactamases. ESBLs are occasioned by single mutation in progenitor (parent) enzymes (a mutation in few amino acids). ESBLs exhibits fundamental changes in important distinguishing factors like **substrate spectra, substrate profile, reactions to inhibitors and isoelectric points**.

Till date over 200 ESBLs are characterised and classified. Still there is no consensus on exact figure. β lactamase have been variously classified over time.

Two commonly used classification schemes are:

- a) **Ambler molecular classification system**
- b) **Bush-Jacoby-Medeiros functional classification system.**

The Ambler molecular classification and the Bush-Jacoby Medeiros functional classification are the two most commonly used classification systems for β -lactamases.

Ambler scheme divides β -lactamases into four major classes (A to D). The basis of this classification scheme rests upon protein homology (amino acid similarity) and not phenotypic characteristics.

The Bush-Jacoby-Medeiros classification scheme groups β -lactamases according to functional similarities (substrate and inhibitor profile). There are four main groups and multiple subgroups in this system. This classification scheme is of much more immediate relevance to the physician or microbiologist in a diagnostic laboratory because it considers β -lactamase and β -lactam substrates that are clinically relevant.

Amber class	Bush group	Characteristic of β lactamases	Number of enzymes
C	1	Often chromosomal enzymes in gram negative but some are plasmid-coded	51
A	2a	<i>Staphylococcal</i> and <i>Enterococcal</i> penicillinase	23
	2b	Broad spectrum β lactamases including TEM-1 and SHV-1, mainly occurring in Gram - negatives	16
	2be	Extended Spectrum β Lactamases	200
	2br	Inhibitor- resistant TEM (IRT) β lactamases	24
	2c	Carbenicillin-hydrolysing enzymes	19
	2d	Cloxacillin (Oxacillin) hydrolysing enzymes	31
	2e	Cephalosporinases inhibited by Clavulanic acid	20
	2f	Carbapenem-hydrolysing enzyme inhibited by Clavulanic acid	4
B	3	Metallo-enzymes that hydrolyse Carbapenems and other β lactams except monobactams. Not inhibited by Clavulanic acid.	24
D	4	Miscellaneous enzymes that do not fit into other groups	9

Table 1.1 Classification of β lactamases**MATERIALS AND METHOD: -****Collection of samples: -**

Multiple urine samples were collected from GMC, Akola. The urinary samples collected are specifically from the patients suffering from urinary tract infection. Around 20 urine samples were collected. They were collected and taken to lab by following all required precautions.

Inoculation of urine sample: -

The collected urine samples were then inoculated on nutrient agar plates. The nutrient agar was prepared by adding 28 gm. of nutrient agar in 1000 ml of distilled water. It was prepared in conical flask, and then the media was heated to dissolve the powdered media completely. It was then sterilized in autoclave at 121°C for 15 min and then allowed to cool. After that the media was poured in sterile petri plates.

A loop full of urine samples were inoculated on the nutrient agar plate. Inoculated plates were kept for incubation in incubator for 37°C for 24 hrs.

Identification of morphologically identical colony: -

Multiple colonies of different bacterial spp. appear on the Nutrient media plate. By observing the colony characters, specific colony was selected. Microscopy of selected colony was done by Gram staining.

Inoculation of identified colony of organism on selective media: -

The selective media for *E. coli* is EMB agar. EMB agar was prepared by adding 35.96 gm. of EMB agar in 1000ml of distilled water. It was then sterilized in autoclave at 121°C for 15. The selected colonies are now inoculated on EMB.

Perform biochemical tests: -

To confirm that the selected microorganism is *E. coli*, various tests are performed. The results obtained by performing various biochemical tests will be cross checked with that of the results from *Bergey's Manual of Systemic Bacteriology*. This will result in identification of the isolate which is being tested.

Antibiotic sensitivity test: -

After the required organism i.e. *E. coli* is obtained, the antibiotic sensitivity profile is tested against various antibiotics like Ampicillin, Gentamycin, Erythromycin, Ciprofloxacin, Chloramphenicol and Amoxiclav by Disc Diffusion Method. To test the antibiotic sensitivity profile, Nutrient agar is prepared by adding 28 gms of nutrient agar in 1000ml of distilled water.

Nutrient broth is prepared by adding 26gm Nutrient broth in 1000ml distilled water. The prepared Nutrient broth is then sterilized in autoclave at 121°C for 15 min and then allowed to cool. After the broth gets cooled, loop full culture of desired organism is added in the broth and kept for incubation overnight.

Then make lawn of the broth on the agar plate with sterile cotton swab. After making a lawn of culture, place the antibiotic discs on the plate with equal distance. Incubate the plates at 37°C for 24 hrs. The results were recorded.

Double disc approximation test: -

The overnight culture suspension of the test isolate was inoculated on the nutrient agar plate using a sterile cotton swab. A disc of Amoxycylav and Ceftazidime were placed 15mm apart. After incubation at 37°C, the presence of synergy between the two discs was interpreted as positive for ESBL production.

Combination disk method: -

The combination disk method uses ceftazidime in the combination of Clavulanic acid was performed for the detection of ESBL. In this test, an overnight culture of the test isolate was inoculated on the agar plate by sterile cotton swab. The Ceftazidime and Clavulanic acid disk were placed 15 mm apart. After incubation at 37°C for 24 hrs, the zone of inhibition was observed against the test organism and was interpreted as positive for ESBL production.

RESULT AND DISCUSSION: -

The urine sample which was collected was inoculated on nutrient agar plates: -

The urine sample inoculated on nutrient media shows growth of multiple microorganisms.

From the plate, morphologically similar looking colony as that of *E. coli* was selected.

Microscopy of selected isolate: -

The microscopy of the isolate shows the appearance as short rods, which is identical to the morphology of *E. coli*

Inoculation on selective media: -

Green metallic sheen coloured colonies appear on EMB agar plate. This indicates that the selected isolate is *E. coli*.

sr. no.	Colony characters	Isolate
1	Size	2 - 4 mm
2	Shape	Circular
3	Margin	Undulated
4	Elevation	Slightly raised
5	Colour	White
6	Opacity	Translucent
7	Texture	Smooth
8	Motility	Non-motile
9	Gram character	Gram negative short rod



Fig1: urine sample on Nutrient agar

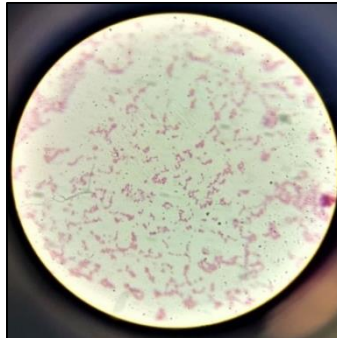


Fig2: microscopy

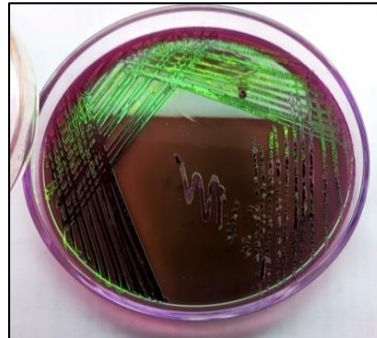


Fig3: inoculation on EMB media

Biochemical test:

[A] IMViC test:-

For IMViC test, indole test and Methyl red test was found to be positive; while Voges Proskauer and citrate test was negative.

[B] Sugar fermentation test:-

The isolate was able to ferment all sugars (Glucose, Sucrose, Mannitol and Lactose) with production of Acid and Gas.

[C] Enzyme test:-

A presence or absence of particular enzyme can be used to identify or distinguish bacteria in laboratory.

Sr. no.	Enzymes	Result
1	Oxidase	Positive
2	Catalase	Positive
3	Urease	Negative
4	Gelatine liquefaction	Positive
5	Amylase	Negative



Fig 5.7: Gelatinase test

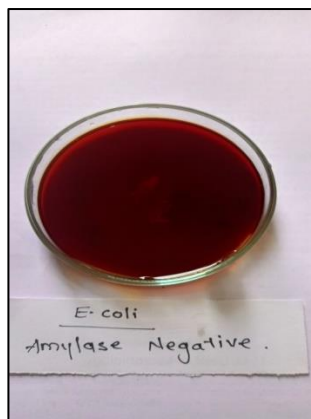


Fig 5.8: Amylase test



Fig 5.9: Urease test



Fig 5.10: Oxidase disc

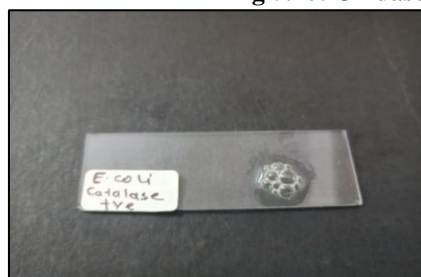


Fig 5.11: Catalase test

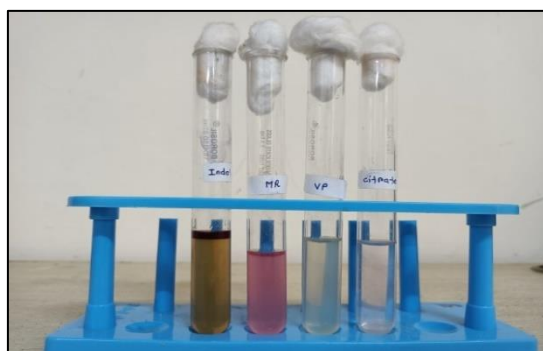


Fig: IMViC test



Fig: Sugar fermentation test

ANTIBIOTIC SENSITIVITY TEST: -

After the confirmation of the isolate as *E. coli*, it is now subjected to antibiotic sensitivity test against various antibiotics. The disc diffusion method was followed while performing the AST. Various antibiotics such as Ampicillin, Gentamycin, Erythromycin, Ciprofloxacin, Chloramphenicol and Amoxycylav. The MHA media was used to check the effectiveness of the antibiotic against the microorganism.

After the incubation of 24 hrs. at 37°C, the zone of inhibition was observed, measured and recorded. The AST of *E. coli* gives an idea of the resistance and sensitivity pattern shown by it.

sr. no.	Antibiotics	sensitive	Resistant
1	Ampicillin	-	Resistant
2	Gentamycin	22 mm	-
3	Erythromycin	11 mm	-
4	Ciprofloxacin	42 mm	-
5	Chloramphenicol	26 mm	-
6	Amoxyclav	-	Resistant

Determination of ESBL producing *E. coli* :-

To determine the presence of the enzyme ESBL produced by the organism, we need to see the susceptibility profile of the microbe against particularly design antibiotics which are specific for ESBL producers. The zone of inhibition more than ≥ 5 mm will indicate the presence of ESBL.

[A] Combination disk method:-

This method is to measure the inhibition zone around a disk of Cephalosporin and around a disk of the same Cephalosporin plus Clavulanate. The activity of extended spectrum Cephalosporins against ESBL producers will be enhanced by the presence of Clavulanic acid.

[B] Double disc synergy test: -

Double disc synergy test was the first test specifically designed to detect ESBL production. In this method, combination of pairs of antibiotics was tested by placing antibiotic disks at distance of 20mm from each other. After 24 hrs. of incubation, if the synergistic effect was present among the antibiotic, or the zone of inhibition was formed, this indicate the organism is ESBL positive.

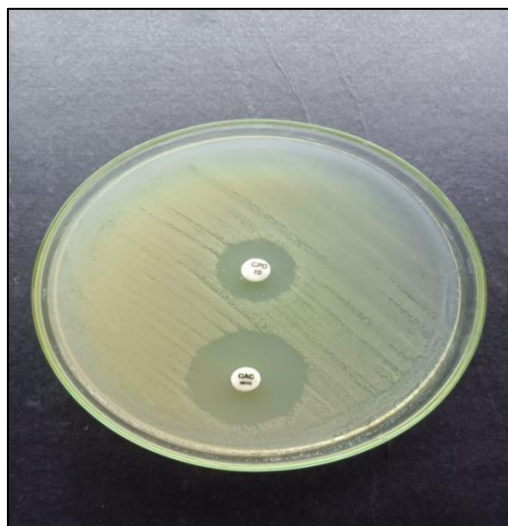


Fig 5.13: - Combination Disc method

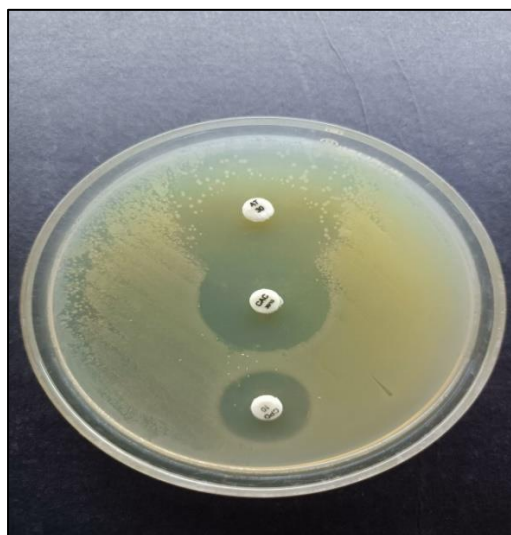
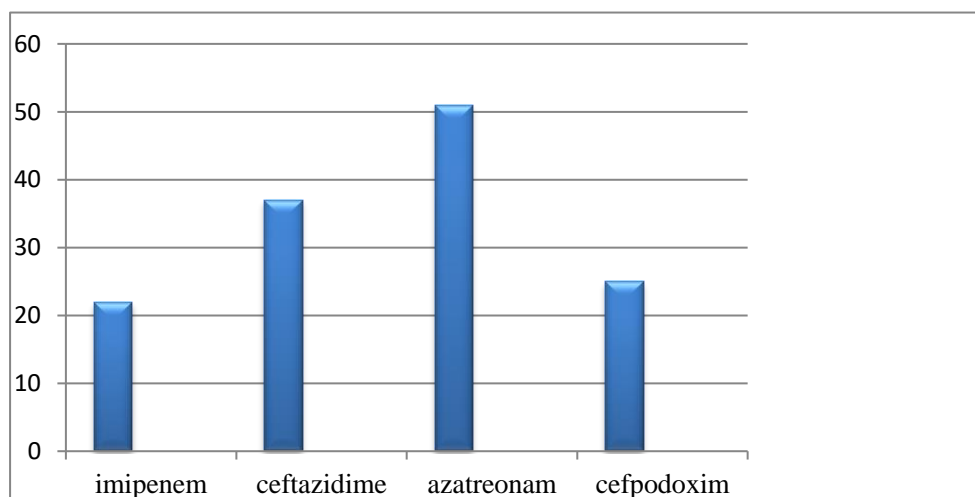


Fig 5.14: - Double Disc Synergy Test.



Sr.no.	Antibiotics	Zone of inhibition	Resistance
1	Imipenem	22 mm	-
2	Ceftazidime	37 mm	-
3	Azatreonam	51 mm	-
4	Cefpodoxime	25 mm	-

DISCUSSION: -

ESBL has become a widespread serious problem. These enzymes are becoming increasingly expressed by many strains of pathogenic bacteria with a potential for dissemination. Presence of ESBL compromises the activity of wide-spectrum antibiotics creating major therapeutic difficulties with a significant impact on the outcome of patients. The continued emergence of ESBLs presents diagnosis challenges to the clinical microbiology laboratories.

In present study, prevalence of ESBL producing *E. coli* was observed. All the isolates were sensitive to Imipenem. The combination disc of Clavulanic acid and Ceftazidime gave prominent zone of inhibition against all 20 samples. Aztreonam gave highest zone of inhibition among all ESBL specific antibiotics used.

CONCLUSION

Alarming rate of drug resistance among uropathogens and high rate of ESBL producing *E. coli* was observed. It is extremely necessary to routinely investigate the drug resistance among all isolates and formulate strict antibiotics prescription policy.

FUTURE PROSPECT:

ESBL shows the multi drug resistance pattern so the awareness and detection of this enzyme is mandatory for the optimal care. ESBL producing organisms can cause the infections and can lead to the life threatening sepsis. In this study ESBL producing *E. coli* is investigated. Awareness should be taken for the critical infection so that the people should be aware of such infection caused by the ESBL producing *Escherichia coli*.

As it is a multi drug resistant it is difficult to detect and treat. Proper antibiotics should be used for the treatment of this infection. Organization such as clinical and laboratory institute should provide proper guidelines for the proper infection control.

While antibiotic resistance is becoming increasingly serious today, there is almost no doubt that more challenging times await us in the future. Resistant microorganisms have increased in the past decades leading to limited treatment option, along with higher morbidity and mortality. *E. coli* is one of the significant microorganism causing major public health problems by acquiring resistance to antibiotics and acting as an opportunistic pathogen of health care associated infections. The production of Extended Spectrum Beta Lactamases (ESBL) is one of the resistant mechanism of *E. coli* against antibiotic.

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