The Pharma Innovation Journal 2019; 8(2): 703-708

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(2): 703-708
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www.thepharmajournal.com
Received: 23-12-2018
Accepted: 25-01-2019

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Antibiotic sensitivity pattern of extended spectrum beta-lactamase

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Abstract
Antibiotic resistance poses a great problem in curing the diseases which are normally cured by use of antibiotics. Antibiotic resistance can affect anyone, in any age and even in any area worldwide. Antibiotic-resistant bacteria are those bacteria which are not controlled or even not killed by an antibiotic. Antibiotic resistance occurs naturally but prolonged use of antibiotics or misuse of antibiotics in human or animals can accelerate this process.
Extended-spectrum beta-lactamase (ESBLs) is an enzyme which is made by some bacteria that are able to hydrolyze extended spectrum of Cephalosporins and also inhibited Clavulanic acid. Extended-spectrum beta-lactamase was first reported in 1983. Extended-spectrum beta-lactamase is that enzyme which is responsible for the cause of hospital-acquired infection. Beta-lactam antibiotic is used to treat the Gram-positive and Gram-negative bacteria, but beta-lactamases produced by Gram-negative organisms. GIT plays an important role in the development of antibiotic-resistant microorganisms in humans and animals. Extended-spectrum beta-lactamase infection may spread through in the environment through contaminated feces in which *E. coli* or other bacteria are present. About 630 samples were collected and after proceeding they all were analyzed by standard microbiological techniques. The result showed that 158 samples were given positive and rest of 472 samples had no growth.

Keywords: Snail, bovine, porcine, physicochemical properties, mucin, mucoadhesives

Introduction
Antibiotic resistance poses a great problem in curing the diseases which are normally cured by use of antibiotics. Antibiotic is a drug which is used to treat bacterial infections, but antibiotics have no effect on viral infection. Antibiotic is a substance which inhibits the growth of other micro-organism. Antibiotics are those medicines which are used to prevent and treat the infectious diseases, but the over use and even misuse of antibiotics has led to the worst effects of antibiotics by developing different resistant strategies by bacteria [1]. The major consequence for antibiotic selection pressure is the emergence of and spread of multi drug resistant pathogens such as Methicilin resistant *Staphylococcus aureus*, Metallo beta lactamases (MBL) *Pseudomonas*, Vancomycin resistant *Enterococcus* (VRE), Multi drug resistant bacteria Mycobacterium tuberculosis (MDR-TB) [2]. Since 1983 among all these the ESBL producing Gram negative bacteria have disseminated across the globe [3].
Penicillins and Cephalosporins are beta lactam antibiotics which act by inhibiting enzymes that are responsible for the cell wall synthesis of bacteria. The most antibiotics were Beta lactam which is used widely to treat the infection which is caused by Gram negative bacteria [4]. There were promoted genetic mutations in the bacteria by continuous and an excessive use which benefitted them to expand spectrum beta lactamases enzymes [5]. There are a group of enzymes called Extended spectrum beta lactamases which conferring resistance to beta lactam ring containing antibiotics such as Penicillins; first-, second- and third-generation Cephalosporins; and Aztreonam (but not the Cephamycins or Carbapenems) and are inhibited by beta lactamases inhibitor such as Clavulanic acid, Sulbactam and Tazobactam [6]. The genes encoding for ESBLS are located on plasmids and thus can be transferred within and between different bacterial species by transformation, conjugation or transduction process [7]. The plasmid mediated transfer of ESBLs have resulted increase resistance to non-beta lactam antibiotics such as Trimethoprim-sulfmethaxozole, Aminoglycosides and Fluroquinolones, Tetracyclines as plasmids may carry genes for conferring multi drug resistance presenting an additional challenge for controlling infections caused by ESBL producing pathogens [8].
Escherichia coli is a Gram negative bacteria which is commonly found in both pathogenic and

non-pathogenic faeces. The normal *E. coli* carry genes for antimicrobial resistance which can be transferred to pathogenic *E. coli*, so the another resistant bacteria are also a reason for concern as they can be cause to circulate of the antibiotic resistant genes [9].

Materials and Methods

Study Design

The present research work was a hospital based cross-sectional study which was conducted in the Microbiology Unit in the different hospitals of the area.

Ethical Consideration

Informed consent was attained from the participant and work approval was taken from the committee of hospital. A term paper of information letter and consent form was prearranged to patients before history of patients was collected. If the patient were children then all history were collected from their parents.

Inclusion and exclusion criteria

The patient's history with complaint of certain points like nausea, abdominal pain or back pain etc was included. Those clinical samples which showed poly-microbial and insignificant growth, incomplete culture form, without proper labeling like date, age, lab number, time and sex were excluded.

Specimen collection

The Mid Stream Urine (MSU) samples (15-20ml) were collected in clean, dry and most important sterilized wide mouthed leak-proof bottle. The instructions which are given by specialist was followed by the patient for perfect result. After collecting the sample from patient sample were labeled properly with lab serial number, age and sex. If sample was not processed at the time of collection then it was important that sample was frozen in 4-6^{o}c and also included preservative in it for best results.

Culture of specimen

The Medias were used as per standard instructions by manufacture company (Himedia). The urine sample was streaked on blood agar (BA) and Mac Conkey agar (MAC). After mixing the urine sample in container, then needed sample was contacted to the centre of the plate, from which the inoculums was spread in a line across the diameter of the plate. The plates were incubated aerobically at 37^{o}c overnight. After incubation the proper results were taken.

Identification of the isolates

The identification of isolates were done by using microbiological techniques. For further re-confirmation we went for performing biochemical tests like Catalase test, Oxidase test, Motility test, Indole test, Citrate utilization test, Triple sugar iron test, Phenyl pyruvic acid test.

ESBL Screening

All isolates were confirmed to ESBL producing members of enterobacteriaceae by growth on Muller Hinton agar. All isolates were inoculated on Muller Hinton agar plates. Antimicrobial susceptibility was determined by the Kirby-Bauer disc diffusion method according to Clinical Laboratory Standard Institute (CLSI) guidelines.

The antibiotics used for determination of collected strains were Ampicillin (10 µg), Cefuroxime (30 µg), Ceftriaxone (30 µg), Cefotaxime (30 µg), Ceftazidime (30 µg), Cefepime (30 µg), Cefoxitin (30 µg), Aztreonam (30 µg), Amoxicillin–Clavulinic acid (20/10 µg), Ampicillin–Sulbactam (10/10 µg), Piperacillin–tazobactam (100/10 µg), Gentamicin (10 µg), Amikacin (30 µg), Ciprofloxacin (5 µg), Imipenem (10 µg), Meropenem (10 µg), Trimethoprim–sulfamethoxazole (1.25/23.75 µg). Norfloxacin (10 µg) and Nitrofurantoin (300 µg), Ceftazidime-clavulanic acid, were tested against isolates from different types of clinical samples [13].

Susceptibility Testing: ESBL sensitivity was establish by having following methods in susceptibility testing

1. **Disk Diffusion Method:** ESBL screening was performed by using Cefodoxime, Cefatazidime (CAZ), Azetreonam (AT), Cefotaxime (CTX) and Ceftriaxone (CTR) according to CLSI guidelines.
2. **ESBL Disc Confirmation:** Enterobacteriaceae suspected to be producers of ESBLs enzymes were taken to the following confirmation test:-

1. Combination Disc Test (CDT)

For each test discs containing cephalosporin alone (Cefotaxine, Ceftazidime, Cefepime) and in combination with Clavulanic acid were applied. The inhibition zone around the cephalosporin disc combined with Clavulanic acid was compared with the zone around the disc with the cephalosporin alone. The test is positive if the inhibition zone diameter is ≥5 mm larger with Clavulanic acid than without it. Cephalosporin and around a disk of the same cephalosporin plus Clavulanate. Depending on the disk type, a difference of ±5 mm between the two diameters or a zone expansion of 50% are considered as indicating ESBL production. Sensitivity and specificity for this methods were first reported to be 96% and 100% respectively.

2. Double Disk Synergy Test (DDST)

Now a days Epsilometer test (E-test) is an exponential gradient method of determination of antimicrobial resistance. The E-test has been developed to provide a direct quantification of microbial suscebtibility of microorganisms. The systems are the only instruments in the market that offer simultaneous automation of overnight, rapid sand specialized panels that were used for both gram positive and gram negative bacteria. The Siemens; Micro Scan technology was introduced as the first advanced technology based on the sound microbiology principles of true minimum inhibitory concentrations (MIC) testing. This principle remains the most sensitive method to determine bacteria's susceptibility to antibiotics.

Quality control

The quality control is a very useful tool for any laboratory and equipments like refrigerator, incubator, autoclave and hot air oven. The temperature of incubator, hot air oven and refrigerator was observed in daily bases for finding best results. The reagents and media which are used in laboratory frequently monitored for their manufacture, expiry date and proper storage. When any media was prepared in laboratory then it was properly labeled with preparation date. The quality of media was ensure by incubating one plate of each lot for sterility and using standard control strains for performance testing.

Results

The total samples 630 were collected and proceed and analyzed by standard microbiological techniques. In this study, 630 samples were collected in which 158 samples were positive and 472 samples with no growth.

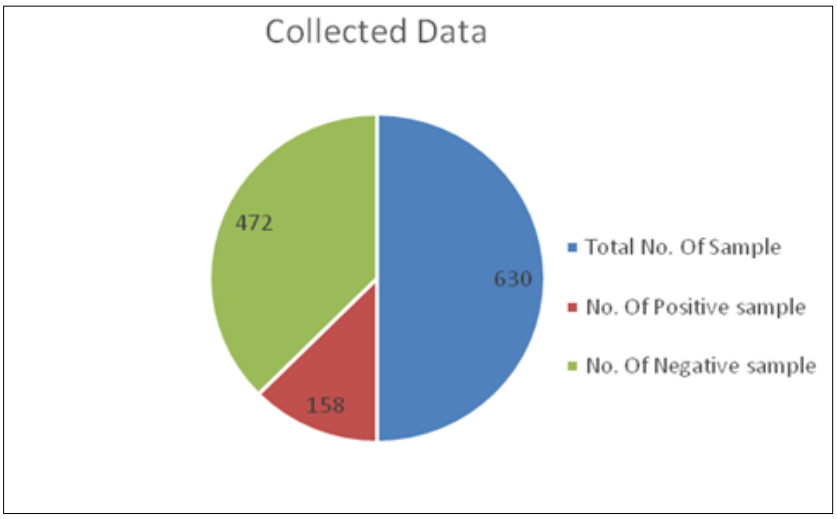

Fig 1: Numbers of collected total samples, positive and negative samples

1. Distribution of organisms isolated:- The total numbers of samples were 630 in which *E. Coli* (n=55), *K. pneumoniae* (n=36), *P. aeruginosa* (n=28), *Staphylococcus aureus* (n=15), *Staphylococcus (CONS)* (n=10), *Enterococcus* (n=8), *Acinetobacter* (n=6), *Proteus* (n=1)

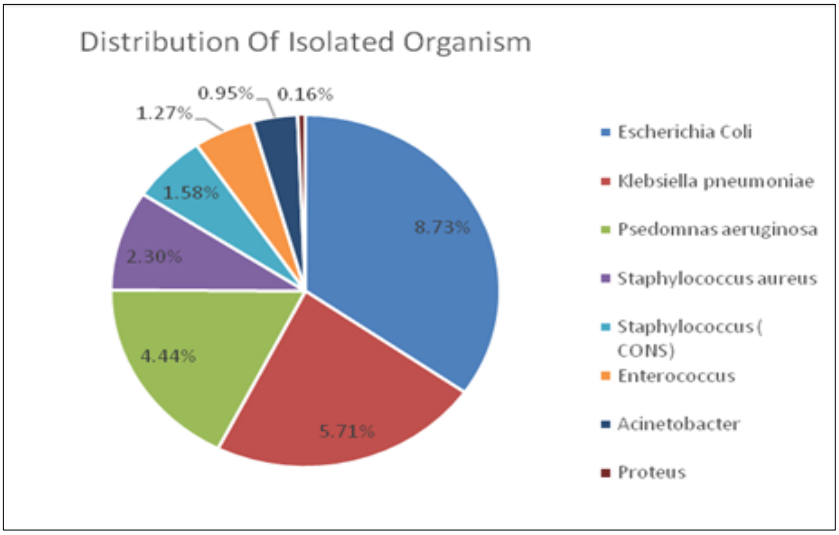

Fig 2: Distribution of isolated organisms

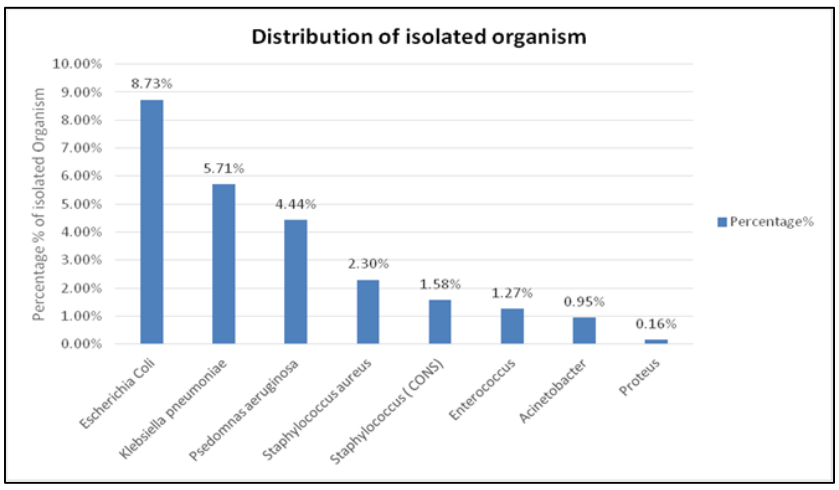

Fig 3: Percentage of isolated organisms having 630 total numbers of samples

1. **Distribution of Enterbacteriaceae:-** From total numbers of cases (n=630) having 97 number of Enterobacteriaceae members include Escherichia coli (n=55), Klebsiella Pneumonia (n=36), Acinetobacter (n=2) and Enterobacter (n=4).

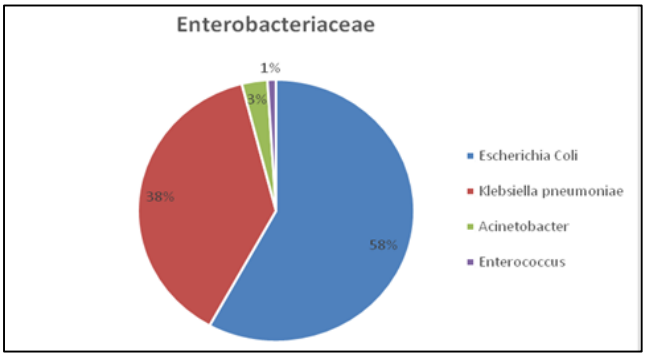

Fig 4: Percentage of Enterobacteriaceae members

2. **Distribution of Enterobacteriaceae's ESBL producers:-** In this data some members of Enterobacteriaceae were ESBL producers like *Escherichia coli*, *Klebsiella pneumonia*. The total numbers of *E. coli* (n=55), in which having 21.8% ESBL producers and total numbers of *Klebsiella pneumonia* (n=36) in which having 11.1% ESBL producers.

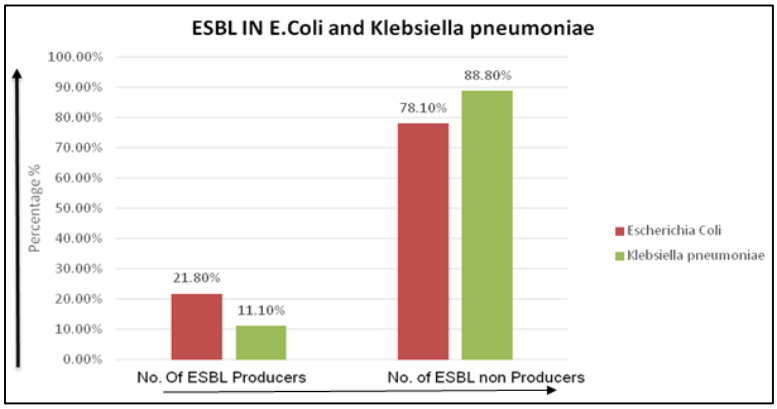

Fig 5: Percentage of ESBL producers and non-producers

3.1 Antibiotic sensitivity and resistance pattern in *Escherichia Coli* (ESBL)

The total number of *Escherichia Coli* (n=55) in which having 12 were ESBL producer, which was identified by following the phenotypic detection test for ESBL. In this sensitivity and resistance pattern of *Escherichia coli* having 3rd generation Cephalosporins alone and with Lavulanic acid, Sulbactum and Tazobactum combination detect the ESBL.

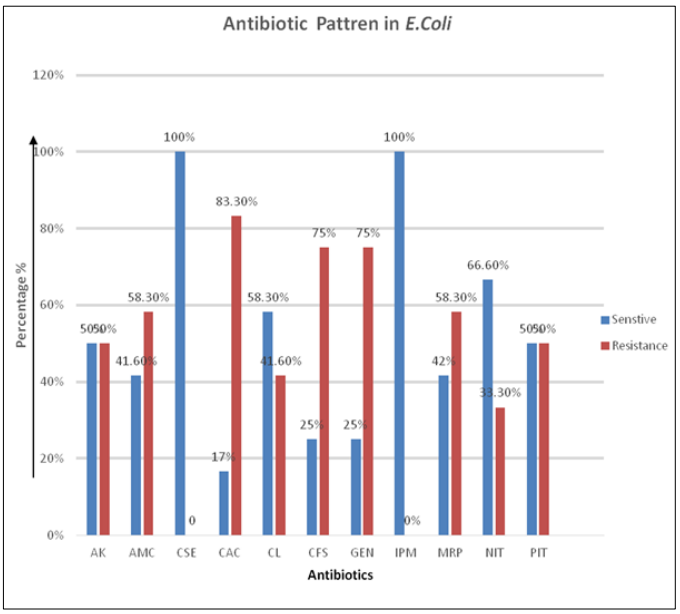

Fig 6: Antibiotic Sensitivity and Resistance pattern of *E. coli*

2.1 Antibiotic sensitivity and resistance pattern *Klebsiella pneumonia:-* The total numbers of *Klebisella pneumonia* (n=36) in which having 4 were ESBL producer, which was identified by following the phenotypic detection test for ESBL. By using the antimicrobial susceptibility test. In this sensitivity and resistance pattern of *Klebsiella pneumonia* having 3rd generation Cephalosporins alone and with Clavulanic acid, Sulbactum and Tazobactum combinations detect the ESBL.

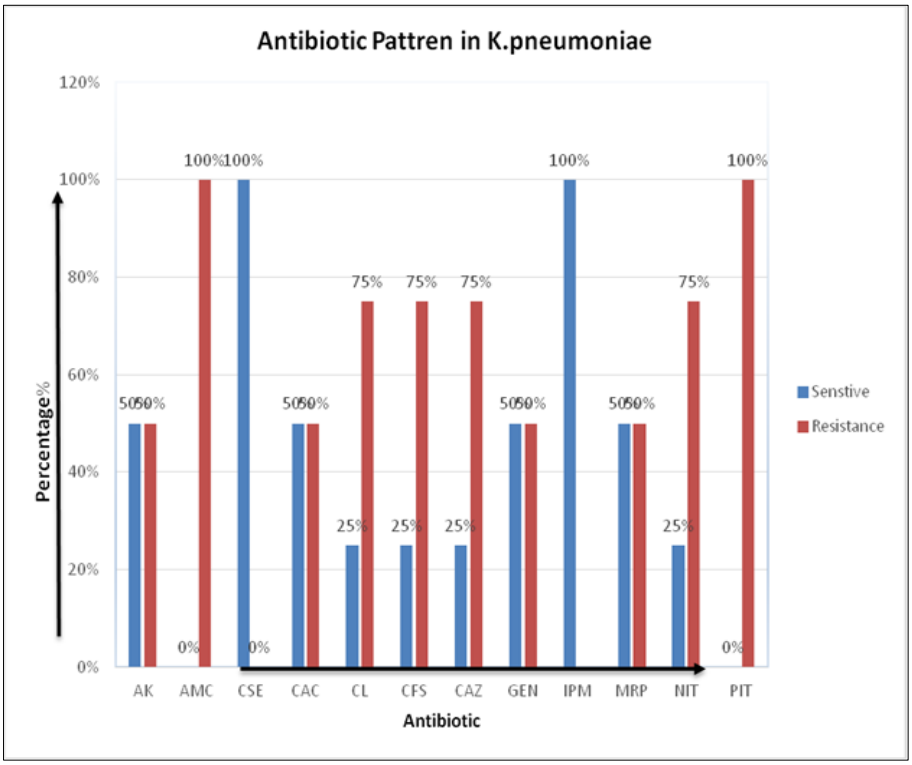

Fig 7: Antibiotic Sensitivity and Resistance pattern of *Klebesilla pneumonia*

Conclusion:- The majority of ESBL, producers were sensitive to Imipenem, Piperacillin-Tazobactum, Amikacin, Amoxyclav, Ceftriaxone EDTA Sulbactum and Cefoperazone / sulbactum. The Screening of *Enterobacteriaceae* for ESBL production is essential for better antibiotics selection and preventing its further emergence and spread. In resource-limited settings, combination disk method can be implemented for screening and confirming ESBL production.

Discussion

IN this study collected samples were taken from various areas and from different patients. The results of pathogenic microorganisms were identified by doing cultures and biochemical tests and their antibiotic susceptibility pattern was performed by the help of combination disk method (For ESBL in gram negative bacteria).

The total were 630 samples and proceed with proper microbiological techniques and only 158(25.07%) samples were shows growth. A total of Enterobacteriaceae (n=97) isolated,

it include the *E. coli* (n=55), *K. pneumoniae* (n=36), Acintobacter spp.(n=2) and Enterobacter spp.(n=4). In this isolated microorganisms the ESBL producers in Enterobacteraceae having *E. coli* (n=12, ESBL producer) and *K. pneumoniae* (n=4, ESBL producers). The ESBL phenotypic detection was performed by followed the antibiotic susceptibility pattern in which include the different methods and techniques for detection of ESBL producers.

In this study the detection of ESBL producers is identified by Combination disk method in which the combination of Clavulanic acid, Sulbactum and Tazobactum with 3rd Cephalosporins antibiotics having ≥5 zone of inhibition as compare to alone 3rd generation Cephalosporins.

Increasing antimicrobial resistance is often associated with a high selective pressure, but cannot be accountable in this particular case. Despite a low level of antimicrobial consumption there is an influx of these resistant bacteria to the society. One route of transmission and many researchers have found ESBL producing bacteria in foodstuff and animal husbandry. Even more alarming is the persistence of these genes in the environment; in treated wastewater from hospitals and community, wastewater sludge, river water and sediment and wild animals. Also some other treatment options are available like first choice antibiotic therapy include carbapenems (for example, against ESBL producers) Piperacillin-Tazobactum might be an alternative against ESBL- producers that are susceptible.

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