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## Extended – Spectrum $\beta$ -Lactamase Producing *Klebsiella* Species among Clinical Isolates.

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### ABSTRACT

Extended – Spectrum Beta-Lactamase producing strains among clinical isolates has been steadily increasing over the past years. Microorganisms responsible for UTIs such as *Escherichia coli* and *klebsiella* 5pp have the ability to produce ESBLs in large quantities. All the strains of *klebsiella* species in this study were collected from routine clinical samples confirmed by biochemical and other tests. Stock cultures of these strains were maintained in nutrient agar Medium. All samples were done in Naryana Medical College and Hospital, Nellore. The study was to know the prevalence of extended spectrum  $\beta$  lactamase in multidrug resistant (MDR) *klebsiella* species isolated from different clinical samples the wide spread use of antibiotics in hospitals an emergence of multidrug resistant organisms of low virulence like *klebsiella* causing were significantly  $p < 0.05$  as a serious opportunistic infections. Development of an infection control policy and hospital antibiotic prescribing guide should follow next. The Petri-dish has long inspired our admiration for ESBL producers. It is time to extend our appreciation to the patients who are the ultimate sufferers.

**Keywords:** *Escherichia coli*, *klebsiella* Urinary Tract Infection and Antibiotics.

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## INTRODUCTION

Extended – Spectrum Beta-Lactamase were first found in Europe (1980 s). ESBLs mediate resistance to cephalosporin antibiotics. They have become a wide spread problem, not only in klebsiella species (*Klebsiella pneumoniae*) but increasing in salmonella paratyphi species. The occurrence of extended spectrum Beta –Lactamases (ESBLs) in enterobacteria that possess includible Bush group I Chromosomal Beta lactamases is increasingly reported worldwide. The incidence of ESBL producing strains among clinical isolates has been steadily increasing over the past years. Microorganisms responsible for UTIs such as *Escherichia coli* and *klebsiella 5pp* have the ability to produce ESBLs in large quantities. These enzymes are plasmid borne and confer multidrug resistance [1]. Making Urinary tract infections difficult to treat with Bronchopneumonia, Multiple abscess formation in the lungs (Friedlanders pneumonia) Difficult to treat. B – Lactom antibiotics are a broad class of antibiotics including penicillin derivatives, basically any antibiotic agent containing a B – Lactam nucleus in its molecular structure. They are the most widely used group of antibiotics available [2]. Beta lactam antibiotics which are named for the Beta lactam ring in their chemical structure, including the penicillins, cephalosporin's and related compounds. These agents are active against many gram positive, gram negative and anaerobic organisms [3]. The Beta Lactam antibiotics exert their effect by interfering with the structural cross linking of peptidoglycans in bacterial cell walls [4]. Action of all Beta – lactam antibiotics involves the Beta- lactam ring fitting into the active site of the one of the enzymes involved in the formation of peptidolycan. These antibiotics disrupt cell wall synthesis thus as cells are formed and begin to generate a cell wall the presence of these antibiotics will result in weak deformed cell walls. Minor shifts in osmolarity an result in rupture of the cell. The bactericidal activity of the penicillins is after related to their ability to bigger membrane associated autolytic enzymes that destroy the cell walls. Other minor mechanisms of action include inhibition of bacterial endo peptidase and glycosidase enzymes involved in bacterial cell growth. There is also recent evidence suggesting the penicillins may inhibits RNA synthesis in some bacteria causing death without any lyses, but the significance of these observations remains to be proven. This group of antibiotics exerts little or no effect on bacteria that are not rapidly growing. Beta Lactam antibiotics are indicated to the prophylaxis and treatment of bacterial infections caused by susceptible organisms. Beta- Lactam antibiotics were mainly active only against gram positive bacteria: the development of broad spectrum Beta- Lactam antibiotics active against gram-negative organisms has increased the usefulness of the B-Lactam antibiotics. Beta –Lactamase is a type of enzyme produced by some bacteria that is responsible for their resistance to beta-lactam antibiotics like penicillins cephalosporins, cephamycins and carbapenems. These antibiotics have a common element in their molecular structure, a four atom ring known as a beta-lactam.

The beta-lactamases are the major defense of gram negative bacteria against beta-lactam antibiotics. Beta –lactamases can be broadly divided into enzymes with a serine residue at the active site, similar to bacterial penicillin binding proteins (PBPS), from which they probably evolved, and the metallo enzymes with zinc ion as a co-factor. The Beta- lactamases are enzymes produced by bacteria to hydrolyse (destroy) beta- lactam antibiotics such as penicillins, cephalasoposins and carbapenems. These enzymes catalyse the hydrolysis of Beta –

lactam ring to effectively destroy the antibiotics activity and enables bacteria to survive in the presence of these drugs. This is major mechanism of resistance to beta- lactam antibiotics in gram negative bacteria. Extended spectrum beta-lactamases (ESBLs) ,Plasmid-mediated AMPc enzymes and Carbapenem hydrolyzing beta- lactamases. Beta lactamases are found in the periplasmic space of gram negative and gram positive bacterial cell wall. Emergence of resistance of Beta-lactam antibiotics began even before the first B-lactam pencillin was developed. The first Beta-lactamase was identified in Escherichia coli prior to the release of pencillin for use in medical practice [5]. TEM and SHV type ESBLs are most often found in E.Coli and K. pneumoniae, however they have also been found in most of the species and other genera of Enterobacteriaceae[6]. TEM – 1 is the most commonly encountered B- lactamase in Gram negative bacteria. TEM -1 is able to hydrolyse pencillins and early aphaosporins such as cephalothin and carbapencillins. The majority of these ESBLs are derived from the wide spread broad-spectrum B- lactamases TEM-1 and SHV-1. There are also new families of ESBLs including the CTX-M and OXA type enzymes as well as novel, on related Beta –Lactamases. Several different methods for the detection of ESBLs in clinical isolates have been suggested. ESBLs have become widespread throughout the world and are now found in a significant percentage of Escherichia coli and klebsiella prenumoniac strains in certain countries. They have also been found in other Entero bacteriaceae strains and psudomonas aeruginosa. Extended spectrum B- lactomases (ESBLs) and AMPC B- lactamases are of increasing clinical concern. ESBLs are most commonly produced by Klebsiella [7]. Escherichia coli but may also occur in other –gram negative bacilli. There after typically plasmid medicated[8]. Clavulanic acid susceptible enzymes that hydrolyzepenicillins; extended spectrum cephelosporins: cefotaxime. Ceftriaxone. Ceftazidime; cefepime and others) and azetreonam. AMPC class B- Lactamases demonstrated or presumed to be chromosmally or plasmid mediated, have been described in pathogens. EX: Klebsiella pneumoniae. Eschesichia coli, salmonella SPP: proteus mirabilis; citrobacter freendii; Acinobactor; Enterobacter spp and p. aeruginosa[9].

## MATERIALS AND METHODS

Media: 1. Nutrient agar 2. Mac conkeys agar 3. Mueller-Hinton agar. Sterile cotton swabs, 0.5McFarland standard inoculums *Bacterial strains are* Klebsiella strains isolated from various clinical specimens.

**Clinical Specimens:** In this project work Klebsiella species isolated from: 1. Sputum, 2. Urine, 3. Pus, 4. Cerebrospinal fluids, 5. Blood, 6. Stool were studied.(shows table no:1)

**Antibiotics:** Cephotaxime, Ceftazidime, Amikacin, Cefixime, Cefepime, Amoxycillin, Cefoperazonc + sulbactum, Amoxycillin/clavulanic acid and Cephotaxime. All the strains of klebsiella species in this study were collected from routine clinical samples confirmed by biochemical and other tests. Stock cultures of these strains were maintained in nutrient agar Medium.

**Maintenance of stock cultures:** For maintenance of stock cultures only purpose culture were used. The colonies from confirmed Klebsiella isolates were taken. The claries were stabbed

into the vials containing nutrient agar medium by using sterile straight inoculation wire and incubated at 37<sup>0</sup> c in BOD incubator for 24hrs after incubation the vials were stored in refrigerator at 4 – 8<sup>0</sup>c. The stock cultures were sub cultured on Mac conkey's Agar for further testing.

**Composition of Mac conkeys agar Media :** Peptone : 20 g / litre, Sodium taurocholate: 5.0g/ litre, Lactose 10g/ litre, Neutral red: 0.04 g/ litre, Agar: 20.0 g/ litre and Ph at 25<sup>0</sup>c : 7.4 +/- 0.2.

**Composition of Mueller-Hinton agar medium[10]:**-Beef infusion: 300 g/ litre, Casein acid hydrolysate : 17.5 g/ litre, Starch 1.5 g/ litre, Agar : 17 g/ litre, Ph at 25<sup>0</sup>c: 7.4.

For preparation of inoculum only pure culture were used with a loop single colony from the subculture plates were transferred to peptone water and incubated at 37<sup>0</sup>c for 2 hrs till light to moderate turbidity was developed corresponding to 0.5 McFarland standards.

**Peptone water composition:** Peptic digest of animal tissue: 10 g/ litre, Sodium chloride : 5 g/ litre and Ph at 25<sup>0</sup>c : 7.4.

**The Antibiotic susceptibility test:**The Mueller- Hinton agar were inoculated with cultured peptone water using sterile non-toxic cotton swabs and spread on Mueller-Hinton agar surface. The antibiotic discs were placed with centre at least 20mm apart. The plates were incubated at 37 c for overnight at 37 c for overnight and observed for sensitivity. The complete inhibition zones were measured and diameters of the zones recorded to the nearest millimeter and interpreted as Sensitive, Intermediate sensitive and Resistant according to NCCLS criteria.

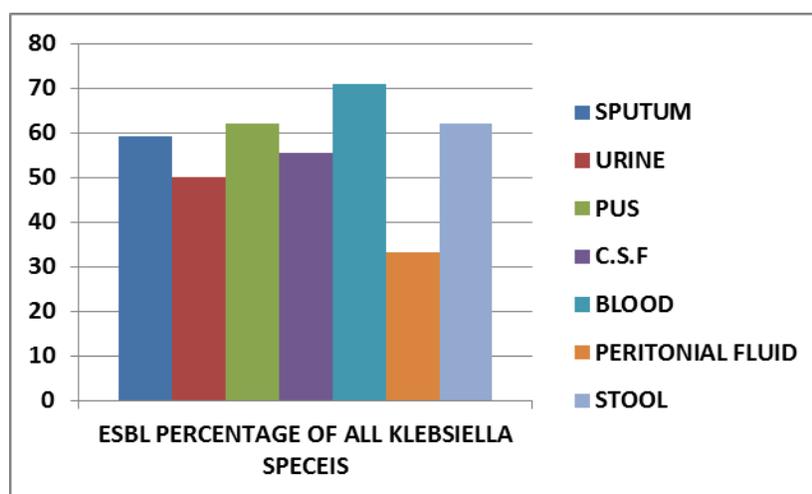
**Double disc synergy test (DDST):** was done to determine synergy between a disc of augmentin (20µg amoxicillin and 10µg clavulanic acid) and 30 µg disc of each 3<sup>rd</sup> generation of cephalosporin antibiotics. Mueller-Hinton agar plates were prepared and inoculated with standardized inoculum (10<sup>5</sup> McFarland tube CFU / ml and) to form a lawn culture. 30µg disc of each 3 GC antibiotic was placed on the agar at a distance of 25mm centre to centre from augmentation disc- E coli ATCC 25922 was used as positive control- ESBL production was interpreted if if the inhibition zone around the test antibiotics disc increased towards the augmentation disc or if neither discs were inhibited where the two antibiotics diffuse together. In double –disc synergy test enhancement of the zone of inhibition of cephalexin (Oxymino Beta Lactam antibiotic) caused by the synergy of the clavulanate in the amoxicillin – clavulanate disc is positive result. Isolates found to be resistant (or) with decreased susceptibility to 3<sup>rd</sup> generation cephalosporins were selected for the presence of ESBL. The results were interpreted as positive when any of the 3 following criteria was fulfilled. Inhibition zone around the test antibiotic showed a clear extension towards augmentin disc. If neither disc were inhibited by itself but bacterial growth was inhibited between the two discs. Broadening inhibitory zone of the third generation cephalosporins towards the disc of augmentin. The strains were considered non-ESBL producing if they did not show the above criteria.

## RESULTS

The study was to know the prevalence of extended spectrum  $\beta$  lactamase in multidrug resistant (MDR) klebsiella species isolated from different clinical samples the wide spread use of antibiotics in hospitals has led to an emergence of multidrug resistant organisms of low virulence like klebsiella causing serious opportunistic infections.(shows Figure:1) Klebsiella pneumoniae is an important organism cause for nosocomial infections and infections due to ESBL producing k. pneumoniae are of concern as 3GC are commonly used for treatment of infections due to gram negative organisms.The study was undertaken to determine sensitivity of klebsiella species to various 3GCs and to know the prevalence of ESBLs producing klebsiella species in clinical samples. A total of 100 klebsiella species were tested for ESBL production by Disk diffusion method of Kirby bauer technique. (Double disk synergy test). In screening test for detection of the test organism is whether ESBL producer or ESBL non-producer the following antibiotics are used as Cephalexin(30  $\mu$ g), Ceftazidime ( 30  $\mu$ g), Amikacin ( 30  $\mu$ g), Cefixime ( 05  $\mu$ g) Cefepime ( 30  $\mu$ g) and Amoxicillin ( 20  $\mu$ g). (Shows Table:1).

**Table:2 TOTAL ESBL PERCENTAGE OF ALL KLEBSIELLA SPECIES FROM ALL**

SL.NO	SAMPLES	TOTAL KLEBSIELLA SPECIES SCREENED FOR ESBL PRODUCTION	NO. OF KLEBSIELLA SPECIES ESBL POSITIVE	PERCENTAGE OF ESBL POSITIVITY
1.	SPUTUM	22	13	59.3%
2.	URINE	22	11	50%
3	PUS	29	18	62.06%
4.	C.S.F.	09	05	55.5%
5.	BLOOD	07	05	71%
6.	PERITONIAL FLUID / SYNOVIAL FLUID	03	01	33.3%
7.	STOOL	08	05	62%



**Figure 1: ESBL PERCENTAGE OF ALL KLEBSIELLA SPECIES FROM ALL CLINICAL SPECIMENS**

Test organism was considered to ESBL producer if the organism resistant to at least two of the 3GCs it is NCCLS – 2004 recommendation for detection of suspected ESBL producing gramnegative bacteria. In this method Amikacin and Amoxycillin and cefoperazone + sulbactam antibiotics also used because all most all klebsiella species are sensitive to Amikacin as well as all klebsiella species are resistant to Amoxicillin. ESBL produced by the isolates is inactivated by sulbactam. Doubl disk synergy test for ESBL producing klebsiella species was species was conducted with center to centre distance of 25mm and 30 mm distance bet ween these but results were negative in case of where distance was 30 mm test organism was considered to produced ESBL if the zone size around the cephotoxine plus Amoxycillin/ clavulanic acid increased where synergism will be seen

### CONCLUSION

ESBL producing organisms pose a major problem for clinical therapeutics. The incidence of ESBL producing strains among clinical isolates has been steadily increasing over the past few years resulting in limitation of therapeutic options. Initially restricted to hospital acquired infections, they have also been isolated from infections in outpatients. Major outbreaks involving ESBL strains have been reported from all over the world, thus making them emerging pathogens. The routine susceptibility tests done by clinical laboratories fail to detect ESBL positive strains and can erroneously detect isolates sometimes to be sensitive to any of the broad spectrum cephalosporin like cefotaxime, ceftazidime, ceftriaxone. With the spread of ESBL producing strains in hospitals all over the world, it is necessary to know the prevalence of ESBL positive strains in a hospital so as to formulate a policy of empirical therapy in high risk units where infections due to resistant organisms. A knowledge of resistance pattern of bacterial strains in a geographical area will help to guide the appropriate and judicious antibiotic use. Infection control precautions like barrier nursing, cohorting of patients and nurses, contact precautions through the use of disposable gloves, gowns, and strict attention to hand washing are essential to limit its spread. Development of an infection control policy and hospital antibiotic prescribing guide should follow this generation. Education of medical and nursing staffs, patients, visitors and medical students through handouts, posters and meetings could play an important part. ESBL producers are intrinsically resistant to all cephalosporins and aztreonam Interpretative comments can accompany microbiology reports to underline this fact. Co-resistance to quinolones and aminoglycosides are common. Quinolone antiobiotics are strong selectors of ESBL producers. ESBLs becoming an increasing problem in hospital and community setting, screening for the presence of these resistant pathogens (like MRSA screeining) would ultimately become a necessity, especially in units with high antiniotic use. These would include high dependency units, post-operative wards, intensive care units, haematology, oncology, burn wards, orthopaedic and transplant centers. Screening for ESBL in large number of patients is a technical as well as financial challenge. A robust screening policy and an effective standard operating procedure would be crucial to minimize cost and confusion. Several specimens like rectal swabs, as well as urine, stool and sputum are tested in some centres to screen for resistant gram negative bacilli (GNB). The choice and number of specimens in an institutional setting am ultimately depend on several factors like patient profile and resource availability. The use of antibiotic (gentamicin, ciprofloxacin, cefpodoxime)

incorporated agar in microtitre plates may facilitate cost effective screening for resistant GNBs in laboratories with large sample load. Resistant isolates can then be subjected to confirmatory tests to identify the presence of ESBLs. The development of evidence based guidelines for the management of ESBL positive infections would require the performance of double blinded randomized controlled trails (RCT). At present there does not seem to exist any significant evidence based recommendations about several aspects in the management of ESBL related infections such as monotherapy versus combination therapy, optimal duration of therapy, best practice in preventing patient to patient spread in hospital settings. The management of ESBL requires a multi-disciplinary approach. CO-ordinated participation of microbiologists, clinicians, nursing personal, hospital infection control team is essential. Therapeutic decision making requires a sound appreciation of clinical perspective. Potential for screening exists but it must be tailored to the institutional need and patient profile. The Petri-dish has long inspired our admiration for ESBL producers. It is time to extend our appreciation to the patients who are the ultimate sufferers.

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