ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 6, Issue, 06, pp.7145-7148, June, 2014

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

ISOLATION OF EXTENDED SPECTRUM B-LACTAMASES (ESBL) PRODUCING *ESCHERICHIA COLI* AND *KLEBSIELLA* SP. FROM DIFFERENT TYPES OF SAMPLES IN TERTIARY CARE HOSPITAL IN GURGAON, INDIA

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ARTICLE INFO

Article History:
Received 14th March, 2014
Received in revised form
29th April, 2014
Accepted 06th May, 2014
Published online 25th June, 2014

Key words:
Pseudomonas spp,
Antimicrobial susceptibility,
ESBL.

ABSTRACT

The present study was conducted to isolation of Extended Spectrum Beta Lactamase (ESBL) producing in gram`s negative bacteria specially in Eschericia coli and Klebsiella pneumonia in different clinical samples received at the department of Laboratory medicine, Alchemist hospital, Gurgaon, india. Aerobic culture was performed in automated analyzer vitek-2 and isolate from blood agar and mackonky agar media. Antimicrobial susceptibility testing was carried out using the Kirby-Bauer agar diffusion method and ESBL production was detected on Mueller Hinton agar by double-disk synergy technique and vitek 2. A total of 180 isolates were collected from 493 samples. From these 180 samples two types of organisms were isolated**:** *Escherichia coli* and *klebsiella* sp. In some samples these two organisms become resistant to many ß lactam antibiotics like cefazoline, ceftriaxone, Aztreonam, Ceftazidime, Ampicillin etc. drug sensitivity test done by disc diffusion method as well as automated vitek-2. Percentage of ESBL positive *E.coli was* 70.5 % and *Klebsiella* sp. was 86.1 % and percentage of ESBL negative *E.coli was* 29.5 % and ESBL negative *Klebsiella* sp. was 14.1 %. However, theses finding shows prevalence of ESBL positive is more in Klebsiella species and negative is more in Escherichia coli on our hospital.

INTRODUCTION

Extended spectrum ß-lactamase (ESBL)-producing organism stance a distinctive challenge to clinical microbiologists, Physicians and infection control professionals. ESBLs are enzymes capable of hydrolyzing penicillins, broad-spectrum cephalosporins and monobactams, and are generally derived from TEM and SHV-type enzymes. ESBLs are frequently located on plasmids that are transferable from strain to strain and between bacterial species. While the prevalence of ESBLs is not known, it is clearly increasing, and in many parts of the world 10–40 % of strains of Escherichia coli and Klebsiella pneumoniae express ESBLs. ESBL-producing Enterobacteriaceae have been responsible for many epidemics of infection throughout the world and stance challenging infection control problems. The clinical outcomes data indicate that ESBLs are clinically significant and, when detected, indicate the need for the use of appropriate antibacterial agents. Inappropriately, the laboratory detection of ESBLs can be complex and, at times, misleading. Antibacterial choice is often complicated by multi-resistance. Many ESBL producing organisms also express AmpC ß-lactamases and may be co-transferred with plasmids mediating aminoglycoside resistance. In addition, there is an increasing association between ESBL production and fluoroquinolone resistance. Although in in vitro tests ESBLs are inhibited by ß-lactamase inhibitors such as clavulanic acid, the activity of ß-lactam/ß-lactamase inhibitor combination agents is influenced by the bacterial inoculum, dose administration regimen and specific type of ESBL present. Currently, carbapenems are regarded as the drugs of choice for treatment of infections caused by ESBL-producing organisms. Unfortunately, use of carbapenems has been associated with the emergence of carbapenem-resistant bacterial species such as Stenotrophomonas sp. or Pseudomonas sp.

Extended spectrum β-lactamases (ESBL) producing organisms are an increasing problem for practitioners dealing with infectious diseases. The ESBL represent a major group of ß-lactamase that have the ability to inactivate ß-lactam antibiotics containing an oxyimino group such as third generation cephalosporins and monobactams (aztreonam) and are inhibited by ß-lactamase inhibitors. Gram negative bacteria, especially members of the Enterobacteriaceae family such as

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Klebsiella pneumoniae and *Escherichia coli*, produce these enzymes. Over 80% of cases of serious infections seeking admission of tertiary care hospitals in India are Gram negative infections.

MATERIALS AND METHODS

The present study was conducted to isolation of Extended Spectrum Beta Lactamase (ESBL) producing in gram`s negative bacteria specially in Eschericia coli and Klebsiella pneumonia in different clinical samples (Urine, Pus, Sputum, BAL and ET) received at the Department of Laboratory medicine, Alchemist hospital, Gurgaon, India. Bacteria identification and conformation by biochemical study by Aerobic culture was performed in automated analyzer vitek-2 and inoculation in ship blood agar and mackonky agar media. Antimicrobial susceptibility testing was carried out using the Kirby-Bauer agar diffusion method and ESBL production was detected on Mueller Hinton agar by double-disk synergy technique and Automated vitek-2 from bioMérieux, France.

RESULTS

During the study period of 6 months, a total of 180 isolates were collected from 493 samples. From these 180 samples, two types of organisms were isolated**:** *Escherichia coli* and *klebsiella* sp. In some samples these two organisms become resistant to many ß lactam antibiotics like cefazoline, ceftriaxone, Aztreonam, Ceftazidime, Ampicillin etc. Percentage of ESBL positive *E.coli was* 70.5 % and *Klebsiella* sp. was 86.1% and percentage of ESBL negative *E.coli was* 29.5 % and ESBL negative *Klebsiella* sp. was14.1 % (Table 1).

Table 1. Characterization and identification of isolates from different ICU and IPD samples

Samples	*Escherichia coli*	*Klebsiella* sp.	Total bacterial isolate
Urine	58(64%)	33(36%)	91
Pus	18(66. 6%)	09(33. 3%)	27
Sputum	04(33. 3%)	08(66. 6%)	12
BAL	11(40%)	17(60%)	28
ET	04(20%)	18(80%)	22
Total	95(55%)	85(45%)	180

Extended spectrum ß-lactamase (ESBL) producing organisms are an increasing problem for practitioners dealing with infectious diseases. Based on various tests 52.7 % *E.coli* isolates were identified as and 47.2 % as *Klebsiella* species. These organisms were selected further to test ESBL producer and non producer by standard disk diffusion method (Table 2).

Table 2. ESBL positive and negative isolates

Organism	ESBL phenotypic positive	ESBL phenotypic negative	Total
E.coli	67(70.5%)	28(29.5%)	95
Klebsiella	73(86.1%)	12(14.1%)	85
Total	140(77.5%)	40(22.2%)	180

Out of 95(52.7%) *isolates* 58 isolated from urine, 18 from pus, 4 from sputum, 16 from Broncho Alveolar Lavage (BAL fluid), 4 from Endotracheal secretion. A total of 85(47.2%) *Klebsiella* species isolated among which 33 were isolated from urine, 8 from sputum, 9 from pus, 12 from BAL fluid, 18 from ET (Table 3). While determining the resistance profile of ESBL produces the resistant pattern determined is as Penicillin (100%), Fluoroquinolones (100%), Aminoglycosides (60%), Beta lactamase inhibitor (90%), Cephalosporins (100%), Lepopeptides (0%), Carbapenems (25%), Monobactam (100%) and Nitrofurantoins (67%) (Table 3).

Table 3. Resistance profile of ESBL producers

S.No.	Antibiotic groups	Antibiotic groups
1	Penicillin	100%
2	Fluoroquinolones	100%
3	Aminoglycosides	60%
4	ß lactamase inhibitor	90%
5	Cephalosporins	100%
6	Lepopeptides	0%
7	Carbapenems	25%
8	Monobactum	100%
9	Nitrofurantoins	67%

Phenotypic Confirmatory test Initial screening was followed by phenotypic test using ceftazidime and ceftazidime with clavulanic acid. In E.coli Out of 95 isolates of 67 isolates were found to be ESBL positive and 28 isolates were ESBL negative. In *Klebsiella* sp. 73 isolates were detected positive and 12 isolates were ESBL negative. Vitek ESBL Test For confirmation, these isolates were reconfirmed by Vitek-2.

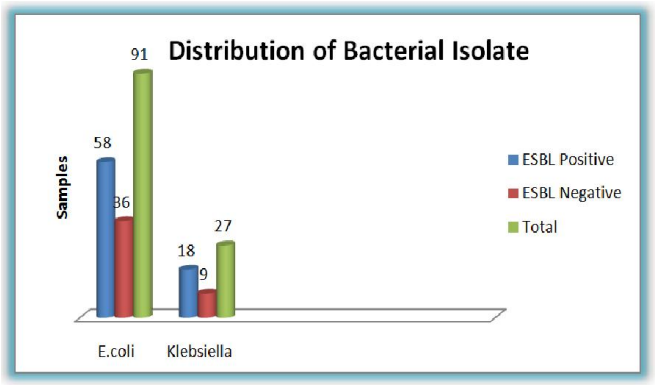


Figure 1. Distribution of Bacterial Isolate within samples

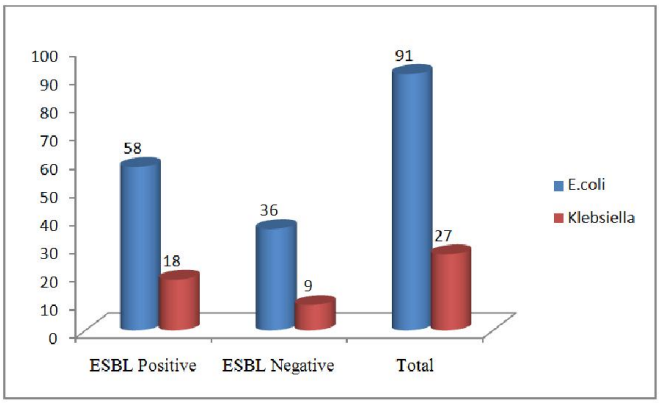
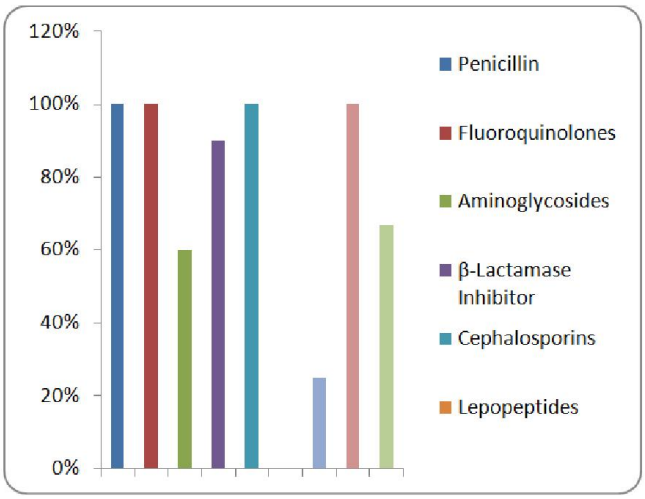


Figure 2. ESBL positive and negative isolates



With the help of the cards-ID GN(Identification card for gram negatives) and GN90(sensitivity card for gram negatives). A predetermined reduction in growth in wells containing clavulanate compared to the drug alone indicates the presence of ESBLs.

DISCUSSION

The ESBL phenomenon began in Western Europe, most likely because expanded spectrum ß-lactam antibiotic were first used there clinically. However it did not take long before ESBLs had been detected in United States and Asia. In the United States occurrence of ESBL production in Enterobacteriaceae ranges from zero to twenty five percent depending on the institution. Among isolates of *Klebsiella pneumoniae*, the percentage of ceftazidime resistance ranges from five to ten percent for non-intensive care unit (non-ICU) and ICU isolates, respectively. The *Escherichia coli* and *Klebsiella* species are the leading causes of serious infections. After the initial description of ESBL production by *Klebsiella* species strains in 1983 and *Escherichia coli* in 1987 resistance to broad spectrum cephalosporins was increasingly reported.

In the present study among 493 samples of ICU and IPD, examined during this study, 180 isolates were isolated as and *Klebsiella* sp. Among which 140 (77. 7%) are ESBL Positive *E.coli* isolates, from which 67(70. 5%) are ESBL positive and 28(29. 5%) are ESBL negative *E.coli* , 73(86. 1%)are ESBL positive *Klebsiella* sp. and 12(14. 1%) are ESBL negative *Klebsiella*. 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 (U Chaudhary, R. aggarwal). In the United states in 1990 to1993 a survey of the intensive care units (ICUs) of 400 Hospitals recorded an increase from 3.6 % to 14.4 % in ESBL producing strains of *Klebsiella* sp. By 1994 the center for Disease control and Prevention National Nosocomial Infections Surveillance System(NNISS)reported that 8% of *Klebsiella* sp. had ESBLs with producers predominately from a few large centers. A 1995-96 study in Richmond, Virginia reported 1. 5% of isolates produced ESBLs. TEM-10, TEM-12 and TEM-26 seem most common in the USA . In Europe as of 1995, ESBLs occur in 20 to 25% of *Klebsiella* sp. from patients in ICUs, although they have been found in up to 30%-40% in France. TEM-3 seems to be most common in France. (Mary S. Farkosh, MT (ASCP), SBB). While determining the resistance and sensitivity profile of ESBL producers Penicillin group drugs are found to be 100% resistance, fluoroquinolones was also found to be 100%, Aminoglycosides was found to be 60% resistant, ß lactamase inhibitors was found to be 90% resistant, Cephalosporins-100% resistant, lepopeptides was not resistant it was 100% sensitive, carbapenems was 25% resistant, Monobactams was found to be 100% resistant and Nitrofurantoins was found to be 77% resistant. In the present study the resistance of ESBL producing strains towards Aminoglycosides group (gentamicin and amikacin) was found to be 60% towards fluoroquinolones 100%. Similar results quoted by Jyoti Sharma *et al.* () showed the resistance conferred by ESBL producers as 60-100% towards gentamicin 40-96% towards amikacin and 33-92% towards ciprofloxacin. In present study Cephalosporins was found to be 100% resistant. Where as 50% resistance was found in Mary S. Farkosh, MT study. A knowledge of resistance pattern of bacterial strains in a geographical area will help to guide the appropriate and judicious antibiotic use. While comparing the results of this study with previous studies it was concluded that rates of ESBL positive strains vary greatly worldwide and within geographic areas and are rapidly changing over time. Moreover the resistant pattern also increasing rapidly. As the drugs which were found to be sensitive for ESBLs earlier now they were resistant. For example Gentamicin and Tobramycin (Aminoglycosides group) were found to be sensitive earlier but now a days they are almost found to be resistant. But Amikacin and Netilmycin are still sensitive for ESBLs. Of all the available antibiotics Carbapenems are the most effective and reliable as they are highly resistant to the hydrolytic activity of all ESBL enzymes, due to the trans-6 hydroxy ethyl group. Antibiotics of lepopeptide group (polymixin B and colistin) are often effective as the drugs of this group were still 100% sensitive. The utility of penicillins, cephalosporins, and aztreonam in treating serious infections due to these organisms is uncertain due to reports of treatment failure despite apparent in vitro susceptibility.

Conclusion

ESBLs are an example of the increasing number and diversity of enzymes that inactivate ß-lactamtype antibacterials. The combination of these enzymes with other resistance traits give strong testimony as to the resilience of microbes and their ability to adapt to their environment. Our ability to successfully treat infections due to these increasingly resistant organisms demands a multifactorial approach combining continued research and development of novel classes of antibacterials, more prudent use of existing agents and an increased emphasis on more effective infection control measures.

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