Original Research Article

Prevalence of ESBL Producing Gram Negative Bacilli in a Tertiary Care Hospital

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Abstract

Introduction: Rapid emergence of ESBL producers in gram negative bacilli has put forward a major challenge worldwide in their detection as well as treatment options for clinicians. Hence for proper management of these resistant bacteria it is so important and essential to correctly identify them. Antimicrobial resistance is now proclaimed as the most important challenge worldwide being faced by humanity in its fight against infectious diseases. Extended Spectrum β-Lactamases (ESBLs) producing bacteria are increasing in number and causing more severe infections because of their continuous mutation and multidrug resistance property which make its difficulty in therapeutic treatment. The aim of this study is to determine the prevalence of extended spectrum beta lactamase (ESBL) producing Gram negative bacteria causing infections among in- and out-patients in a tertiary health care facility. **Methodology:** This study was conducted at a tertiary care hospital in which gram-negative bacilli which were isolated from patients' samples. This study was conducted in Department of Microbiology at T.S. Misra Medical college& hospital, Amausi, Lucknow, Uttar Pradesh. The duration of study was over a period of two year from January 2019 to 2021. **Results:** This result of this study revealed that, 53.9% percent of the bacterial isolates which showed resistant to the third generation cephalosporins were observed to be as ESBL producers. **Conclusion:** ESBL-producing bacteria are a strain of multidrug-resistant pathogens that are increasing rapidly and becoming a major problem in the region of infectious diseases. It is necessary to report ESBL production along with the routine antibiotic susceptibility reporting, it will help the clinicians in prescribing proper antibiotics for treatment.

Keywords: ESBL Producers, Gram Negative Bacteria, Bacterial Isolates, ß-Lactamases.

Introduction

Gram – Negative Bacilli (GNB) are rapidly developing resistance against antibiotics. This expanding problem has been reported to be due to the organisms' ability to acquire and transmit plasmids, to mutate, and presence of other mobile genetic elements encoding resistance genes.[1] The emergence of resistance against Beta-lactamase antibiotics has resulted in a major clinical crisis as these antibiotics are one of the most widely prescribed worldwide. [2] In the early 1980s, the third generation cephalosporins were introduced and these new drugs were a big leap against ß-lactamase-mediated bacterial resistance to antibiotics. In 1983, a report was published about plasmid-encoded ß-lactamases which were capable of hydrolyzing the extended-spectrum cephalosporins.[3] Mutations within the structural genes encoding the older enzymes were reported. This mutation gave rise to derivatives that possessed an extended substrate profile compared with that of the parental enzymes. These new enzymes were given the name Extended-spectrum beta lactamases (ESBLs). These new enzymes were derivatives of older enzymes and developed capabilities to hydrolyze a broader spectrum of β-lactam drugs.[4] The unregulated widespread use of the third generation cephalosporins and aztreonam was believed to be the major cause of the mutations in these enzymes, leading to the emergence of ESBL7. K. pneumoniae was the first in which ESBL production was reported in 1983 occurred and in 1987, it was also found in E. coli.[5] Since its first detection in Germany in 1983, ESBLs have diversified themselves throughout the world. ESBL producing strains are reported to be Klebsiella pneumoniae, Klebsiella oxytoca and Escherichia coli.[4] Other organisms such as Enterobacter spp., Salmonella, Morganella morganii, Proteus spp., Serratia marcescens and Pseudomonas aeruginosa also produce ESBL but to a lower frequency.[6] ESBLs have more than 300 variants.

Risk factors associated with ESBL are old age (> 65 years), male gender, previous use of β- lactam antibiotics and fluoroquinolones amongst others [6,7].

The infections caused by ESBL-producing organisms are urinary tract infections, peritonitis, cholangitis, intra-abdominal abscess, pneumonia, and catheter-associated blood stream infections which are similar to infections caused by gram negative bacilli.[8] These infections have very few treatment options as these are usually multidrug-resistant. Thus, these lead to increased morbidity,

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mortality and health care associated costs especially in the Intensive-care units.**[7]**

The extended–spectrum cephalosporins and fluoroquinolones are broad– spectrum antibiotics and are the drugs of choice for infections caused by various Gram-negative pathogens [8].

It is difficult to find out drug resistance by conventional antimicrobial susceptibility methods, which lead to uncontrolled spread of ESBL producing organisms. Therefore, in developing countries, it becomes extremely important to find out these resistant organisms as there is excessive use of antibiotics, over the counter sell of third generation cephalosporins and lack of adequate antimicrobial resistance surveillance8. Increased morbidity and mortality in ESBL-producing Gram negative bacterial infections have been found to be due to inappropriate or delayed antimicrobial treatment [9].

Multidrug resistance, difficulty in detection and treatment, and increased mortality are the major problems associated with ESBLs. Therefore, it is extremely important to detect these ESBL enzymes and to spread awareness for better patient care. The judicious use of antimicrobial agents and improved infection control methods must become health care priorities.

Materials & Methods

Study Population: This study was conducted at a tertiary care hospital in which gram negative bacilli which have been isolated from patients samples both IPD as well as OPD and causality, that showed resistance against the third generation cephalosporins as per the standard of Clinical Laboratory Standards Institute (CLSI) guidelines, included in this study .

Study Area: This study was conducted in Department of Microbiology at T.S. Misra Medical college& hospital, Amausi, Lucknow, Uttar Pradesh.

Study Duration: The duration of study was over a period of two year from January 2019 to 2021.

Data Collection: In this study we were received different samples such as pus, sputum, tracheal aspirate, cerebrospinal fluid, ascitic fluid, pleural fluid, blood and urine, tracheal aspirate and high vaginal swab from different department. All samples were included either from OPD or IPD during the period of study. After transportation of samples to the Microbiology Department, culture was done on Blood Agar &MacConkey's Agar & incubated at 37ºC & reading was taking after 24-48 hours according to latest CLSI guidelines. Bacterial culture was identified on the basis of Gram staining & biochemical reactions. After that gram negative bacteria were tested for ESBL production by using the double-disk approximation test.

Double-Disk Approximation Test

For double disk approximation test, we were taking overnight culture suspension of the bacterial isolate which was compared with 0.5 McFarland's standard was inoculated by using cotton swab on the surface of a Mueller Hinton Agar plate.

The combination-disk test using both cefotaxime and ceftazidime, in combination with clavulanic acid, was placed for the detection of ESBL according to the CLSI guidelines. The overnight culture suspension of the bacterial isolate which was compared to 0.5 McFarland's standard was inoculated by using a sterile cotton swab on the surface of a Mueller Hinton Agar plate. The Cefotaxime (30 µg) and cefotaxime-clavulanic acid (30 µg/ 10 µg) disks were put it on MHA on the difference of nearby 20 mm.

Then incubating overnight at 37°C & reading was taking, a ≥ 5-mm increase in the zone diameter for either antimicrobial agent which were tested in combination with clavulanic acid vs. its zone when tested alone, was interpreted as positive for Extended spectrum beta-lactamases (ESBL) production.

Data Analysis: Data was analyzed by using Microsoft Excel.

Results

We were included 384 IPD, OPD & causality samples. Out of 384 samples, 62.7% were received from OPD (62.7%) then IPD (29.4%) & causality. We were received different samples like urine 74.5%, 12.5% pus, 5.7% blood, 3.1% endotracheal aspirate, 1.5% sputum,1.5% body fluids & 0.5% CSF & High vaginal swab. This study observed that isolated bacteria from culture 41.1% Escherichia coli which was most common then Kelbsiella (28.3%), Enterobacter spp.(17.4%), Cithrobacter spp. (5.9%),Pseudomonas aeruginosa (3.6%) &Acinetobacter spp. (3.3%). Overall, 53.9% percent of the bacterial isolates which were resistant to the third generation cephalosporins were observed to be as ESBL producers which were obtained from the OPD, IPD & causality cases.

Table 1: Distribution of cases according to sample received from different department

Samples from	Number	Percentage
IPD	113	29.4%
OPD	241	62.7%
Emergency	30	7.9%
Total	384	100%

Table 2: Distribution of cases according to samples

Sample	Number	Percentage
Urine	286	74.5%
Blood	22	5.7%
Pus	48	12.5%
Endotracheal Aspirate	12	3.1%
Sputum	6	1.5%
CSF	2	0.5%
High vaginal swab	2	0.5%
Body fluids	6	1.5%
Total	384	100%

Table 3: Distribution of cases according to organism isolated from different samples

Organisms	Number	Percentage
Escherichia coli	158	41.1%
Klebsiellaspp	109	28.3%
Pseudomonas areuginosa	14	3.6%
Citrobacter spp.	23	5.9%

Enterobacter spp.	67	17.4%
Acinetobacter spp.	13	3.3%
Total	384	100%

Table 4: Distribution of cases according to sample ESBL positive and negative isolates

Outcome	Number	Percentage
ESBL positive	207	53.9%
ESBL negative	177	46.1%
Total	384	100%

Discussion

The production of ESBLs has been reported to cause resistance against extended spectrum cephalosporins [10]. The nosocomial outbreaks have been reported to be caused by ESBL-producing organisms [11-13]. Most of these outbreaks have been limited to high-risk patient care areas such as ICUs, oncology units, etc., but, in 1999, an outbreak was reported in a nursing home [14]. Therefore, these resistant organisms are not only threatening to intensive care units or tertiary hospitals but also to primary care hospitals.

Recent studies showed an increase in the occurrence of ESBL production among the members of Enterobacteriaceae [15]. A study on uropathogens reported 26.6% of isolates of Klebsiella pneumoniae, Escherichia coli, Enterobacter, Proteus and Citrobacter spp. were ESBL producers [16]. Another study showed that 48.3% of their cefotaxime resistant gram-negative bacilli were ESBL producers [16]. A study from Coimbatore documented ESBL production to be 41% in E. coli and 40% in K. pneumoniae [17]. Similarly, study by Mathur et al reported ESBL production to be 62% in E. coli and 73% in K. pneumoniae [18].In the present study, ESBL production was observed to 41.1% in E. coli and 28.3 % in K. pneumonia, Pseudomonas 3.6%, Acinetobacter 3.3%, Citrobacter 5.9% &Enterobacter 17.4% isolates. Finding of lower ESBL production in K. pneumoniae in the present study was contrary to the findings in previous studies [16-17]. It was further observed in the present study that ESBL production among E. coli and K. pneumoniae isolates was more frequently detected by the combination disk method than the double disk approximation test. The use of the combination disk method for the phenotypic confirmation of ESBL production among Enterobacteriaceae has been, therefore, proposed by Clinical Laboratory Standards Institute (CLSI) .

In comparison to Enterobacteriaceae, in Pseudomonas spp. ESBL production is in reduced amount because their resistance is mediated by various other mechanisms. These mechanisms were the production of metallo-beta-lactamases, lack of drug penetration due to mutations in the porins, and the loss of certain outer membrane proteins and efflux pumps [18-20]. Though CLSI proposed the double disk approximation method for testing ESBL production among the Pseudomonas aeruginosa isolates but significant differences between the ESBL detection rates of the two methods could not be established in the present study. This non-establishment between two methods can be attributed to the relatively small number of isolates in the present study.

Just like a study from Coimbatore [21], it was observed in the present study that a majority of the isolates of Enterobacteriaceae were susceptible to imipenem and piperacillin-tazobactam. In both the studies, amikacin also showed good activity against gram-negative bacteria. It was observed in our study that co-resistance to amoxicillin-clavulanate, gentamicin and ciprofloxacin was very common and there was multi-drug resistance among the E. coli and K. pneumoniae isolates rather than other.

The early detection of ESBL producing organisms is extremely important in controlling hospital infections as these organisms are the commonest nosocomial pathogens. Multidrug resistant Enterobacteriaceae are the most common isolates in most of the laboratories. It is a challenge to control these multidrug resistant organisms. In the present study, this challenge was further increased by the co-existence of the resistance to β-lactams, aminoglycosides and fluoroquinolones. It has been reported that Carbapenems are the most effective treatment options among all the available antimicrobial agents against infections caused by the ESBL producing isolates [15]. The overuse of carbapenems should be avoided as it may lead to resistance in gram-negative organisms. The labs, which are not equipped with molecular methods, should regularly detect ESBLs by conventional methods as genotyping is not more informative for the treatment.

Conclusion

This study concludes that ESBL-producing organisms are a breed of multidrug-resistant pathogens. These have increased extremely rapidly and have become a major problem in the area of infectious diseases. It is extremely important to report ESBL production along with the routine sensitivity reporting. This will help the clinicians in prescribing proper antibiotics. Piperacillin-tazobactam and imipenem are the most effective against ESBL producing organisms.

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