Original Research Article

Prevalence and Antimicrobial Susceptibility Pattern of ESBL Producing Gram Negative Bacilli: An Institutional Based Study

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Abstract

Introduction: The production of extended-spectrum- β lactamases (ESBLs) is an important mechanism for resistance to the third generation cephalosporins. Awareness and the detection of these enzymes are necessary for optimal patient care.**Methodology:** The total of 96 isolates which were isolated from the different samples tested for ESBL production. This study was conducted in Department of Microbiology, Heritage institute of Medical Sciences.**Result:** This result of this study revealed those 69.7% organisms which were ESBL producers & 30.2% organisms which were non ESBL producers.**Conclusion:** It is essential to report ESBL production along with the routine sensitivity reporting, which will help the clinician in prescribing the proper antibiotics.

Keywords: ESBL, Antibiotic Susceptibility Test, β lactamases.

Introduction

It is well known that antibiotic resistance among Gram-negative bacilli (GNB) is a rapidly expanding problem. It is due to the organisms' ability to mutate, to acquire and transmit plasmids and other mobile genetic elements encoding resistance genes.[1] Beta-lactamase antibiotics are considered as the most widely prescribed antibiotics. The resistance of these agents has caused a major clinical crisis.[2] In 1890s, the introduction of the third generation cephalosporins into clinical practice was signaled as a major breakthrough in the fight against ß-lactamase-mediated bacterial resistance to antibiotics. The first report of plasmid-encoded ß-lactamases capable of hydrolyzing the extended-spectrum cephalosporins was printed in 1983.[3] Mutations within the structural genes encoding the older enzymes had arisen and giving rise to derivatives that possessed an extended substrate profile compared with that of the parental enzymes. Therefore, these new enzymes were given the name Extended-spectrum beta lactamases (ESBLs). They reflect the fact that they were derivatives of older enzymes and had a new capability to hydrolyze a broader spectrum of βlactam drugs.[4] The first reported Extended-spectrum beta-lactamase (ESBL) producer detected in 1983 in K. pneumoniae. It was followed by the detection of an ESBL producer in E. coli in 1987.[5] Meanwhile their early description in Germany in 1983, ESBLs have diversified and spread globally.

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Infections produced by ESBL-producing Gram negative bacteria are related with increased morbidity and mortality. It is linked to inappropriate antimicrobial treatment.[6] ESBL production consists of several risk factors. these factors include old age (> 65 years), male gender, previous use of β-lactam antibiotics and fluoroquinolones amongst others.[6,7] In Nigeria, extended–spectrum cephalosporins and fluoroquinolones are mostly used as broad– spectrum antibiotics. It remains the drugs of choice to treat infections caused by various Gram negative pathogens.[8] The use of antibiotics in Nigeria is unregulated and over the counter sales of antibiotics without prescriptions are common.[9-11] These indicate that ESBL producing organisms may be very common in Nigeria and indeed reports of ESBL–producing Gram negative bacteria exists.[11]

Materials & Methods

Study Population: The total of 96 isolates which were isolated from the different samples tested for ESBL production.

Study Area: This study was conducted in Department of Microbiology, Heritage institute of Medical Sciences.

Study Duration: This study was done over a period of two years.

Data Collection

The gram-negative bacilli which were isolated from the patients, which showed resistance to the third generation cephalosporins as per the Clinical Laboratory Standards Institute (CLSI) guidelines, were included in this study. They were isolated from various specimens such as pus, sputum, tracheal aspirate, cerebrospinal fluid, ascitic fluid, pleural fluid, blood and urine which were received in our lab during the study period. The isolates were identified, based on the standard bacteriological techniques and were tested for ESBL production by using the double-disk approximation test which was described by Jarlier et al and the combination disk method which was recommended by CLSI.

Combination Disk Method

The combination-disk test using ceftazidime in combination with clavulanicacid, was performed for the detection of ESBL according to the CLSI guidelines.

In this test, an overnight culture suspension of the test isolate which was adjusted to 0.5 McFarland's standard was inoculated by using sterile cotton swab on the surface of a Mueller Hinton Agar plate. The ceftazidime (30 µg) and ceftazidime-clavulanic acid (30 µg/ 10 µg) disks were placed 20 mm apart. After incubating overnight at 37°C, 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 ESBL production.

Data Analysis

Data were analysed by using Microsoft Excel.

Results

In this study we were included total 96 cases who had infection of Gram-negative bacilli.53.2% were male & rest were female. Prevalence of age group was 31.5% in 21-40 age groups. We found that 30 samples were received from General Medicine followed by surgery (25%), OBG (21.8%), Pediatrics (15.6%) & Orthopedics (6.25%). We had received 50% urine sample, 15.6% stool, 9.3% pus, fluid &tissue. We observed that the isolates which have found in different samples were Escherichia coli 50% followed by Klebsiella pneumonia 40.6%, then others. This study also observed antibiotic susceptibility test in which most prevalent susceptible & resistant antibiotic was Amoxcycillin –Clavulanate&Nalidixic acid respectively. This result of this study revealed those 69.7% organisms which were ESBL producers & 30.2% organisms which were non ESBL producers.

Table 1: Distribution of cases according to Gender

Gender	No. of cases	Percentage
Male	51	53.2
Female	45	46.8
Total	96	100

Table 2: Distribution of cases according to Age

Age	No. of cases	Percentage
<20	15	15.6
21-40	30	31.5
41-60	24	25
>60	27	28.1
Total	96	100

Table 3: Distribution of cases according to Department

Department	No. of cases	Percentage
OBG	21	21.8
General Medicine	30	31.2
Surgery	24	25
Pediatrics	15	15.6
Orthopaedics	6	6.25
Total	96	100

Table 4: Distribution of cases according to Sample

Sample	No. of cases	Percentage
Urine	48	50
Tissue	9	9.3
Stool	15	15.6
Pus	9	9.3
Sputum	6	6.2
Fluid	9	9.3
Total	96	100

Table 5: Distribution of cases according to Isolates

Organisms	No. of cases	Percentage
Escherichia coli	48	50
Enterobacterspp	3	3.1
Proteus spp	2	2.1
Shigellasonni	1	1.1
Acenetobacter spp.	3	3.1
Klebseilla pneumonia	39	40.6
Total	96	100

Table 6: Distribution of cases according to Antibiotic susceptibility test

Antibiotics	Susceptible	Resistant
Amikacin	84	12
Ampicillin	9	87
Amoxcycillin –Clavulanate	90	6
Ampicillin-sulbactam	42	54

Aztreonam	30	66
Azithromycin	30	66
Cefepime	24	72
Cefuroxime	24	72
Ceftriaxone	18	78
Cefotaxime	18	78
Chloramphenicol	63	33
Nalidixic Acid	15	81
Ofloxacin	36	60
Piperacillin	57	39
Piperacillin-Tazobactam	68	28

Table 7: Distribution of cases according to ESBL Producers

ESBL Producers	No. of cases	Percentage
+	67	69.7
-	29	30.2
Total	96	100

Discussion

The resistance to extended spectrum cephalosporins is mostly mediated by the production of ESBLs. It has been reported in US that a number of nosocomial outbreaks which were caused by ESBL-producing organisms.[12-14]Though, most of the outbreaks were limited to high-risk patientcare areas such as ICUs etc. The first report of an outbreak in nursing homes found in the literature in the year 1999.[15]Thus, the threat of these resistant organisms is not limited to intensive care units only.An Indian study revealed that uropathogens such as Klebsiella pneumoniae, Escherichia coli, Enterobacter, Proteus and Citrobacter spp., had 26.6% of the isolates which were ESBL producers.[16] Another study from Nagpur found that 48.3% of their cefotaxime resistant gram-negative bacilli were ESBL producers.[16]

It is also reported that ESBL production was 41% in E. coli and 40% in K. pneumoniae.[17] Similarly Mathur et al, showed 62% of the E. coli and 73% of the K. pneumoniae isolates were reported to be ESBL producers.[18]

This study also revealed that 50% of the E. coli and that 39 % of the K. pneumoniae isolates were ESBL producers. Though E. coli were more often reported as ESBL producers in other studies. Our study also observed that ESBL production was more common among the E. coli isolates in comparison to the K. pneumoniae isolates.

ESBL production is less than Enterobacteriaceae in Pseudomonas spp. The reason could be their resistance is mediated by various other mechanisms such as lack of drug penetration due to mutations in the porins and the loss of certain outer membrane proteins and efflux pumps.[19]

This study found that ESBL production among E. coli and K. pneumoniae isolates was more often detected by the combination disk method than the double disk approximation test.

Though results could not document any significant differences between the ESBL detection rates of the two methods in Pseudomonas aeruginosa, the CLSI recommends the double disk approximation method for testing ESBL production among the Pseudomonas aeruginosa isolates.

The reason behind the Our failure to detect the better performance of the double disk approximation test could be due to the relatively small number of isolates which were tested in our study.

Our study observed that mostly isolates were susceptible to imipenem and piperacillin-tazobactam. Similarly, findings were found by a study from Coimbatore.[20] In both the studies, amikacin also showed good activity against gram-negative bacteria. We studied the occurrence of multi-drug resistance among the E. coli and K. pneumoniae isolates and found that co-resistance to amoxicillin-clavulanate, gentamicin and ciprofloxacin was very common.

ESBL producing organisms is the commonest nosocomial pathogens. It is important to detect and treat them as early as possible. Since ESBL production is very common among the nosocomial pathogens, early detection will surely help in controlling hospital infections. Enterobacteriaceae are the common isolates in most of the laboratories. Majority of these isolates are multi-drug resistant. To make a control of these multidrug resistant organisms is a therapeutic challenge. This complexity is enhanced further by the co-existence of the resistance to β-lactams, aminoglycosides and fluoroquinolones, as observed in the present study. Of all the available antimicrobial agents, carbapenems are the most active and reliable treatment options for infections which are caused by the ESBL producing isolates.Though, the misuse of carbapenems may lead to resistance in gram-negative organisms. The routine detection of ESBLs by conventional methods should be carried out in every lab where molecular methods cannot be performed, as genotyping is not more informative for the treatment.

Conclusion

It can be concluded that the ESBL-producing organisms are a breed of multidrug-resistant pathogens that are increasing quickly and becoming a major problem in the area of infectious diseases. It is important to report ESBL production along with the routine sensitivity reporting. It will help the clinicians in prescribing proper antibiotics.

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