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PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY OF URINARY TRACT INFECTION CASES AND FINDING OF ESBL AND AMPC β-LACTAMASES PRODUCING ESCHERICHIA COLI AND KLEBSIELLA PNEUMONIAE IN A TERTIARY CARE HOSPITAL MIZORAM

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ABSTRACT

Introduction: Resistance to broad spectrum -lactams mediated by extended spectrum -lactamases (ESBL) and AmpC -lactamases enzymes is a growing threat worldwide. **Aim:** The aim of the study was to detect the prevalence and antimicrobial susceptibility of ESBL and AmpC -lactamase producing Escherichia coli and Klebsiellapneumoniae isolated from Urinary Tract infection **Materials and Methods:** A total of 288 isolates comprising of 180 Escherichia coli and 108 Klebsiellapneumoniaeisolated from various clinical samples were included. ESBL was detected by Phenotypic Confirmatory Disc Diffusion Test (PCDDT) and Double Disk Synergy Test (DDST). AmpC detection was done by AmpC disk test. **Results:** Out of 180 Escherichia coli, and 108 Klebsiellapneumoniaeisolates 91(50.5%) and 63(58.3%) were confirmed to be ESBL producers by PCDDT and 81(45%) and 57(52.7%) by DDST respectively. AmpC was detected in 35(19.4%) of Escherichia coli and 33(30.5%) of Klebsiellapneumoniae isolates. Co-production of ESBL and AmpC was detected in 6(3.3%) Escherichia coli and 11(10.18%) of Klebsiellapneumonia isolates. Majority of ESBL producers were from blood in both organisms. Multi drug resistance (MDR) was seen in 79.1% of ESBLEscherichiacoli and 63.5% of ESBLKlebsiellapneumoniae isolates. MDR was seen in 28(96.5%) of AmpC producing Escherichia coli and all AmpC producing Klebsiellapneumoniae isolates. **Conclusion:** It is essential to report ESBL and AmpC beta lactamase production along with routine susceptibility which will aid the clinicians in prescribing antibiotics.Strict adherence to the hospital antibiotic policy and good infection control practices would go a long way in curtailing the menace of drug resistance.

KEYWORDS :

INTRODUCTION

The rapid global dissemination of Enterobacteriaceae harboring plasmid borne extended- spectrum β-lactamases (ESBL) and plasmid mediated AmpC β-lactamases represents a significant clinical threat. [1, 2]The predominant mechanism for resistance to β lactam antibiotics in gram negative bacteria is by synthesis of β-lactamases. Among the β-lactamases the production of ESBLs and AmpC β-lactamases are the most common[3]

ESBLs are plasmid-mediated β-lactamases that are capable of efficiently hydrolyzing penicillin, narrow and broad spectrum cephalosporins and monobactams (aztreonam), but they do not hydrolyze cephamycin or carbapenems (imipenem, meropenem). β-Lactamase, inhibitors such as clavulanic acid, sulbactam and tazobactam generally inhibit ESBL producing strains. They have evolved from genes of TEM-1, TEM- 2 or SHV- 1 by mutation that alter the amino acid configuration around the active site of these β-lactamases rendering them susceptible to hydrolysis by these enzymes. There are also new families of ESBLs, including the CTX-M and OXA-type enzymes as well as novel unrelated β-lactamases. ESBL producing isolates are most commonly found in Klebsiella pneumoniae (K. pneumoniae) and Escherichia coli. (E. coli). [1]

AmpC β-lactamases are primarily chromosomal and plasmid-mediated and are resistant to β-lactamase inhibitors such as clavulanic acid but can hydrolyze cephamycin. Plasmid mediated AmpC β-lactamases (PMABLs) have evolved by the movement of chromosomal genes on to plasmids and are found in E. coli, K. pneumoniae, Salmonella spp, Proteus mirabilis, Citrobacter freundii, Enterobacter aerogenes which confer resistance similar to their chromosomal counterparts. Carbapenems are one of the antibiotics of last resort for many bacterial infections such as E. coli and K. pneumoniae producing AmpC and ESBL. [4]

These organisms are responsible for a variety of infections like urinary tract infections, septicaemia, hospital acquired pneumonia, intra-abdominal abscess, brain abscess and device related infections and are typically associated with multidrug resistance. Treatment failures after instituting β-lactam antibiotic therapy for infections caused by ESBL producing gram negative bacilli have been reported. [5]It has been demonstrated that ESBL and AmpC production by infecting organisms adversely affects the clinical outcome. Distinguishing between the AmpC and the ESBL producing organisms has epidemiological significance and it may have a therapeutic importance as well. [6] Moreover, these strains are no longer confined to the hospital environment, but of late are being isolated from the community at increasing frequencies.[7,8]Therefore, it is necessary to know their prevalence so as to enable the clinician to select appropriate antibiotic regimen at the earliest. The routine susceptibility tests performed by clinical laboratories fail to detect these strains making treatment options difficult. With this background the current study was conducted to determine the prevalence of ESBL and AmpC β-lactamases in E. coli and K. pneumoniae which were isolated from various clinical samples from both in-patients and out-patients who attended a tertiary care hospital in north-west India.

MATERIAL AND METHODS

A total of 288 consecutive, non-repetitive isolates comprising of 108 K. pneumoniae and 180 E.coli were recovered from different clinical samples between January 2018 and May 2022. (Table 1) The isolates were identified by standard biochemical methods.

Antimicrobial susceptibility testing

Antibiotic susceptibility of the isolates was done by Kirby

Bauer disc diffusion method following the Clinical and Laboratory Standards Institute (CLSI) guidelines[9] using commercially available discs (HiMedia, Mumbai, India). Cefepime(30μg), Ceftriaxone(30μg), Ceftazidime(30μg), Cefoxitin(30μg), Amikacin(30μg), Gentamicin(10 μg), Cefuroxime(30μg), Ciprofloxacin(5μg), Doxycycline(30μg), Meropenem(10μg), Norfloxacin(10μg), Nitrofurantoin(300μg) and Cefoperazone/ Sulbactam(75/10μg),

Screening for ESBLs and AmpC β-lactamases

As per CLSI recommendation, isolates showing resistance (zone ≤ 22mm for ceftazidime and ≤ 25mm for ceftriaxone) by disc diffusion method were considered potential ESBL producers and further preceded for confirmation.[9]

Isolates showing resistance to cefoxitin (inhibition zone < 18mm) by disc diffusion method were considered potential AmpC producers and further tested for presence of AmpC β-lactamase enzyme by AmpC disk test.

Detection of ESBLs and AmpC β lactamases

The Phenotypic Confirmatory Disc Diffusion Test (PCDDT)

All strains that were potential ESBL producers were subjected to confirmation using the PCDDT as recommended by CLSI. [9] A disc of cefotaxime (30μg) and ceftazidime (30μg) alone and a disc of cefotaxime/clavulanic acid (30 μg/10 μg) and ceftazidime/clavulanic acid (30 μg/10 μg) were placed independently 30 mm apart center to center on a lawn culture of 0.5 McFarland turbidity of the test isolate on Muller Hinton Agar (MHA) plate and incubated for 18-24 hours at 35°C. A ≥ 5 mm increase in zone diameter for either antimicrobial tested in combination with clavulanic acid versus its zone when tested alone confirmed ESBL production. (Figure 1)

Double Disc Synergy Test

A 0.5 McFarland suspension of the test isolate was swabbed on MHA plate and 30 μg antibiotic discs of ceftazidime, ceftriaxone and cefotaxime were placed on the plate 15 mm (center to center) from the amoxycillin/clavulanate (20μg/10 μg) (augmentin) disc and incubated at 37°C for 18-24 hrs.. Clear extension of the edge of the inhibition zone of any of these cephalosporin discs towards the augmentin disc was interpreted as positive for ESBL production. (Figure 2)

AmpC disk test

Lawn cultures of ATCC E. coli 25922 were prepared on MHA plate and a 30 μg cefoxitin disc was placed on the inoculated surface of the agar. A sterile plain disc moistened with sterile saline (20μL) and inoculated with several colonies of the test organism was placed besides the cefoxitin disk almost touching it. After overnight incubation at 35°C, the plates were examined for either an indentation or a flattening of the zone of inhibition, indicating enzymatic inactivation of cefoxitin (positive result), or the absence of a distortion, indicating a negative result. [10](Figure 3)

Quality Control: Every batch of media prepared was checked for sterility for 24 hours. CLSI reference strains of ESBL positive K. pneumoniae ATCC 700603 and ESBL negative E.coli ATCC 25922 were included in the study.

Statistical Analysis - Chi square test was applied for analysis of categorical data. All statistical calculations were done by using MedCalc Statistical Software, version 14.12.0(MedCalc Software bvba, MedCalc Ostend, Belgium). P <0.05 was taken as significant for interpretation.

RESULTS

Out of 288 non-repetitive isolates that were included in the study, 180 were E.coli and 108 were K. pneumoniae. The number of ESBL and AmpC β lactamase producers detected by screening test was 250 and 145 respectively.

Out of 250 screen positive isolates, 154(61.6%) were confirmed as ESBL producers. DDST detected 138(55.2%) ESBL producers while all 154 were detected by PCDDT. (Table 2)Ten strains of E. coli and six strains of K. pneumoniae were not detected as ESBL producers by DDST. ESBL production was seen in 91/180(50.5%) of E. coli and 63/108(58.3%) of K. pneumoniae. .Distribution of ESBL producers from various Urinary Tract Infection Cases. Maximum number of ESBL producers was isolated from blood accounting for 80% and 82.14% of E. coli and K. pneumoniae respectively.

Detection of AmpC β-lactamases

Out of 145 screen positive isolates, 68/145(46.89%) were confirmed as AmpC β-lactamase producers by AmpC disk test. AmpC β-lactamase production was seen in 35/180(19.4%) of E. coli and 33/108(30.5%) of K. pneumoniae isolates. (Table 1)

Coproduction of ESBL and AmpC β-lactamases

Among the 154 ESBL positive isolates, 17 also tested positive for AmpC β-lactamase. Co-production of ESBL and AmpC was observed in 17/288(5.9%) isolates. It was higher in K. pneumoniae (10.2%) than in E. coli (3.3%).

Antimicrobial Sensitivity pattern

A wide spectrum of antimicrobial resistance pattern to various antimicrobial agents was detected in ESBL positive E. coli and in K. pneumoniae. (Figure 4, 5) Both E. coli and K. pneumoniae strains showed a high degree of resistance to 4th generation cephalosporin cefepime accounting for 91.7% and 94% respectively. Least resistance was seen with meropenem in E coli isolates accounting for 1.2%. However among K. pneumoniae isolates the resistance was 14.3%. Among the urinary E. coli isolates a high resistance of 89.2% was seen with norfloxacin.

A high multidrug resistance (MDR) of 79.1% and 65.2% respectively was observed among ESBL producing strains of E. coli and K. pneumoniae. MDR was significantly higher in ESBL E. coli strains than non-ESBL strains. (P 0.036) Multidrug resistance was seen in 28/29(96.5%) of AmpC producing E. coli and 22/22(100%) of K. pneumoniae isolates.

DISCUSSION

With the spread of ESBL and AmpC producing strains all over the world, it is necessary to know the prevalence of these strains in hospitals. The overall prevalence of ESBL in the present study was 154/288(53.5%). ESBL was detected in 58.3% of K. pneumoniae and 50.5% of E. coli strains.

The prevalence of ESBL among clinical isolates varies greatly worldwide and in geographical areas and is rapidly changing over time. Reports of ESBL detection among clinical isolates of E. coli range between 20%and 80.6% and those among K. pneumoniae ranges between 20% and 86.7%. [11, 12, 13] Variation in the detection rates within and across the states could be due to the differences in the methodology used in these studies. Also it may be due to different patterns of antibiotic use & differences in the selection of organisms for the study. The PCDDT which is recommended by CLSI for phenotypic confirmation of ESBL among E. coli and K. pneumoniae was found to be more sensitive than DDST test. PCDDT detected 154/288(53.5%) of all the ESBL producers while DDST detected only 138/288(47.9%). The DDST lacks sensitivity because of the problem of optimal disc space and the proper storage of clavulanic acid containing discs. Similar observation has been reported by other studies. [14,15]

Techniques to identify AmpC β lactamase producing isolates are available but are still evolving & are not yet optimized for the clinical laboratory.[4] Due to lack of reliable detection methods, their exact prevalence is unknown. Various studies have reported prevalence of AmpC between 2.2% 37.5%. [16, 17, 18] The overall prevalence of AmpC β lactamases in the present

study was 23.6%. Among E. coli it was 19.4% while it was 30.5% among K. pneumoniae isolates. High level of AmpC production is typically associated with in vitro resistance to 3GC's and cephamycins leading to clinical treatment failures with broad spectrum cephalosporins.[19,20]

Co-production of both ESBL and AmpC was observed in (17/288) 5.9% of isolates. It has been stated that AmpC β-lactamases when present along with ESBL can mask the phenotype of the latter. [4] Thus the coexistence of AmpC and ESBL in the same strain may give false negative results for detection of ESBL. When ESBL production is suspected, but the confirmatory test is negative, the strain should be screened for presence of AmpC β-lactamases.

Due to widespread use of antibiotics, MDR E. coli and K. pneumoniae strains isolated are increasing that poses severe challenges to public health. In the present study MDR was seen in 79.1% of E. coli and 63.5% of K. pneumoniae isolates of ESBL producing strains. Resistance of ESBL producing isolates to 3GCs among E.coli was found to coexist with resistance to two or more antibiotics such as amikacin (P 0.03), gentamicin (P 0.01),cefepime(P 0.00006), cefoxitin(P 0.0002) and doxycycline(P 0.02).while in ESBL K. pneumoniae resistance was seen with doxycycline (P 0.02) and cefoperazone/sulbactam (P 0.04). This coexistence of multidrug resistance has been reported earlier. [21, 22] Mechanisms of co-resistance are not clear, but one possible mechanism is the co-transmission of ESBL and resistance to other antimicrobials within the same conjugative plasmids. The highest drug resistance was observed for cefepime accounting for 90% in E. coli and 93.4% in K. pneumoniae isolates. Similar high resistance has been observed in other studies in India. [23,24] Resistance to cefepime could be attributed to the high prevalence of CTX-M type ESBLs in these isolates, some of which are capable of hydrolyzing cefepime. [25] Very high drug resistance of 85.9% was seen for norfloxacin in urinary isolates of E. coli. Imipenem was found to be the most effective drug against ESBL E. coli showing a susceptibility of 98.9 % whereas 14.28% of ESBL K. pneumoniae isolates were resistant to imipenem which could be because of carriage of carbapenemase genes.

Multidrug resistance was observed in 28(96.5%) of AmpC producing E. coli and 28/28(100%) of K. pneumoniae isolates. Similar findings have been reported in other studies[26, 27] This emphasizes the need for detecting AmpC β-lactamase in MDR isolates so as to avoid therapeutic failures & nosocomial outbreaks.

Table1. Results of screening and confirmatory tests for ESBL and AmpC production

Name of microorganism	Total No. of isolates	No. of isolates resistant to 3GCs in screening test	No. of isolates positive by PCDDT*	No. of isolates positive by DDS†	No.of isolates resistant to Cefoxitin	No. of isolates positive by Amp disk test
Escherichia coli	180	148	91	81	81	35
Klebsiella pneumoniae	108	102	63	57	64	33
Total	**288**	**250**	**154**	**138**	**145**	**68**

*PCDDT= phenotypic confirmatory disc diffusion test; †DDST= double disc synergy test

The increased ESBL and AmpC producing isolates are indicative of the ominous trend of more and more isolates acquiring resistance mechanisms thus rendering the antimicrobial armamrium ineffective. The high prevalence of these organisms emphasizes the need for early detection of these -lactamases which can help in instituting appropriate antimicrobial therapy & in avoiding the development and dissemination of these multidrug resistant strains. Every health care institution must develop its own antimicrobial stewardship program which is based on the local epidemiological data & international guidelines, to optimize the antimicrobial use among the hospitalized patients and to improve patient outcomes. [28] Preventive measures like a continuous surveillance & strict implementation of infection control practices can go a long way in containing the menace of drug resistance in health care settings.

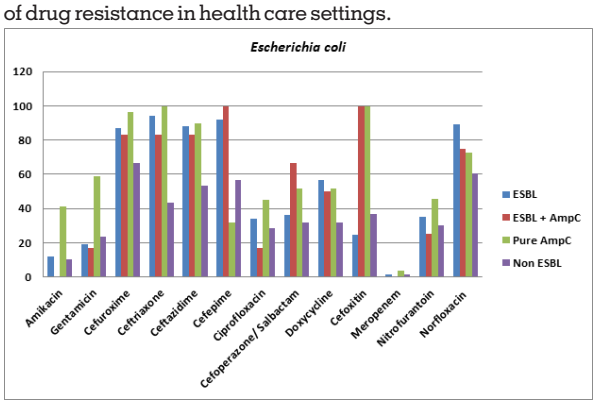

Figure 1: Antimicrobial resistance patterns of clinical isolates of β lactamase and non β lactamase producing Escherichia coli.

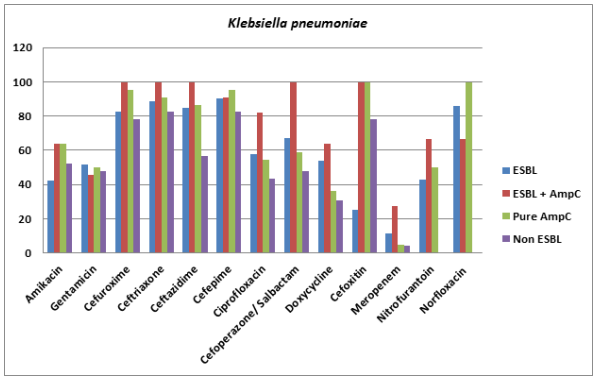

Figure2: Antimicrobial resistance patterns of clinical isolates of β-lactamase and non-β lactamase producing Klebsiella pneumoniae

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