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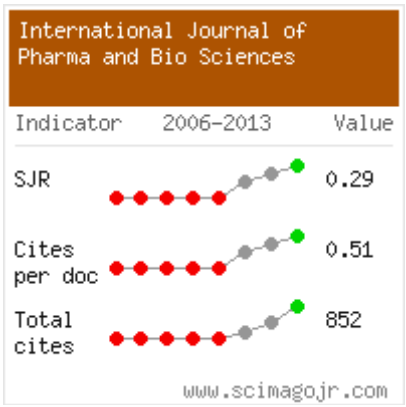

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THE PREVALENCE OF CO-PRODUCTION OF ESBL, AMPC AND METALLO-β-LACTAMASES IN *KLEBSIELLA PNEUMONIAE* ISOLATES, IN A TERTIARY LEVEL HEALTH CARE PROVIDING FACILITY IN HARYANA

MEENU GARG*, RAMA SIKKA, ANTARIKSH DEEP AND UMA CHAUDHARY

Department of Microbiology, Pt. B. D. Sharma PGIMS, Rohtak (Haryana), India.

ABSTRACT

Klebsiella pneumoniae is a pathogen primarily capable of causing urinary tract infections and pneumonia in otherwise healthy people. Most of the strains are frequently resistant to numerous antibiotics, responsible for conferring multidrug resistance. This study was conducted to determine the prevalence of extended spectrum β-lactamases (ESBL), AmpC β-lactamases and metallo β-lactamases (MBL) and their coproduction by phenotypic methods. Out of 100 *K. pneumoniae* isolates, 41%, 45% and 36% were found to be ESBL producers, AmpC producers and MBL producers respectively. Co-production of all three β – lactamases i.e. ESBL, AmpC and MBL was observed in 3 % of the isolates. The high prevalence of β- lactamases emphasises the need for an early detection of the β-lactamase producing organisms, which can help in initiating an appropriate antimicrobial therapy and in avoiding the development and the dissemination of multidrug resistant strains.

KEY WORDS: ***Klebsiella pneumoniae,*** **ESBL, MBL and AmpC β- lactamases**

*Corresponding author

MEENU GARG
Department of Microbiology, Pt. B. D. Sharma PGIMS, Rohtak(Haryana), India.

INTRODUCTION

Klebsiella pneumoniae is a pathogen primarily capable of causing urinary tract infections (UTIs), liver abscess, and pneumonia in otherwise healthy people. However, most infections caused by *K. pneumoniae* are nosocomial in origin and/or seen in those individuals, who are immunocompromised by various underlying conditions. In addition to pneumonia and urinary tract infections, nosocomial infections caused by *K. pneumoniae* include wound infections, infections of intravascular and other invasive devices, biliary tract infections, peritonitis and meningitis[1]. *K. pneumoniae* is second only to *Escherichia coli* as a cause of bacteremia resulting from UTI and of gram-negative bacteremia. The strains responsible for nosocomial infections are frequently resistant to numerous antibiotics as a result of the acquisition of plasmids, responsible for conferring multidrug-resistance[2]. For example, *K. pneumoniae* is one of the most common organisms to carry plasmids encoding extended-spectrum β-lactamases and carbapenemases and bacteremia with such strains is associated with higher rates of treatment failure and death[3]. The production of antibiotic-inactivating enzymes is one of the best known mechanisms of resistance and is typified by the β-lactamases[4]. β-lactamases are the hydrolytic enzymes which cleave the β lactam ring and are the primary mechanism of conferring bacterial resistance to β lactam antibiotics, such as penicillins and cephalosporins[5]. ESBLs are molecular class A or D β-lactamases which are able to hydrolyse oxyimino cephalosporins, have an active site serine with molecular mass of approximately 29000 Da and are generally inhibited by beta lactamase inhibitors such as clavulanic acid, sulbactam or tazobactam[6]. AmpC beta lactamases are cephalosporinases that confer resistance to a wide range of beta lactam drugs, thereby causing a serious therapeutic problem[7]. MBLs, like all β-lactamases, can be divided into those that are normally chromosomally mediated and those that are encoded by transferable genes. Enzymes possess the key zinc coordinating residues of three histidines and one cysteine and accommodates the transferable MBLs IMP, VIM, GIM and SPM-1[8] .The co-existence of different classes of β-lactamases in a single bacterial isolate often poses diagnostic and treatment challenges and often limits the treatment options to fourth-generation cephalosporins or carbapenems. Carbapenamase producing strains leave few (polymyxins) or no options, for managing such infections. The present study was conducted to determine the prevalence of extended spectrum β-lactamases (ESBL), AmpC β-lactamases and metallo β-lactamases (MBL) and their coproduction by phenotypic methods in these isolates.

MATERIALS AND METHODS

The study was conducted in the Department of Microbiology, Pt. B.D. Sharma PGIMS, Rohtak, over a period of one year. In all, a hundred isolates of *K. pneumoniae* were obtained from various clinical samples like sputum, blood, pus, urine and high vaginal swabs (HVS) and these isolates were identified by standard microbiological procedures[9]. Antimicrobial susceptibility testing of these isolates was done by Kirby Bauer disc diffusion method following Clinical and Laboratory Standard Institute (CLSI) guidelines. Discs of the following antimicrobial agents were used: ampicillin (10µg), gentamicin (10µg), amikacin (30µg), amoxicillin/clavulanic acid (20µg/10µg), ampicillin/sulbactam (10µg/10µg), piperacillin/tazobactam (100µg/10µg), ticarcillin/clavulanic acid (75µg/10µg), cefuroxime (30µg), cefepime (30µg), cefotaxime (30µg), ceftriaxone (30µg), ciprofloxacin (5µg), levofloxacin (5µg), ertapenem (10µg), imipenem (10µg), meropenem (10µg), trimethoprim-sulfamethoxazole (1.25µg/23.75µg), aztreonam (30µg), ceftazidime (30µg). In case of urinary isolates, ofloxacin (5µg), norfloxacin (10µg) and nitrofurantoin (300µg) were added.

Detection of β-lactamases

Isolates with reduced susceptibility to third generation cephalosporins were tested for ESBL production as per CLSI guidelines[10]. Isolates showing reduced susceptibility to cefoxitin were tested for elaboration of AmpC[11]. Isolates showing reduced susceptibility to imipenem were tested for MBL production[12].

Detection of ESBL

ESBL production was determined by disc diffusion test (by using ceftazidime/ ceftazidime-clavulanic acid and cefotaxime/ cefotaxime-clavulanic acid discs) as per the CLSI guidelines[10].

Detection of AmpC β- lactamases

AmpC β- lactamase production was determined by AmpC disc test. A lawn culture of *E. coli* ATCC 25922 was prepared on MHA plate. Several colonies of test organism were inoculated on sterile discs (6mm) moistened with sterile saline (20µl). The inoculated disc was placed beside a cefoxitin disc on agar plate. The plates were incubated overnight at 35^0C. A positive test appeared as flattening or indentation of the cefoxitin inhibition zone in the vicinity of the test disc. A negative test was interpreted if an undistorted zone was obtained[11].

Detection of MBL

MBL production was determined by combined disc method using imipenem and ceftazidime discs. Test organism was inoculated on MHA plate following CLSI guidelines. A 0.5M EDTA solution was prepared by dissolving 18.61gm of disodium EDTA.$2H_2O$ in 100ml of distilled water and adjusting it to pH 8 using NaOH. Two 10µg imipenem discs and two 30µg ceftazidime discs were placed on the surface of agar plate and EDTA solution was added to one imipenem and one ceftazidime disc to obtain a desired concentration of 750µg. The zones of inhibition of imipenem, ceftazidime and imipenem-EDTA and ceftazidime-EDTA discs were compared after 16-18 hours of incubation at 35^0C. A positive test was indicated by zone enhancement with EDTA impregnated imipenem and ceftazidime discs. The enhancement of zone size by ≥5mm for ceftazidime EDTA disc as compared to ceftazidime alone and a zone size enhancement by ≥7mm for imipenem EDTA disc as compared to imipenem alone was taken as positive criteria for MBL production[12].

RESULTS

Out of 100 *K. pneumoniae* isolates, 94% isolates showed decreased sensitivity to one or the other third generation cephalosporins**.** Out of these 94 isolates, 41 (43.6%) were found to be ESBL producers. Out of 100 *K. pneumoniae* isolates, 93% were cefoxitin resistant and 36 (38.7%) were found to be AmpC producers. In all, a total of 62% *K. pneumoniae* isolates were resistant to imipenem i.e. screen positive. Out of these 62 screen positive isolates, 45 (72.5%) were MBL producers (Table 1).

Table 1
Prevalence of various β- lactamases

Type of β-lactamase	No.	%
ESBL	41	41
AmpC	36	36
MBL	45	45

Co-production of ESBL and MBL production was seen in 12 (12%) isolates. Co-production of ESBL and AmpC was seen in 9 (9%) isolates. MBL production along with AmpC was observed in 20 (20%) isolates. Co-production of all three β – lactamases i.e. ESBL, AmpC and MBL was observed in 3 (3%) isolates (Table 2).

Table 2
Coproduction of various β-lactamases in K. pneumoniae isolates

Type of β lactamase	No.	%
ESBL + MBL	12	12
ESBL + AmpC	9	9
MBL + AmpC	20	20
ESBL + MBL + AmpC	3	3

DISCUSSION

Traditionally, out of members of family Enterobacteriaceae, *K. pneumoniae*, makes up the majority of the infections caused as a result of elaboration of ESBLs in hospital settings. Infections caused by such Enterobacteriaceae are increasingly being reported from various parts of the globe including India. The various β-lactamases have their origin either in the chromosomal genes or are transferred through plasmids and/or transposons. In the present study, ESBL production was observed in 41% of *K. pneumoniae* isolates. Similar findings were reported by Tumbarello et al, who in their study reported ESBL production in 32.6% of *K. pneumoniae* isolates by double disc synergy test and E test[13]. Paterson et al, in their study reported ESBL production in 18% of *K. pneumoniae* isolates[14]. However, a study conducted by Harada et al, revealed ESBL production in 2.8% of *K. pneumoniae* isolates[15]. Different prevalence rates of ESBL production in *K. pneumoniae* isolates can be attributed to the geographical divide.AmpC β- lactamases, in the present study were detected in 36% of *K. pneumoniae* isolates. Manoharan et al reported AmpC β- lactamase production in 36.5% of *K. pneumoniae* isolates[16]. Hemlatha et al detected AmpC β – lactamase production in 33% of *K. pneumoniae* isolates by boronic acid inhibitor based method[17]. However, Paul et al reported AmpC β - lactamase production in 13.9% of *K. pneumoniae* isolates[18]. Mohamuda et al reported much higher AmpC β-lactamase production in 63.3% of *K. pneumoniae* isolates than that reported in present study[19].MBLs were detected in 45% of *K. pneumoniae* isolates, in the present study. In a study conducted by Chika et al 15.4% of the *K. pneumoniae* strains were found to be MBL producers by disc potentiation test[20]. Khajuria et al reported much higher MBL production with 100% of *K. pneumoniae* isolates being MBL producers[21]. In the present study, the coproduction of ESBL and MBL, and that of ESBL and AmpC enzymes was detected in 12% and 9% *K. pneumoniae* isolates, respectively. Similar prevalence rates have been reported by Oberoi et al, who in their study found the prevalence rates for coproduction of ESBL and AmpC enzymes to be 9.09%[22] . In a study by Hemlatha et al, AmpC β-lactamases were detected in 47.3% isolates, four-fifths of which occurred in combination with ESBLs[17]. In a study conducted by Pai et al, the coproduction of ESBL and AmpC enzymes was detected in 11.1% *K. pneumoniae* isolates.[24] In the present study, the coproduction of MBL and AmpC enzymes was detected in 20% of *K. pneumoniae* isolates. Oberoi et al in their study reported MBL production in 22.7% of the isolates and AmpC production was observed in 2.2% of the isolates. Coproduction of MBL and AmpC was seen in 2.2% of strains which was much lower than the present study[22]. In the present study, the coproduction of ESBL, AmpC β- lactamases and MBL was observed in 3 (3%) *K. pneumoniae* isolates. In a study conducted by Oberoi et al, the coproduction of ESBL, AmpC β- lactamases and MBL in gram negative isolates from a tertiary care hospital in Punjab was found to be 19.04% which was much higher than in our study[22] . Rawat et al conducted a study to determine production of various β- lactamases and coproduction of ESBL, AmpC β- lactamases and MBL was not observed in any of the *K. pneumoniae* isolates[23].

CONCLUSION

The high prevalence of β- lactamases emphasises the need for an early detection of the β-lactamase producing organisms by simple screening methods. The co-production of various β-lactamases, which is quite often plasmid/transposon mediated, may also simultaneously confer resistance to fluoroquinolones and aminoglycosides, thus severely limiting treatment options. Moreover, routine screening for the various β-lactamases can avert releasing of erroneous laboratory reports with pseudo susceptibility to cephalosporins, which can help in initiating an appropriate antimicrobial therapy and in avoiding the development and the dissemination of multidrug resistant strains.

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