Journal of Research in Pharmacy Practice

Original Article

Antimicrobial susceptibility pattern of extended-spectrum beta-lactamase producing *Klebsiella pneumoniae* clinical isolates in an Indian tertiary hospital

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Received: May 2015
Accepted: June 2015

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ABSTRACT

Objective: There is an increased prevalence of extended-spectrum beta-lactamase producing *Klebsiella pneumoniae* (ESBL-KP) worldwide including India, which is a major concern for the clinicians, especially in intensive care units and pediatric patients. This study aims to determine the prevalence of ESBL-KP and antimicrobial sensitivity profile to plan a proper hospital infection control program to prevent the spread of resistant strains.

Methods: KP isolates obtained from various clinical samples were evaluated to detect the production of ESBL by phenotypic methods. Antimicrobial susceptibility profile was also determined of all the isolates.

Findings: Of 223 nonduplicate isolates of *K. pneumoniae*, 114 (51.1%) were ESBL producer and antimicrobial susceptibility profile showed the isolates were uniformly sensitive to imipenem and highly susceptible to beta-lactamase inhibitor combination drugs (67–81%) and aminoglycosides (62–76%), but less susceptible to third generation cephalosporins (14–24%) and non-β-lactam antibiotics such as nitrofurantoin (57%), fluoroquinolones (29–57%), piperacillin (19–23%), and aztreonam (15–24%).

Conclusion: This study found that beta-lactamase inhibitor combinations are effective in treatment of such infections due to ESBL-KP thus these drugs should be a part of the empirical therapy and carbapenems should be used when the antimicrobial susceptibility tests report resistance against inhibitors combinations.

Keywords: Beta-lactamase inhibitor; extended-spectrum beta-lactamase; *Klebsiella pneumoniae*; susceptibility pattern

INTRODUCTION

Klebsiella pneumoniae (KP) is one of the leading causes of nosocomial infections seen worldwide, causing pneumonia, bloodstream infections, urinary tract infections, surgical site or wound infections and meningitis.[1] Increased rate of treatment failure and death associated with infections caused by KP is a major concern for the clinicians especially in intensive care units and pediatric patients.[2] This could be attributed to the unprecedented use of antibiotics in health care set up without proper antibiotic policy which has led to increased prevalence of infections caused by extended-spectrum beta-lactamase producing KP (ESBL-KP). In recent years, outbreaks of infection caused by multidrug-resistant ESBL-KP have been reported throughout the world.[3,4]

Access this article online

Website: www.jrpp.net

DOI: 10.4103/2279-042X.162363

How to cite this article: Singh AK, Jain S, Kumar D, Singh RP, Bhatt H. Antimicrobial susceptibility pattern of extended-spectrum beta- lactamase producing *Klebsiella pneumoniae* clinical isolates in an Indian tertiary hospital. J Res Pharm Pract 2015;4:153-9.

ESBL represent a major group of bacterial beta-lactamases that belong to Bush-Jacoby functional subgroup 2be and Ambler class A, that retain the ability to hydrolyzes penicillin and early cephalosporins such as cephaloridine and cephalothin and in addition hydrolyze one or more oxyimino-cephalosporins such as cefotaxime, ceftazidime, and aztreonam at a rate generally more than 10% that of benzylpenicillin. The subgroup has been derived by amino acid substitutions in the enzymes TEM-1, TEM-2 and SHV-1 of functional subgroup 2b and has broadened the spectrum of the substrate. The subgroup 2be is characteristically sensitive to clavulanic acid, and this feature is used in their detection in laboratories.[5]

The risk factors associated with infection due ESBL-KP are severe underlying illness, long-term treatment with multiple antibiotics, prolonged duration of hospital stay, surgical intervention, instrumentation and presence of indwelling intravenous catheters.[6] Mechanical ventilation and endotracheal intubation are the risk factors associated in infections seen in intensive care units.[7]

Knowledge of present scenario of prevalence and drug resistance helps in the development of antibiotic policy for infection caused by ESBL-KP. Therefore, This study was conducted to determine the prevalence of ESBL-KP and antimicrobial sensitivity profile to plan a proper hospital infection control program to prevent the spread of resistant strains.

METHODS

KP isolates obtained from samples (urine, blood, pus, wound swab, ear swab, sputum, stool, and aspirate) received in the bacteriology laboratory in the department of microbiology, from various inpatient and outpatient departments of Gold Field Institute of Medical Sciences and Research, Faridabad, Haryana, India, during July 2013 to June 2014 were included in the study. Ethical clearance was obtained from the institute and a detailed clinical history, and demographic data of patient was recorded in a preformed questionnaire.

Sample processing

Various samples were collected in their respective sterile container and immediately transported to the laboratory. Urine samples received in the sterile universal container were inoculated on cysteine lactose electrolyte deficient agar media. Blood samples received in brain heart infusion broth were incubated for 16–18 h at 37°C aerobically and then subculture was done on 5% blood agar and MacConkey agar media daily until 7 days before reporting it negative. Other specimens such as pus, wound swab, ear swab, sputum, stool, and aspirate were inoculated on 5% blood agar and MacConkey agar media. All inoculated media were incubated aerobically at 37°C for 16–18 h. Identification of growth as KP was done on the basis of its colonial morphology showing large, mucoid, convex, smooth, lactose fermenting and translucent colony; Gram-staining showing uniformly stained Gram-negative rods of 1–2 × 0.5–0.8 μm size, parallel or bulging sides and slightly pointed or rounded ends; nonsporing; nonmotile in hanging drop preparation; biochemical reactions showing oxidase test negative, catalase test positive, O/F (oxidation/fermentation) test showing glucose fermentation, motility and gas production; nitrate reduction test positive, indole test negative, methyl red test negative, Voges–Proskauer test positive, citrate utilized, urease test positive, lactose fermenter, triple sugar iron agar showing both butt and slant yellow with gas production, lysine decarboxylase test negative. Biochemical reactions were performed by inoculating the colony in a nutrient broth at 37°C for 2–3 h.

Antimicrobial susceptibility test of *Klebsiella pneumoniae*

The test was done by Kirby–Bauer disk diffusion method as recommended by Clinical and Laboratory Standards Institute (CLSI) 2013 guidelines.[8] Antibiotic discs and Mueller-Hinton agar (MHA) media obtained from HiMedia Laboratoriess Pvt. Ltd., India was used for antimicrobial susceptibility testing. Antibiotic discs used were ampicillin (10 μg), piperacillin (100 μg), cefoperazone (75 μg), cefoxitin (30 μg), ceftazidime (30 μg), cefotaxime (30 μg), ceftriaxone (30 μg), cefepime (30 μg), piperacillin-tazobactam (100/10 μg), amoxicillin/clavulanate (20/10 μg), cefoperazone/sulbactam (75/10 μg), ceftazidime/clavulanate (30/10 μg), aztreonam (30 μg), imipenem (10 μg), amikacin (30 μg), gentamicin (10 μg), ciprofloxacin (30 μg), ofloxacin (5 μg), norfloxacin (10 μg), nitrofurantoin (300 μg). The diameter of the zone of inhibitions was measured, and the organism was labeled as sensitive, resistant or intermediate [Table 1]. The quality control of antibiotic sensitivity was done using *Escherichia coli* ATCC 25922 and *E. coli* ATCC 35218 (for β-lactam/β-lactamase inhibitor combination).

Extended-spectrum beta-lactamase detection test

Phenotypic screening and confirmatory test of KP was done for ESBL production as per CLSI 2013 guidelines.[8]

Screening test

Screening test to determine ESBL production was done by using cefpodoxime, ceftazidime, aztreonam, cefotaxime and ceftriaxone antibiotic disc as per CLSI guidelines. Inoculum was prepared from the isolates

in nutrient broth with turbidity equivalent to 0.5 McFarland standards. Lawn culture of the inoculum was done on MHA media using a sterile cotton swab. Antibiotic discs were applied using a sterile forceps on the surface of media and incubated aerobically at 35°C for 18–24 h. Zones of inhibition for interpretation of results used for cefpodoxime, ceftazidime, aztreonam, cefotaxime and ceftriaxone to indicate potential ESBL producer were ≤17 mm, ≤22 mm, ≤27 mm, <27 mm, <25 mm respectively. Quality control strain used was *E. coli* ATCC 25922. Phenotypic confirmatory tests were done only if any strain was detected as ESBL producer by the screening method.

Confirmatory test

Phenotypic confirmation of ESBL production was done by two different methods; double-disk diffusion method and cephalosporin/clavulanate combination discs method.

a. In double-disk diffusion or double-disk synergy test MHA plate were first inoculated by lawn culture with the strain of KP to be tested. Antibiotic disc of ceftazidime, aztreonam, cefotaxime, and ceftriaxone 30 µg each was applied at an approximate distance of 30 mm distance from a centrally placed amoxicillin/clavulanate (20 µg/10 µg) antibiotic disc. The plates were then incubated aerobically at 37°C for 16–18 h. After incubation, zone of inhibition around these four antibiotic discs were examined for an extension of the edge toward the amoxicillin/clavulanate disc, indicating the production of ESBL
b. Cephalosporin/clavulanate combination discs test was done by using cefotaxime (30 µg) and ceftazidime discs (30 µg) with or without clavulanate (10 µg). The strain of KP to be tested was lawn cultured on MHA plate. Antibiotic discs were placed at an appropriate distance from each other and incubated aerobically at 37°C for 16–18 h. After incubation, the zone of inhibition around each disc was measured. A difference in the zone of inhibition of ≥ 5 mm between the cephalosporin discs and their clavulanate containing discs indicates the production of ESBL. Quality control strains used were KP ATCC 700603 and *E. coli* ATCC 25922.

Statistical analysis

Chi-square test was performed using Microsoft Office Excel 2010 for statistical analysis of the data. A $P < 0.05$ were considered statistically significant.

RESULTS

Among isolates obtained from various clinical samples, 223 nonduplicate isolates of KP which showed resistance to oxiimino-cephalosporins were included in this study. Distribution of isolates on the basis of the source is documented in Table 1. KP was obtained in highest number from urine followed by blood, pus, sputum, wound, stool, aspirates and least from ear swab. Among 223 isolates, 161 isolates were obtained from inpatients, and 62 isolates were obtained from outpatients samples.

Table 1: Distribution of *K. pneumoniae* and ESBL-KP based on the source of specimen

Specimen	Inpatients	Outpatients	*K. pneumoniae* (%)	ESBL-KP (%)
Urine	56	24	80 (36)	42/80 (53)
Blood	28	12	40 (18)	26/40 (65)
Pus	30	06	36 (16)	16/36 (44)
Sputum	18	06	24 (11)	14/24 (58)
Wound	12	08	20 (9)	08/20 (40)
Stool	06	04	10 (4.5)	04/10 (40)
Aspirate	09	00	09 (4)	04/09 (44)
Ear swab	02	02	04 (1.8)	00/04 (0)
Total	161	62	223	114

Data are presented as number (%) of isolates. ESBL-KP=Extended-spectrum beta-lactamase producing *K. pneumoniae*, *K. pneumoniae=Klebsiella pneumoniae*

Antimicrobial susceptibility of KP isolated from urine, pus and blood is documented in Table 2. Susceptibility to cephalosporin ranges between 39% and 48% which is quite low. Whereas, beta-lactamase inhibitor combination drugs, piperacillin/tazobactam, amoxicillin/clavulanic acid, cefoperazone/sulbactam, ceftazidime/clavulanate showed susceptibility of 72% to 92%. KP showed 100% susceptibility to imipenem. KP showed a better susceptibility to beta-lactamase inhibitor combination drugs as compared to nitrofurantoin, fluoroquinolones, piperacillin and aztreonam.

Of total isolates of KP 114 (51.1%) were ESBL-KP and 109 (48.9%) were non-ESBL-KP. Among ESBL-KP, maximum number was isolated from blood (65%), followed by sputum (58%) and urine (53%) [Table 1].

Of 161 isolates obtained from inpatients, 96 (59.6%) were ESBL-KP whilst 18 (29%) out of 62 from outpatients were ESBL-KP. ESBL-KP were significantly associated with the inpatients than outpatients as compared to non-ESBL-KP ($P < 0.001$) [Table 3].

Antimicrobial sensitivity pattern of ESBL-KP from urine and blood showed that it was uniformly sensitivity to imipenem, but susceptibility to third generation cephalosporin and non-β-lactam antibiotics was further decreased as compared to non-ESBL-KP [Table 4].

DISCUSSION

Nosocomial infections have been a major challenge for the clinicians worldwide, especially in developing

Table 2: Antimicrobial susceptibility pattern of *Klebsiella pneumoniae*

Antibiotics	Specimen (%)			
	Urine (*n*=80)	Blood (*n*=40)	Pus (*n*=36)	Total samples (*n*=223)
Penicillins				
Piperacillin	26 (32)	12 (30)	16 (44)	92 (41)
β-lactam/β-lactamase inhibitor combinations				
Piperacillin/tazobactam	68 (85)	34 (85)	32 (89)	206 (92)
Amoxicillin/clavulanic acid	58 (73)	28 (70)	26 (72)	164 (74)
Cefoperazone/sulbactam	64 (80)	30 (75)	32 (89)	164 (74)
Ceftazidime/clavulanate	58 (73)	30 (75)	30 (83)	160 (72)
Cephalosporins				
Cefoperazone	18 (23)	10 (25)	14 (39)	100 (45)
Cefoxitin	26 (33)	12 (30)	08 (22)	86 (39)
Ceftazidime	34 (43)	14 (35)	12 (33)	106 (48)
Cefotaxime	26 (33)	10 (25)	14 (39)	92 (41)
Ceftriaxone	34 (43)	14 (35)	14 (39)	96 (43)
Cefepime	34 (43)	14 (35)	16 (44)	90 (40)
Monobactam				
Aztreonam	32 (40)	12 (30)	14 (39)	88 (39)
Carabapenem				
Imipenem	80 (100)	40 (100)	36 (100)	223 (100)
Aminoglycosides				
Gentamicin	52 (65)	24 (60)	22 (61)	172 (77)
Amikacin	54 (68)	28 (70)	30 (83)	182 (82)
Flouroquinolones				
Ciprofloxacin	48 (60)	22 (55)	26 (72)	114 (51)
Ofloxacin	50 (63)	20 (50)	26 (72)	130 (59)
Norfloxacin	52 (65)			
Nitrofuran				
Nitrofurantoin	52 (65)			

Data are presented as number (%) of isolates

Table 3: Distribution of ESBL-KP among inpatients and outpatients' samples

K. pneumoniae	Inpatients (%)	Outpatients (%)	*P*
ESBL producers	96 (59.6)	18 (29)	<0.001
Non-ESBL producers	65 (40.4)	44 (71)	
Total	161	62	

Data are presented as number (%) of isolates. ESBL-KP=Extended-spectrum beta-lactamase producing *K. pneumoniae*, *K. pneumoniae=Klebsiella pneumoniae*

countries. KP is one of the most common causes of nosocomial infections among hospitals in India. Antimicrobial agents are although available for treatment for those infections, but their effectiveness has been compromised due to the emergence of antimicrobial resistance. Increasing resistance to antimicrobial agents has not only increased the prevalence of disease among the hospitalized patients but in turn, it also increases the cost of treatment.

This study was conducted to determine the prevalence of ESBL-KP among the clinical samples from various wards of Gold Field Institute of Medical Sciences and Research. A total of 223 KP isolates were obtained which were resistant to third generation cephalosporins. Among these, 36% of isolates were obtained from urine samples and 18% were obtained from blood samples. This indicates that urinary tract infections are more common as compared to other infections, which may be due to prolonged hospital stay with the presence of indwelling catheters.

Antimicrobial susceptibility pattern of isolates which were resistant to third generation cephalosporins showed a full susceptibility to imipenem (100%) followed by a high susceptibility to beta-lactamase inhibitors combinations (72% to 92%). A low susceptibility pattern was observed for penicillins and cephalosporins. The susceptibility pattern remains almost same when compared between isolates obtained from pus, urine, and blood. Babypadmini and Appalaraju also reported a similar susceptibility of urinary isolates to imipenem and a lower susceptibility to cephalosporins among the hospitalized patients.[9] Similar results were obtained by Menon *et al*. with 100% susceptibility to imipenem and 72.8% susceptibility to cefoperazone-sulbactam.[10] A low susceptibility to penicillins and cephalosporins may be attributed to the irrational use of these antibiotics.

Table 4: Antimicrobial susceptibility pattern of ESBL-KP in urine and blood samples

Antibiotics	Specimen (%)	
	Urine (*n*=42)	Blood (*n*=26)
Penicillins		
Piperacillin	08 (19)	06 (23)
β-lactam/β-lactamase inhibitor combinations		
Piperacillin/tazobactam	34 (81)	18 (69)
Amoxicillin/clavulanic acid	28 (67)	18 (69)
Cefoperazone/sulbactam	30 (71)	20 (77)
Ceftazidime/clavulanate	28 (67)	20 (77)
Cephalosporins		
Cefoperazone	06 (14)	06 (23)
Cefoxitin	06 (14)	04 (15)
Ceftazidime	10 (24)	04 (15)
Cefotaxime	08 (19)	04 (15)
Ceftriaxone	06 (14)	04 (15)
Cefepime	08 (19)	06 (23)
Monobactam		
Aztreonam	10 (24)	04 (15)
Carbapenem		
Imipenem	42 (100)	26 (100)
Aminoglycosides		
Amikacin	32 (76)	18 (69)
Gentamicin	30 (71)	16 (62)
Flouroquinolones		
Ciprofloxacin	12 (29)	12 (46)
Ofloxacin	18 (43)	12 (46)
Norfloxacin	24 (57)	
Nitrofuran		
Nitrofurantoin	24 (57)	

Data are presented as number (%) of isolates. ESBL-KP=Extended-spectrum beta-lactamase producing *Klebsiella pneumoniae*

Out of 223 KP 51.1% *Klebsiella* spp. were ESBL producer by phenotypic confirmatory methods. The prevalence of ESBL-KP varies greatly from country to country and among the institutions within the country. In USA recent data from the MYSTIC program suggests that the prevalence of ESBL-KP has been reduced to a low level of 2.4–4.4%, which is further corroborated with the data from the CDC which suggests that 0.5–1% of hospital-acquired infections were contributed by ESBL producing organisms.[11,12] In Japan, the prevalence of ESBL-KP was found to increase from 0.5% in 2006 to 4.7% in 2010.[13] In Asia, the percentage of ESBL-KP is 26.5% in Korea, 28.4% in Taiwan, 13% in Hong Kong.[14-16] In India, percentage of ESBL-KP ranges from 16% to 73%.[17-20]

ESBL-KP was isolated from all the different types of samples such as blood, urine, sputum, wound, pus, ear, stool, and aspirates. About 65% of the isolates were obtained from blood, whereas 58%, 53%, and 44% were isolated from sputum, urine, and pus respectively. ESBL-KP was obtained in a significant amount of the blood samples which may be alarming because of the serious nature of blood stream infections. Similar findings were observed by Sarojamma and Ramakrishna, Gupta *et al*. and Ananthan and Subha with 57.14%, 69.2%, 92.5%, of ESBL-KP isolates from blood samples respectively.[21-23]

In the present study, the prevalence of ESBL-KP was higher among inpatients (59.6%) as compared to outpatients (29%). This suggests that there may be a higher rate of transmission of the resistant strains in the hospital. However, several studies suggested a high prevalence of ESBL-KP among the outpatients which may be due to excessive use of third generation cephalosporins in the community.[24] However, in the present study the isolates were obtained from both the community and hospital patients, and this may limit the value of the study findings.

ESBL-KP isolates showed a higher degree of antimicrobial resistance as compared to non-ESBL producers. Imipenem, beta-lactamase inhibitor combinations, and aminoglycosides were showed to be the most effective antimicrobials for ESBL-KP isolates obtained from urine and blood samples. Norfloxacin and nitrofurantoin had also showed a moderate degree of efficacy against ESBL-KP. On the other hand, penicillin and cephalosporins showed a lesser degree of susceptibility, which may be due to unprecedented use and over the counter sale of these drugs. Beta-lactamase inhibitor combinations, aminoglycosides, norfloxacin and nitrofurantoin, showed a good susceptibility rates and these drugs may be recommended as a first-line of treatment of infections caused by ESBL-KP wherever suitable. However, carbapenems should be kept as a reserved drug for serious life-threatening infections in order to prevent the development of resistance against them. Goyal *et al*. have reported a higher rate of resistance to ciprofloxacin (93.8%), trimethoprim-sulfamethoxazole 79.1%), gentamicin (66.7%) while low resistance to amikacin (14.7%) among the ESBL isolates.[25] Somily *et al*. had also demonstrated the antimicrobial resistance profile of ESBL-KP and showed a higher degree of susceptibility to amikacin (92.5%) and nitrofurantoin (67.43%).[26] Maina *et al*. demonstrated 99.4% susceptibility to carbapenems, but a higher degree of resistance to gentamicin, ceftazidime and nitrofurantoin among ESBL-KP.[27]

In conclusion, ESBL producing organisms always remain a matter of concern for the clinicians and microbiologists due to their resistance to commonly used antibiotics, leaving the clinicians with very few treatment options. This in turn may increase pressure on the reserved drugs such as carbapenems and may lead to the

development of resistance against them. This study found that inhibitor combinations are also effective in treatment of such infections thus these drugs should be a part of the empirical therapy and carbapenems should be used when the antimicrobial susceptibility test report resistance against inhibitors combinations. While in case of urinary tract infections, empirical therapy should include norfloxacin or nitrofurantoin. As resistance to carbapenem is emerging, its use as a part of empirical therapy should be avoided, and a proper antibiotic policy for every health care institution should be made available to guide the clinicians in order to prevent the development of resistance to these antibiotics.

AUTHORS' CONTRIBUTION

Conceived and designed the experiments: AKS, SJ, RPS. Performed the experiment in the laboratory: DK, HB. Analyzed the data: AKS, SJ. Wrote the first draft of the manuscript: AKS. Contributed to the writing of the manuscript: AKS, SJ, RPS. Agree with manuscript results and conclusions: AKS, SJ, DK, RPS, HB. Made critical revisions and approved final version: AKS. All authors reviewed and approved of the final manuscript.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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