Print ISSN: 2319-2003 | Online ISSN: 2279-0780

IJBCP International Journal of Basic & Clinical Pharmacology

doi: 10.5455/2319-2003.ijbcp20130111

Research Article

Third generation cephalosporin-resistance in *Klebsiella pneumoniae* isolates: an emerging threat

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Received: 19 November 2012
Revised: 13 December 2012
Accepted: 14 December 2012

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ABSTRACT

Background: Newer generation cephalosporin-resistance among *Klebsiella pneumoniae* organisms has increased recently. Present study is undertaken to find incidence, antimicrobial susceptibility and prevalence of extended spectrum beta-lactamase (ESBL) in *K. pneumoniae* isolates in a tertiary care hospital.

Methods: Prospective study was carried out between June to December 2011. Samples of pus, blood, urine, cerebro-spinal fluid, stool, peritoneal, pleural and synovial fluid were collected from indoor and outdoor patients for isolation and antimicrobial susceptibility pattern of *K. pneumoniae* in the department of microbiology, G.R. Medical College Gwalior, M.P. Ceftazidime resistant *K. pneumoniae* were subjected to Phenotypic Confirmatory Disc Diffusion Test (PCDDT) and Double Disc Synergy Test (DDST) for detection of ESBL.

Results: Out of 2480 samples collected a total of 530 *K. pneumoniae* were isolated and subjected to antimicrobial susceptibility. Antibiotic sensitivity to imipenem, cefoperazone, amikacin and ofloxacin were 82, 74, 73 and 72% respectively whereas sensitivity to ceftizoxime, ceftriaxone cefotaxime, ceftazidime ranged between 47-50%. *K. pneumoniae* were found to be resistant to ampicillin, co-trimoxazole, doxycycline and gentamicin, by 91, 82, 54 and 50% respectively. Among third generation cephalosporins *K. pneumoniae* were least sensitive (47%) to ceftazidime. About 33 and 32% of the ceftazidime resistant strains were found to be ESBL positive by PCDDT and DDST respectively.

Conclusions: This study has shown that prevalence of ESBL producing *K. pneumoniae* is the most important reason for increased resistance to third generation cephalosporins. There is need to carry out tests for detection of ESBL producing bacteria routinely.

Keywords: Antimicrobial-resistance, Clinical isolates, *Klebsiella pneumoniae*, Third generation- cephalosporins

INTRODUCTION

A variety of nosocomial and community acquired infections such as pneumonia, urinary tract infections, septicemia, soft tissue infections are caused by *K. pneumoniae*, one of the most deadly pathogen of enterobacteriaceae group.[1] The emergence of multidrug resistance among gram negative bacteria from the genus *Klebsiella* has increased. Several strains of *Klebsiella* which demonstrated similarities in DNA homology are known. These are (1) *Klebsiella pneumoniae*, (2) *Klebsiella ozaenae*, (3) *Klebsiella rhinoscleromatis*, (4) *Klebsiella oxytoca*, (5) *Klebsiella planticola*, (6) *Klebsiella terrigena*, and (7) *Klebsiella ornithinolytica*. Since *K. pneumoniae* species is most prevalent in hospital acquired infections hence is medically most important. These bacteria may spread horizontally among patients in a particular department by environmental vectors.[2] Newer cephalosporins, fluoroquinolones, aminoglycosides and carbapenems are the class of drugs most effective for the treatment of infections caused by *K. pneumoniae*. Rising resistance to these drugs is a notable global threat.[3-5] One of the reason for development of resistance to third generation cephalosporins is production of extended spectrum beta lactamase (ESBL) enzyme which hydrolyses oxyimino-beta-lactam in third generation cephalosporins like cefotaxime, ceftriaxone, ceftazidime and ceftizoxime.[6] The incidence of ESBL-producing strains among clinical *K. pneumoniae* isolates have steadily increased over the past years and it varies from

institution to institution.[7] Therefore present study is undertaken to evaluate incidence, antimicrobial susceptibility and prevalence of ESBL in *K. pneumoniae* isolates from different clinical samples in a tertiary care hospital.

METHODS

Sample collection and identification of K. pneumoniae

Prospective study was carried out between June to December 2011. Samples of pus, blood, urine, cerebrospinal fluid, stool, peritoneal, pleural and synovial fluid were collected from indoor and outdoor patients for isolation and antimicrobial susceptibility pattern of *K. pneumoniae* in the department of Microbiology, G. R. Medical College, Gwalior, (M.P.) These samples were inoculated on Mac Conkey's agar and nutrient agar followed by the identification of the isolates based on their cultural characteristics and their reactions in standard biochemical tests.8 In case of blood sample blood was incubated at 37°C overnight in Brain Heart Infusion broth.

Antimicrobial agents

Isolated *K. pneumoniae* were tested for antimicrobial susceptibility by the Kirby bauer disc diffusion technique[9] according to the Clinical and Laboratory Standard Institute (CLSI) guidelines on Muller Hinton agar by paper disks impregnated with antibiotics (Span Diagnostics Limited, Surat, India): Penicillins: ampicillin 10µg, Cephalosporins: cefoperazone 75 µg, ceftriaxone 30 µg, ceftazidime 30 µg, cefotaxime 30 µg, ceftizoxime 30 µg, Carbapenems: imipenem 10 µg, Aminoglycosides: amikacin 30 µg, gentamicin 10 µg, Quinolones: ofloxacin 5 µg, levofloxacin 5 µg, ciprofloxacin 5µg, Tetracyclines: doxycycline 30 µg and co-trimoxazole 25 µg.

Detection of Extended- spectrum beta-lactamase (ESBL) production

1. Phenotypic Confirmatory Disc Diffusion Test (PCDDT)

Lawn culture of the organism was made and 3rd-generation cephalosporin: ceftazidime (30 µg) disc and ceftazidime + clavulanic acid (30 µg + 10 µg) disc were placed with 25 mm apart. An isolate showing increase of ≥5 mm in zone of inhibition for ceftazidime + clavulanic acid compared to ceftazidime was confirmed as ESBL producer.[10]

2. Double Disc Synergy Test (DDST)

The isolated colonies were inoculated in peptone water at 37°C for 2-6 h. The turbidity was adjusted to 0.5 McFarland standards and lawn culture was made on Mueller-Hinton agar using sterile swab. Amoxicillin + Clavulanic acid disc (20/10 µg) was placed in the centre of plate. Both side of Amoxicillin + Clavulanic acid disc, a disc of cefotaxime (30 µg) and ceftazidime (30 µg) were placed with centre to centre distance of 15 mm to centrally placed disc. The plate was incubated at 37°C overnight. ESBL production was interpreted as the 3rd-generation cephalosporin disc inhibition was increased towards the Amoxicillin + Clavulanic acid disc or if neither discs were inhibitory alone but bacterial growth was inhibited where the two antibiotics were diffused together.[11]

RESULTS

Incidence of K. pneumoniae in clinical samples

Out of 2480 samples collected, a total of 530 *K. pneumoniae* were isolated. Hospital prevalence of *K. pneumoniae* was found to be 21%. Among these 530 microorganisms isolated and studied 66% were from the indoor and 34% from outdoor samples. The sample wise prevalence of *K. pneumoniae* was 31, 21, 19, 18 and 15% from blood, pus, urine, cerebrospinal fluid and miscellaneous (include pleural, peritoneal, synovial fluid and stool) respectively (Table 1).

Table 1: Sample wise distribution of *K. pneumoniae* isolates.

Type of Sample	No. of samples	Indoor isolates	Outdoor isolates	Total isolates
Pus	888	65	124	189
Urine	812	82	76	158
Blood	349	99	8	107
CSF	356	63	2	65
Miscellaneous	75	6	5	11
Total	**2480**	**315**	**215**	**530**

Antimicrobial susceptibility pattern of K. pneumoniae isolates

K. pneumoniae isolated from different samples showed antimicrobial sensitivity range to imipenem 72-94%, cefoperazone 69-90%, amikacin 67-78% , ofloxacin 55-89%, levofloxacin 44-67%, ciprofloxacin 42-69%, gentamicin 35-64%, ceftizoxime 43-54%, ceftriaxone 40-54%, ceftazidime 30-64%, Cefotaxime 32-60%. Antibiotic resistance of *K. pneumoniae* ranged between 87-95% to ampicillin, 67-91% to co-trimoxazole, 39-65% to gentamicin and 37-61% to doxycycline (Table 2).

Table 2: Antibiotic sensitivity (% age) of clinical isolates of *K. pneumoniae.*

Antibiotic	Pus	Urine	Blood	CSF	Miscellaneous	Mean
Imipenem	82.5	72.1	84.6	94.4	66.7	81.8
Cefoperazone	69.3	76.6	83.1	71	88.9	73.9
Ceftizoxime	53.9	50.6	43.1	50.4	44.4	50.8
Ceftriaxone	47.6	53.8	40	48.6	44.4	48.7
Cefotaxime	32.3	54.4	58.5	59.8	55.5	48.1
Ceftazidime	30.7	52.5	55.3	64.5	33.3	47.2
Amikacin	68.3	78.5	73.9	76.6	66.0	73.4
Ofloxacin	66.7	62.3	89.2	86.9	55.5	72.1
Levofloxacin	61.9	44.3	65	50.4	66.7	54.1
Ciprofloxacin	42.3	48.7	63.1	69.1	55.5	52.5
Gentamicin	35.4	64.5	61.5	53.3	55.5	51.3
Doxycycline	43.9	38.6	63.1	50.4	44.4	46
Co-trimoxazole	11.3	9.1	30	12.5	33.3	17.8
Ampicillin	10.6	11.4	3	4.7	11.1	8.7

Miscellaneous group includes: pleural, peritoneal, synovial fluid and stool samples.

Antimicrobial class wise sensitivity of K. pneumoniae isolates

Among 3 most commonly used antibiotics class of drugs against *K. pneumoniae* namely cephalosporins, aminoglycosides and quinolones, aminoglycoside and fluoroquinolones class showed almost equal antimicrobial sensitivity and was higher (62%) as compared with that of cephalosporin class (55%) (Table 3).

Table 3: Antibiotic class wise sensitivity (%age) of *K. pneumoniae* isolates.

Sample	Cephalosporins	Aminoglycosides	Fluoroquinolones
Blood	58.9	65	68.9
Urine	61.4	72	51.9
Pus	43.6	52	57
CSF	56	68	69.2
Mean	**55**	**62.5**	**62**

Cephalosporins include: Cefoperazone, Ceftriaxone, Ceftazidime, Cefotaxime, Ceftizoxime
Aminoglycosides include: Amikacin, Gentamicin
Quinolones include: Ofloxacin, Levofloxacin, Ciprofloxacin

ESBL positive strains

Ceftazidime resistant *K. pneumoniae* were screened for ESBL enzyme by PCDDT and DDST methods. Out of 257 ceftazidime resistant *K. pneumoniae* about 88 (33%) and 87 (32%) were found ESBL positive by PCDDT and DDST respectively. Fifty three (60%) out of 88 ESBL producing *K. pneumoniae* were from indoor samples whereas only 35 (40%) were from outdoor samples. Majority of indoor ESBL positive isolates were from medical intensive care unit and surgery wards.

DISCUSSION

The emergence of gram negative bacterial species with acquired resistance to various broad spectrum beta lactams is becoming a worldwide clinical problem. *K. pneumoniae* did not show 100% sensitivity to any of the antimicrobial used in the present study. The sensitivity pattern shown was in decreasing order to imipenem, cefoperazone, amikacin, ofloxacin, levofloxacin, ciprofloxacin, gentamicin, ceftizoxime, ceftriaxone, cefotaxime and ceftazidime respectively. Overall antimicrobial group wise mean sensitivity to cephalosporins (55%) was less than aminoglycosides (62.5%) and quinolones (62%). Resistance against third generation cephalosporins in present study highlights the most alarming situation of highly diverse antibiotic resistance rate. This can pose serious negative impact in low economy country like India where infectious diseases hold a major health challenge. Overuse of ceftriaxone, cefotaxime, ceftazidime and ceftizoxime has been revealed in the present study this may be associated with development of resistance to β-lactam antibiotics as reported earlier.[12] To explore cephalosporin resistance *K. pneumoniae* were tested for ESBL production by two methods. About 33% of the ceftazidime resistant *K. pneumoniae* were found to produce ESBL by PCDDT and 32% by DDST. Similar trend was observed in study conducted at other tertiary care hospital.[13] The PCDDT test was found to be an inexpensive, simple and more sensitive alternative, for the detection of ESBL than DDST which is in accordance with earlier reports.[14] Prevalence of ESBL production is the most important reason for resistance against novel cephalosporins. As emphasized by various authors prevalence of ESBL positive strain is reported in several regions of India and it varied from one hospital in particular region to another.[15-17] Wide spread use of third generation cephalosporins causing mutation in genes producing

enzymes and ESBL has been produced by mutation in TEM1, TEM2, SHV1 in *K. pneumoniae*.[18] Routine testing for these gene might help in prevention of spread as well as colonization of patients hospitalized for more than 10 days by ESBL positive strains.[19]

Antimicrobial susceptibility to cefoperazone (74%) was higher as compared to other cephalosporins might be due to its limited use and different chemical structure so it is likely that it is not hydrolysed by ESBL as reported earlier.[20] The other causes for resistance against *K. pneumoniae* among different third generation might be due to production of Amp C beta lactamases[21], production of porins[22] or some other unknown mechanism. Therefore tests to detect these should be performed on routine basis.

In the present study, high resistance to other drug classes like aminoglycosides, quinolones may be due to plasmid responsible for ESBL production which frequently carry gene encoding resistance to other antibiotic so the options for treatment of ESBL producing organism is very limited. Since these plasmids are easily transmitted among different members of the *Enterobacteriaceae*, accumulation of resistance genes results in strains which contain multi-resistant plasmids.[23]

Even years after discontinuation of ceftazidime and other extended-spectrum cephalosporins, continued colonization of patients by ESBL-producing *Klebsiella* strains has been observed.[24] Recently the use of antibiotics from the class carbapenems as last resort drugs for treating infections due to ESBL-producing organisms has increased. In this study 82% of *K. pneumoniae* strains were found to be sensitive to imipenem. The increased resistance to carbapenems is reported due to production of *Klebsiella pneumoniae* carbapenemase (KPC)[25] an enzyme which hydrolyses carbapenems and will have a serious threat to public health in society. There is need of special laboratory standard techniques to screen KPC as it cannot be detected by routine laboratory methods.

Due to rapid emergence of multidrug resistant organisms, the therapeutic options are becoming limited therefore; in the near future an urgent need for implementation of strict hospital infection control measures to prevent the nosocomial spread of drug resistant *K. pneumoniae* is needed. These include strict adherence to basic epidemiological standards for maintenance and care of urinary catheters, tracheostomies, wounds along with good hand-washing practices[26], regulation of antibiotic use in the hospital to prevent misuse and overuse of antibiotics and to collect nosocomial infection surveillance data. Hospital infection prevention and control programmes led in the past have resulted in considerable improvements in the management and control of these infections.[27]

CONCLUSION

This study has shown that prevalence of ESBL producing *K. pneumoniae* is the most important reason for increased resistance against third generation cephalosporins. Findings of this single centre study give an idea of the increasing threat of resistance around a tertiary care hospital which cannot be generalized in larger perspective. Due to cost factors genetic studies are not feasible with the large number of isolates. Routine detection of ESBL-producing microorganisms is required to be done by each laboratory to control the spread of these infections and also to institute proper therapeutic strategies that ensure reduced patient stay, morbidity and cost per day in the hospital.

Funding: No funding sources
Competing interests: There are no competing interests to declare

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doi: 10.5455/2319-2003.ijbcp20130111
Cite this article as: Kothari S, Mishra V, Ranjan N, Singh A. Third generation cephalosporin-resistance in Klebsiella pneumoniae isolates: an emerging threat. Int J Basic Clin Pharmacol 2013;2:56-60.