ORIGINAL ARTICLE

Prevalence of Extended-Spectrum Beta-Lactamases (ESBL) Production Among *Escherichia coli* and *Klebsiella pneumoniae* Isolates in a Rural Tertiary Care Hospital of Haryana

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Abstract

Background: Extended-spectrum beta-lactamase producing *Enterobacteriaceae*, the most common being *Escherichia coli* and *Klebsiella pneumoniae* are considered as the most dangerous one with hardly any antibiotic left for treatment of infections caused by these bacteria. There is paucity of reports from rural hospitals especially from India in this regard. ***Purpose:*** To find out the prevalence of ESBL producing *E. coli* and *K. pneumoniae* isolates from patients with both HAI and CAI in a rural set up and to find out a reliable and cost-effective method for detection of ESBL production. ***Material and Methods:*** A total of 300 *Enterobacteriaceae* isolates, *E. coli* (n=252) and *K. pneumoniae* (n=48) isolated during the study period were analysed. 55.7% isolates were obtained from patients with community acquired infections and 44.3% were from hospital acquired infections. These isolates were subjected to ESBL detection by Kirby-Bauer disc diffusion method using third generation cephalosporin discs, HiCrome ESBL agar, Vitek 2 system and double disc synergy test (DDST). ***Results:*** Considering DDST results as gold standard, a total of 222 (74%) isolates comprising of 196 *E. coli* and 26 *K. pneumoniae* were phenotypically confirmed as ESBL producers. Comparing this result, other methods viz. HiCrome ESBL agar and Vitek 2 system were equally sensitive and specific for ESBL detection. Antibiogram of the ESBL-producing isolates showed higher resistance rates to cephalosporins other non-cephalosporin group of antibiotics viz. ampicillin, amoxyclav, aztreonam, ciprofloxacin, co-trimoxazole and nalidixic acid. The ESBL producers showed low degree of resistance against aminoglycosides and carbapenem group of antibiotics. ***Conclusion:*** The present study showed high prevalence of ESBL positivity among the *E. coli* and *K. pneumoniae* isolates. HiCrome agar for detection of ESBL production could be a simple and cost-effective method.

Key Words: ESBL, *Escherichia coli*, *K. pneumoniae*, DDST

Introduction

Antimicrobial resistance (AMR) is a growing public health threat worldwide and is one of the most common priority areas defined (1). Among the rapidly increasing different types of AMR, extended-spectrum â-lactamase producing *Enterobacteriaceae* (ESBL-E), most common being *Escherichia coli* and *Klebsiella pneumoniae* are considered as the most dangerous one with hardly any

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antibiotic left for treatment of infections caused by these bacteria (1). ESBLs are group of â-lactamases, encoded by plasmids which possess the ability to disintegrate third generation cephalosporins and aztreonam but are inhibited by clavulanic acid. They are derived from genes for TEM-1, TEM-2, or SHV-1 by mutations that alter the amino acid sequence around the active site of these â-lactamases (2). Most of the ESBL producing bacteria are multidrug resistant since the plasmids responsible for ESBL production carry genes encoding resistance to other drug classes for example, aminoglycosides (3). Thus, antibiotic options available for the treatment of ESBL-producing organisms are becoming limited. Carbapenems are the treatment of choice for serious infections due to ESBL-producing organisms, yet carbapenem resistance has been reported (1).

ESBL-E have been responsible for numerous outbreaks of infection throughout the world and pose challenging infection control practices in the hospital settings. These bacteria are being increasingly isolated from hospital acquired (HAI) and community acquired infections (CAI). As high as 88.3% of its prevalence have been reported from studies from India (4,5). Another south east Asian country i.e., Pakistan reported 52% prevalence rate of ESBL producing bacteria (6). Burden of ESBL producing bacteria is higher in developing countries however this problem is not confined only to these countries, it has been reported from developed countries such as USA and Switzerland with prevalence rate as high as 15% (7,8).

Many reports on ESBL production among *E. coli* and *K. pneumoniae* from clinical isolates have been available from urban hospitals from both developed as well as developing countries. However, limited reports from rural hospitals especially from India are available. Thus, the present study was aimed to find out the prevalence of ESBL producing *E. coli* and *K. pneumoniae* isolates from patients with both HAI and CAI in a rural set up and to find out a reliable and cost-effective method for detection of ESBL production.

Material and methods

The present study was a cross-sectional one which included a total of 300 strains of *E. coli* and *K. pneumoniae* isolated from clinical specimen viz. urine, sputum, pus, stool, etc. from both inpatients and outpatients processed in bacteriology laboratory, SGT Hospital, Gurugram, Haryana. Patient consent was taken from all the patients from whom *E. coli* and *K. pneumoniae* were isolated and detailed about the objectives of the study.

The isolates were subjected to antimicrobial susceptibility testing using the following antibiotic discs and interpreted as per CLSI guidelines (9): ampicillin (10 µg), amoxicillin-clavulanic acid (20/10 µg), piperacillin/tazobactam (100/10 µg), amikacin (30 µg), gentamicin (10 µg), cefotaxime (30 µg), ceftazidime (CAZ, 30 µg), ceftriaxone (CTR, 30 µg), aztreonam (30 µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), co-trimoxazole (25 µg), nitrofurantoin (300 µg), ertapenem (10 µg), meropenem (10 µg) and imipenem (10 µg). All the antibiotic discs were procured from Himedia, Mumbai, India. Bacterial isolates showing resistant to cefotaxime and ceftazidime were considered as potential ESBL producers as per CLSI 2019 guidelines (9,10). These isolates were further subjected to Hicrome ESBL agar for ESBL screening and Double disk synergy test for phenotypic confirmation of ESBL production.

Hicrome ESBL agar (Himedia, Mumbai, India) is based on an agar with a mixture of antibiotics designed specifically to enable the selective growth of ESBL producing *Enterobacteriaceae* and chromogenic substrates for direct identification of the most frequently encountered ESBL-E (11). The bacterial strains were inoculated on this medium and incubated at 37°C for 18-24 hours. The interpretation of the result was based on colour of the colonies: *E. coli*: pink and *K. pneumoniae*: bluish green. Each sample was tested using the VITEK 2 system (BioMerieux).

Double disk synergy test (DDST) was considered as gold standard for phenotypic ESBL detection as per CLSI (9). Two pairs of antibiotic discs, ceftazidime (30 µg) and ceftazidime plus clavulanic acid (30 µg plus 10 µg) discs as first pair and cefotaxime (30µg) and cefotaxime plus clavulanic acid, an inhibitor of ESBL (30 µg plus 10 µg) discs as second pair were placed at a distance of 25 mm, centre to centre, on a plate of Mueller Hinton agar inoculated with the bacterial inoculum (turbidity of 0.5 MacFarland). Phenotypic confirmation of ESBL production was based on zone diameters e"5 mm larger around ceftazidime + clavulanic acid disc and cefotaxime + clavulanic acid disc compared to zone diameters around ceftazidime and cefotaxime discs respectively (9,10).

Sensitivity and specificity for various ESBL detection methods were evaluated considering DDST as gold standard. Chi-square test was done to evaluate the statistical significance for ESBL positivity rate and antimicrobial resistance rates for various antimicrobials.

p <0.05 was taken as statistically significant.

Results

A total of 2867 clinical specimens were received in the Bacteriology laboratory from both inpatient and outpatient departments of a tertiary care hospital in Haryana during June and July 2019. Of the total clinical specimens, 300 isolates of *Enterobacteriaceae* (*Escherichia coli*, n=252 and *Klebsiella pneumoniae*, n=48) were isolated from 295 specimens (five out of 295 specimens yielded both *E. coli* and *K. pneumoniae*). These isolates were collected from 164 and 131 patients attending OPD and IPD respectively. Majority of the isolates were obtained from urine 231 (77%), followed by pus and swabs 25 (8.3%), stool 20 (6.7%), blood 15 (5%), respiratory specimens 08 (2.7%), and body fluid 1 (0.3%). Of the patients attending OPD, 141 *E. coli* and 26 *K. pneumoniae* were isolated while 111 *E. coli* and 22 *K. pneumoniae* were isolated from patients attending IPD (*Table 1*). A total of 179 isolates were from patients with community acquired infections (CAI) and 121 isolates were from patients with hospital acquired infections (HAI). CAI was defined by a positive bacterial culture obtained from patients attending outpatient departments or within 48 h of hospital admission from hospitalized patients without any prior history of hospitalization or antibiotic treatment in the last 30 days (11).

Of the 300 bacterial isolates, 226 (75.3%) isolates were resistant to atleast two members of third generation cephalosporins (cefotaxime or ceftriaxone or ceftazidime) in Kirby-Bauer disc diffusion method were considered as potential ESBL producers. While 222 (74%) isolates were screened potential ESBL producers by using HiCrome ESBL agar and Vitek 2 system. All the isolates were further subjected to double disc synergy test; 222 (74%) of the isolates were confirmed to be ESBL producers (*E. coli*, n= 196 and *K. pneumoniae*, n=26) by this method (*Table 2*). Comparison of these methods considering DDST result as gold standard revealed HiCrome ESBL agar and Vitek 2 system as equally sensitive and specific to detect ESBL producers as that of DDST. While screening test for ESBL detection using third generation cephalosporin discs was 100% sensitive to detect ESBL producers however the specificity was

Table 1: Distribution of Various Clinical Specimens from which E. coli and K. pneumoniae were Isolated

Clinical specimens	*E. coli* (n=252)		*K. pneumoniae* (n=48)	
	OPD No. (%)	IPD No. (%)	OPD No. (%)	IPD No. (%)
Urine	125 (49.6)	72 (28.6)	22 (45.8)	12 (25)
Pus and swabs	4 (1.6)	18 (7.1)	00	3 (6.3)
Stool	12 (4.8)	8 (3.2)	00	00
Blood	00	12 (4.8)	01 (2.1)	02 (4.2)
Respiratory specimens	00	00	03 (6.3)	05 (10.4)
Body fluid	00	01 (0.4)	00	00
Total	141 (56)	111 (44)	26 (54.2)	22 (45.8)

Table 2: Comparison of Various Methods for Detection of ESBL Production Among E. coli and K. pneumoniae Isolates

Bacterial isolates	Resistance to third generation cephalosporins No. (%)	Growth on HiCrome ESBL agar No. (%)	Vitek 2 Syste No. (%)	Double disc synergy test No. (%)
E. coli (n=252)	200 (79.4)	196 (77.8)	196 (77.8)	196 (77.8)
K. pneumoniae (n=48)	26 (54.2)	26 (54.2)	26 (54.2)	26 (54.2)
Total (n=300)	226 (75.3)	222 (74)	222 (74)	222 (74)
Sensitivity (%)*	100	100	100	-
Specificity (%)*	95.1	100	100	-

*Sensitivity and specificity were calculated considering DDST results as the gold standard

Table 3: Distribution of ESBL Producing E. coli and K. pneumoniae Isolates from Patients Attending OPD and IPD Identified by Double Disc Synergy Test

Clinical specimens	Bacterial isolates	ESBL producing *E. coli* and *K. pneumoniae*		Statistical comparisons *p* value
		OPD No. (%)	IPD No. (%)	
Urine	*E. coli* (n=197)	95 (48.2)	60 (30.5)	<0.001
	K. pneumoniae (n=34)	12 (35.3)	8 (23.5)	NS (0.5)
Pus and swabs	*E. coli* (n=22)	3 (13.6)	15 (68.2)	0.002
	K. pneumoniae (n=03)	00	3 (100)	NS (0.2)
Stool	*E. coli*(n=20)	06 (30)	6 (30)	NS (0.9)
	K. pneumoniae (n=00)	00	00	-
Blood	*E. coli* (n=12)	00	10 (83.3)	0.01
	K. pneumoniae (n=03)	00	01 (33.3)	NS (0.4)
Respiratory specimens	*E. coli* (n=00)	00	00	-
	K. pneumoniae (n=08)	00	02 (25)	NS (0.3)
Body fluid	*E. coli* (n=01)	00	01 (100)	NS (0.4)
	K. pneumoniae (n=00)	00	00	-
Total (n=300)		116	106	-

NS=Not significant (p>0.05); p values not significant are shown in parenthesis

Table 4. Comparison of Antibiotic Resistance Profile of Extended-Spectrum â-Lactamases (ESBL) Producing and Non-producing Escherichia coli and Klebsiella pneumoniae

Antibiotics (potency)	*E. coli* and *K. pneumoniae*		Test of significance *p* value
	ESBL (n=222) No. (%)	Non-ESBL (n=78) No. (%)	
AMP (10 µg)	222 (100)	70 (89.7)	<0.001
AMC (20/10µg)	165 (74)	25 (32.1)	<0.001
PIT (100/10 µg)	36 (16.2)	00	<0.001
AK (30 µg)	19 (8.6)	00	<0.001
GEN (10 µg)	28 (12.6)	03 (3.8)	0.03
CTX (30 µg)	222 (100)	00	<0.001
CAZ (30 µg)	217 (97.7)	00	<0.001
CTR (30 µg)	222 (100)	02 (2.6)	<0.001
AT (30 µg)	210 (94.6)	1 (1.3)	<0.001
CIP (5 µg)	158 (71.2)	17 (21.8)	<0.001
COT (25 µg)	165 (74.3)	25 (32.1)	<0.001
ETP (10 µg)	40 (18)	00	0.02
IPM (10 µg)	55 (24.8)	00	0.002
MRP (10 µg)	46 (20.7)	00	0.008
NA (30 µg)*	144 (82.3)	24 (42.9)	<0.001
NIT (300µg)*	56 (32)	3 (5.4)	<0.001

AMP- Ampicillin, AMC- Amoxyclav, PIT- Piperacillin/Tazobactam, AK- Amikacin, Gen- Gentamicin, CTX- Cefotaxime, CAZ- Ceftazidime, CTR- Ceftriaxone, AT- Aztreonam, CIP- Ciprofloxacin, COT- Co-trimoxazole, ETP- Ertapenem, IPM- Imipenem, MRP- Meropenem, NA- Nalidixic acid, NIT- Nitrofurantoin

*Only uropathogens (175 ESBL isolates and 56 non-ESBL isolates) were additionally tested against Nitrofurantoin and nalidixic acid

found to be 95.1% (*Table 2*). ESBL producing isolates were more frequently isolated from IPD 79.7% (106/133) than OPD 69.5% (116/167); $p<0.05$). Correspondingly, among the bacterial isolates, the proportion of ESBL positivity was significantly higher 82.6% (100/121) in HAI isolates than 68.2% (122/179) in CAI isolates ($p<0.05$). There were significant differences in the ESBL producing *E. coli* isolation rate from blood, urine, pus and swabs among OPD and IPD, $p<0.05$ (*Table 3*).

ESBL-producing isolates showed significantly higher resistance rates to cephalosporins (resistance rates ranging from 97.7 to 100%) and other non-cephalosporin group of antibiotics viz. ampicillin, amoxyclav, aztreonam, ciprofloxacin, co-trimoxazole and nalidixic acid (resistance rates ranging from 71.2 to 100%). While other group of antibiotics such as piperacillin/tazobactam, aminoglycosides and carbapenems showed low degree of resistance i.e., resistance rates ranging from 8.6 to 24.8%. For nitrofurantoin, moderate degree of resistance was exhibited by ESBL producers (32%) (*Table 4*). Non-ESBL-producers also exhibited low to high degree of resistance against various antibiotics. Ampicillin resistance rate was higher i.e., 89.7% whereas low degree of resistance was shown against aztreonam, gentamicin, ceftriaxone and nitrofurantoin (resistance rates ranging from 1.3 to 5.4%) while moderate degree of resistance was shown towards amoxyclav, ciprofloxacin, co-trimoxazole and nalidixic acid (resistance rates ranging from 21.8 to 42.9%). All the non-ESBL producers were susceptible to piperacillin/tazobactam, amikacin, cefotaxime, ceftazidime and carbapenems (*Table 4*).

Discussion

Prevalence of both HAI and CAI due to ESBL producing *E. coli* and *K. pneumoniae* has been increasingly reported worldwide including India leaving very few to very few therapeutic options for treatment of such infections. In the present study which is conducted in a rural tertiary care hospital also reported high prevalence rate of ESBL production among *E. coli* and *K. pneumoniae* i.e., 74%. In our study majority of the isolates were obtained from urine specimen (78.8%) which is in accordance with study from central India (12). A multicentric survey from India reported prevalence of ESBL among *E. coli* isolated from clinical specimens as 68% (13). Other studies conducted in tertiary care hospitals from India reported high prevalence rate of ESBL production among *E. coli* and *K. pneumoniae* ranging from 87 to 88.3% (4,5). In contrast to the present finding, a low overall prevalence (33.3 to 36.8%) of ESBL production among *E. coli* and *K. pneumoniae* has been reported from India and Saudi Arabia (14,15). Earlier ESBL producing bacteria were considered as nosocomial pathogens and were isolated from patients with HAI. In the recent past these bacteria are being increasingly isolated from patients with CAI too. This type of trends was seen in the present study, the prevalence of ESBL producing *E. coli* and *K. pneumoniae* was higher in HAI compared to CAI.

Four methods for detection of ESBL production among *E. coli* and *K. pneumoniae* were assessed in the present study. The results obtained in DDST, as recommended by CLSI as phenotypic confirmatory test was considered as gold standard. Considering this, all the three methods i.e., use of third generation cephalosporins, HiCrome ESBL agar and Vitek 2 system can be used for ESBL detection except for specificity of method using third generation cephalosporins. ESBL **sc**reening method using third generation cephalosporin discs, a CLSI recommended method could detect all the ESBL producing isolates however, the specificity was found to be 95.1%. A study from India reported 81 out of 102 (79.4%) gram negative bacterial isolates resistant to third generation cephalosporins was detected to be ESBL producer by DDST (16). The high sensitivity of this ESBL screening method in the present study could be due to the use of three different third generation cephalosporin discs, as per CLSI recommendation atleast resistant to two third generation cephalosporins to consider potential ESBL producers. Whereas HiCrome agar, a chromogenic ESBL screening medium used in the present study reporting 100% sensitivity and specificity for ESBL detection. A study from south India evaluated HiCrome ESBL agar for detection of ESBL producing bacteria which showed 100% sensitivity with one isolate showing false positive when the result was compared with DDST results (17). Similar results were obtained in Vitek 2 system for detection of ESBL producers.

The antibiotic susceptibility pattern of the ESBL producing isolates showed high co-resistance with other non-cephalosporin group of antibiotics when compared to non-ESBL producing isolates. This could be due to the location of plasmids responsible for ESBL production which also carry genes encoding resistance to other drug classes (3). High resistance rates against antibiotics such as ampicillin, amoxyclav, aztreonam, ciprofloxacin, co-trimoxazole and nalidixic acid were observed which is

also evident in studies from India and abroad, resistance rate ranging from 59 to 86.7% (10,18,19). Low resistance rate i.e., 8.6% to 12.6% against amikacin and gentamicin was observed. A multicentric study from India also reported similar findings and possible reasons such as lesser use of this antibiotic in community practice and its injectable route were addressed (10).

Due to increase prevalence of ESBL producing gram negative bacterial isolates, use of carbapenems (last resort antibiotics) has increased in clinical practice leading to emergence of carbapenem resistance bacteria. In the present study, the prevalence of potential carbapenemase producing *E. coli* and *K. pneumoniae* was found to be 18% considering the resistance rate against Ertapenem (as per CLSI 2019 guidelines). A study from north India reported prevalence of carbapenemase producing *E. coli* and *K. pneumoniae* to be 14.9% and 11.9% respectively (20). A very low prevalence rate (4%) of carbapenemase producing *E. coli* have been reported in a study from south India (21). Non-ESBL producing isolates also showed low to moderate degree of resistance to non-cephalosporin antibiotics which is evident in other studies also (18).

Conclusion

The finding of the present study highlights the high prevalence of antibiotic resistance especially co-resistance to non-cephalosporin antibiotics among the ESBL producing *E. coli* and *K. pneumoniae* in a rural set up. This clinically signify which group of antibiotics shall be given to patients to effectively cure the clinical condition and prevent mortality and morbidity. This study also compares various ESBL detection methods and thereby identifying the method which will be reliable and cost effective in a resource poor set up of rural hospital. Prevalence of both HAI and CAI due to ESBL producing *E. coli* and *K. pneumoniae* has been increasingly reported worldwide including India leaving very few to very few therapeutic options for treatment of such infections.

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Conflicts of Interest

There are no conflicts of interest.

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