Phenotypic detection of ESBL among E.coli and Klebiella pneumoniae by CLSI guidelines in a Teaching Hospital MIMS, Mandya

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Abstract

Introduction: Escherichia coli and Klebsiella pneumoniae cause a wide range of infections. Multidrug-resistance strains carrying resistance genes have become a growing problem worldwide. The ESBLs have emerged distinctly, especially in Escherichia coli and Klebsiella pneumoniae.

Material and Methods: 250 non repetitive isolates of E. coli and K. pneumoniae were isolated from different clinical samples (pus, urine, sputum, blood) for the study. Each isolate was tested for production of ESBL by CLSI recommended PCT

Results: 133 E. coli and 117 K. pneumoniae were isolated where 65.2% of ESBL were detected.

Conclusion: All the clinical samples growing gram negative bacteria should be tested for ESBL, production. Considering the grave scenario of antibiotic resistance in our country, it is high time that all clinical laboratories start detecting these enzymes routinely and accurately.

Keywords: Escherichia coli, Klebsiella pneumoniae, ESBLs, CLSI.

Introduction

Antimicrobial resistance is a global health security emergency that is rising due to emergence of microorganisms that are no longer treatable because of their resistance to virtually all available antibiotic treatment options.

In 1990s, there was increasing appearance of extended spectrum β-Lactamase, presently metallo β-Lactamase prevalence is increasing.in current situation, multi-drug resistant bacteria have spread widely which is being seen through various mechanisms, of which resistance by production of enzymes are most common.

Emergence of antibiotic resistance in bacteria is a major concern in health care system. Bacteria have evolved different mechanisms of drug resistance. Resistance to β-Lactam antibiotics in gram negative bacilli is primarily mediated by β-Lactamases.

CDC estimates that in United States, more than two million people are sickened every year with antibiotic resistant infections, with at least 23,000 dying every year. ESBL(Extended Spectrum β-Lactamases) producing Enterobacteriaceae were first reported in 1983 from Germany & since then a steady increase of these strains have been reported worldwide.

Bush, Jacoby & Medeiros have classified β-Lactamases according to substrate profiles.

The prevalence of ESBLs in India has now reached epidemic proportions, ranging from 62% to 100% in *Escherichia coli* and *Klebsiella spp*. respectively as observed in the 10 Indian medical centre SENTRY study.

ESBL, AmpC β-Lactamases & Carbapenemases are major β-Lactamases released by pathogenic gram negative bacilli.

β-Lactamases are the commonest cause of bacterial resistance to β-Lactam antimicrobial agents. Their spread destroyed the utility of benzylpenicillin against *Staphylococci* and has hugely undermined that of ampicillin against *Enterobacteria* and *Haemophilus* and *Neisseria spp*. New enzymes and new modes of production of old enzymes now threaten the value of extended-spectrum cephalosporins against enterobacteria. Further β-Lactamases, some of which will become important in the future, are described in most issues of *Antimicrobial Agents and Chemotherapy* and in other journals.

The current β-Lactamase classifications have reached a high level of complexity, making them less accessible to clinicians, infection control professionals, hospital management and politicians. From the clinical perspective, a revised comprehensible nomenclature scheme is therefore needed. The term extended-spectrum b-lactamases (ESBLs) has reached a broader audience over time, but is currently restricted to functional class 2be/molecular class A, clavulanic acid inhibited enzymes with activity against extended-spectrum cephalosporins. The proposed new classification expands the definition of ESBL to other clinically important acquired β-Lactamases with activity against extended-spectrum cephalosporins and/or carbapenems. The classical class-A ESBLs have been designated ESBLA in this classification, whereas plasmid-mediated AmpC and OXA-ESBLs are classed as miscellaneous ESBLs (ESBLM). Lastly, the carbapenemases have been designated ESBLCARBA, ESBLs with hydrolytic activity against carbapenems. We believe that this simplified classification may encourage new groups of healthcare professionals to

engage in the effort to prevent the spread of acquired β-Lactamases.

Aims and Objectives

1. Phenotypic detection of ESBL enzyme producing *E.coli* & *K pneumoniae*.
2. To know the prevalence of β-Lactamase producing *E.coli* & *Klebsiella pneumoniae*.

Material and Methods

All samples were routinely cultured on MacConkey and blood agar plates. These plates were routinely incubated at 37˚C aerobically and after overnight incubation, they were checked for bacterial growth. The organisms were identified by their colony morphology, staining characters, pigment production, motility and other relevant biochemical tests as per standard laboratory methods of identification.

Isolation and identification of organisms

Suspected Gram negative organisms were identified by colony characteristics, motility, oxidase reaction, citrate utilization, indole and gas production and sugar fermentation reactions .Triple sugar iron agar was used for H2S production, sugar fermentation.

Maintenance and preservation of culture strains

Organisms grown in appropriate media for 18 hours were preserved in a nutrient agar slant at 2-8° C in a refrigerator.

Samples (Sputum, pus, urine, blood, ear discharge) received for culture and sensitivity testing to microbiology laboratory of MIMS will be processed as following.

The samples were plated onto blood agar and Macconkey agar. The Macconkey agar plate showing lactose fermenting (pink) colonies were confirmed to be E coli and Klebsiella pneumoniae by series of biochemical tests.

Clinical isolates of E. coli & Klebsiella pneumonia identified as per above mentioned procedure was be selected for the study.

Quality Control (QC) organisms used for the study:
E. coli ATCC 25922
Klebsiella pneumonia ATCC 700603

Modified Kirby-Bauer sensitivity testing method was used for this purpose. Muller Hinton agar media was used, which has PH 7.2-7.4. Media was transfer in to 90 mm diameter sterile Petri dishes to a depth of 4 (four) mm. The surface was lightly and uniformly inoculated by cotton swab in three directions rotating the plate approximately 60°, to ensure even distribution. Prior to inoculation, the swab stick was dipped into bacterial suspension having visually equivalent turbidity to 0.5McFarland standards. The swab stick was then took out and squeezed on the wall of the test tube to discard extra suspension. Inoculated plates were incubated at 37 °C for 24 hours. On the next day, plates were read by taking measurement of zone of inhibition.
Testing for ESBL production

a. Screening test

Following antibiotic disks will be placed on MHA after performing a lawn culture of the test organism, incubated at 35ºC for 24 hours. The zone diameter is measured after 24 hours.

Antibiotic	Zone size for possible ESBL producing strains
Aztreonam(30µg)	≤27mm
Cefotaxime(30µg)	≤27mm
Cefpodoxime(30µg)	≤22mm
Ceftazidime(30µg)	≤22mm
Ceftriaxone(30µg)	≤25mm

Screen positive isolates are further tested for confirmation of ESBL production

b. CLSI phenotypic confirmatory test

By disk diffusion method: After performing a lawn culture of the test organism, a ≥5mm increase in zone diameter for cefotaxime(30µg)& ceftazidime(30µg) tested in combination with clavulanic acid(CA) versus its zone when tested alone confirms an ESBL producing organism.

Results

Table 1: Distribution of *E. coli* and *K. pneumoniae* isolates in males and females

Organism	Gender		Total
	Female	Male	
E. coli	72(54.1%)	61(45.9%)	133(53.2%)
K. pneumoniae	31(26.5%)	86(73.5%)	117(46.8%)
Total	103(41.2%)	147(58.8%)	250(100%)

Table 2: Distribution of *E. coli* and *K. pneumoniae* isolates in males and females

Organism	Gender		Total
	Female	Male	
E. coli	72(54.1%)	61(45.9%)	133(53.2%)
K. pneumoniae	31(26.5%)	86(73.5%)	117(46.8%)
Total	103(41.2%)	147(58.8%)	250(100%)

Above table shows distribution of isolates in males and females, where *E. coli* have been isolated 0with female preponderance. 117 *K. pneumoniae* are isolated with male preponderance.

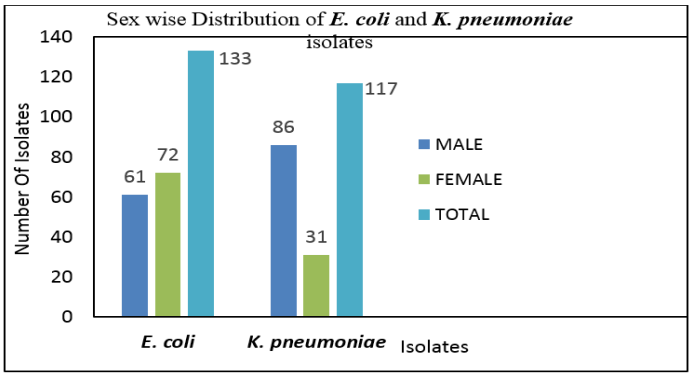

Fig. 1: Shows Sex wise Distribution of *E. coli* and *K. pneumoniae* isolates

Table 3: Age distribution of patients with *E. coli* and *K. pneumoniae* isolates

Age in years	Organism		Total
	E. coli	*K. pneumoniae*	
1-30 days	0	5(4.3%)	5(2%)
1-12 months	1(0.8%)	1(0.9%)	2(0.8%)
1-2 years	2(1.5%)	4(3.3%)	6(2.4%)
3-5 years	5(3.7%)	2(1.7%)	7(2.8%)
6-10 years	3(2.3%)	2(1.7%)	5(2%)
11-15 years	4(3%)	0	4(1.6%)
16-20 years	10(7.5%)	5(4.3%)	15(6%)
>20 years	108(81.2%)	98(83.8%)	206(82.4%)
Total	133(100%)	117(100%)	250(100%)

Above table shows age distribution and isolate distribution in patients studied, where majority of samples 206(82.4%) were received from patients aged more than 20yrs.

17 (12.8%) samples were in age between 11-25 years from urine of females with UTI.

Only *K. pneumoniae* was isolated from neonates (blood culture and sensitivity).

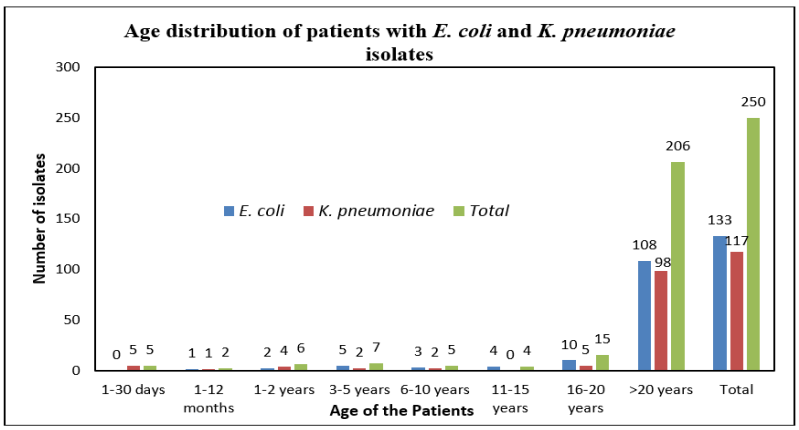

Fig. 2: Age distribution of patients with E. coli and K. pneumonia isolates

Table 4: Sample wise distribution of isolates

Organism	PUS	Sputum	Urine	Blood	Total
E. coli	50(37.6%)	13(9.8%)	70(52.6%)	0	133(100%)
K. pneumoniae	46(39.3%)	50(42.7%)	15(12.8%)	6(5.2%)	117(100%)
Total	96(38.4%)	63(25.2%)	85(34%)	6(2.4%)	250(100%)

Out of 250 samples studied, majority of samples 96(38.4%) were from pus, 85(34%) were from urine and 63(25.2%) from sputum. *E.coli* was isolated 133 out of 250 samples, 70(52.6%) from urine samples majority were young females suffering from UTI.

K. pneumoniae was isolated from 117 out of 250 samples, 50(42.7%) from sputum samples, 46(39.3%) from pus samples and 15(12.8%) from urine samples.

From blood samples only *K. pneumoniae* was isolated in 6 cases.

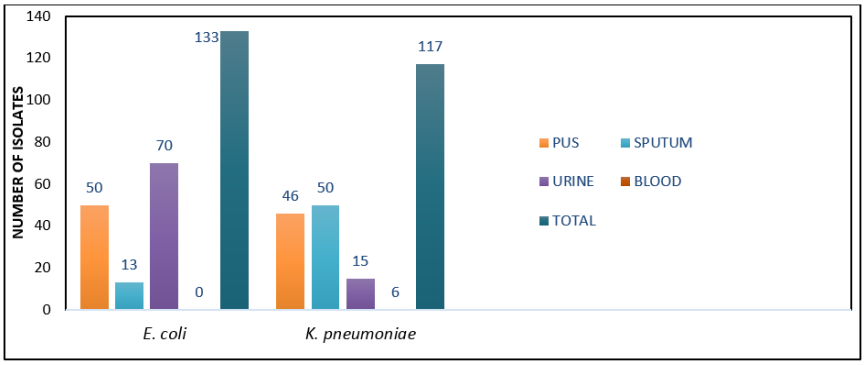

Fig. 3: Sample wise distribution of isolates

Table 5: Distribution of isolates showing ESBL positive by screening and confirmatory test

Organism	ESBL by Screening		ESBL Confirmed	
	Negative	Positive	Negative	Positive
E. coli (n=133)	25(18.8%)	108(81.2%)	45(33.8%)	88(66.2%)
K. pneumoniae (n=117)	29(24.8%)	88(75.2%)	42(35.9%)	75(64.1%)
Total(n=250)	54(21.6%)	196(78.4%)	87(34.8%)	163(65.2%)

Above table shows detection of ESBL. Out of 133 *E. coli* isolated, 108(81.2%) screened positive and 88(66.2%) were confirmed ESBL by CLSI recommended method and out of 117 *K. pneumonia* isolated, 88(75.2%) screened positive and 75(64.1%) confirmed positive for ESBL.

Discussion

Table 1 shows gender distribution of isolates where female preponderance of *E. coli* can be attributed to UTI caused by the organism.

In the present study, a total of 250 strains are studied, of which 133 are *E. coli* and 117 are K pneumoniae.

Most of the isolates are from sample collected from patients aged over 20 years as shown in table 1, which can be attributed to their environmental exposure and outdoor activity.

Of the manual ESBL confirmation methods, disk augmentation displayed the overall highest positivity rate, whereas E test detected ESBL production in only 2/26 isolates and yielded a high number of non-determinable results.

Rastogi V, et al. and Garrec H, et al. reported higher rates (100% and 93% respectively) of incidence than our study.

While study by Rodrigues C, et al., Babypadmini S, et al. and Bhattacharjee A, et al. reported the rate to be 40% to 62.5% with the CLSI recommended Phenotypic confirmation method, which is lower than our study rates.

It is assumed that this high rate of ESBLs by phenotypic method, may be due to mutation of first two parent gene TEM-1, SHV-1and newer most prevalent gene CTX-M in the world. CTX-M gene now the most common in E.coli in community and it may be due to over use of ceftriaxone or due to fecal carriage and transfer gene by horizontal transmission.

Isolates that test positive with screening test but negative on confirmatory test should be looked for CMY-2 and FOX-5 Amp C genes.

In India, high prevalence of ESBL producing *Klebsiella* strains has been reported by various groups. Reported frequency of ESBL producing *Klebsiella* spp. from India ranged between 6 and 87 per cent.

Conclusion

ESBLs producing Gram-negative bacteria are an increasing public health problem worldwide because of their resistance to β- lactam antibiotics. ESBL, gene are typically carried on transferable plasmids or are part of the bacterial chromosome. This enzyme which have been detected primarily in *E. coli and K. pneumoniae* but were also found in other Gram negative bacteria, including non-fermenters and members of the family Enterobacteriaceae.

Because resistance to broad spectrum antibiotic ß lactams is linked to various molecular mechanisms

involving a wide variety of enzymes, detection has become diagnostic challenge in clinical laboratories.

In our study, since, detection of ESBL is over 80%, this study indicates that routine ESBL detection should be made imperative and empirical use of third generation cephalosporins must be discouraged.

In most cases, the standard disk diffusion tests are effective. ESBL detection in routine laboratories are done by disk diffusion methods. Considering the grave scenario of antibiotic resistance in our country, it is high time that all clinical laboratories start detecting these enzymes routinely and accurately.

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