ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 10, Issue 4, 2017

Online - 2455-3891
Print - 0974-2441

Research Article

PREVALENCE OF VARIOUS BETA-LACTAMASES AMONG GRAM-NEGATIVE BACILLI IN URINARY ISOLATES FROM PATIENTS IN A TERTIARY CARE HOSPITAL OF NORTHERN INDIA

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Received: 20 May 2016, Revised and Accepted: 16 January 2017

ABSTRACT

Objective: Urinary tract infections are considered among the most common infections, occurring either in the community or health-care setting. We are left with very few options for the treatment due to rapid development of antibiotic resistance among the organisms. To find out the prevalence of various types of β-lactamases among urinary isolates.

Methods: Seven antibiotic discs (HiMedia) were placed in combinations and approximation in a particular sequence on a 90 mm diameter Mueller-Hinton agar plate.

Results: Out of a total 165 urinary isolates, 66 (40%) isolates were positive for extended spectrum β-lactamase (ESBL) production, AmpC β-lactamases (AmpC) activity was present in 31 (18.78%) isolates, co-production of both ESBL and AmpC was seen in 16 (9.69%) isolates, 3 (1.81%) isolates produced metallo β-lactamase (MBL), 2 (1.21%) isolates produced both MBL, and ESBL and 1 (0.60%) isolates were positive for inducible third generation cephalosporin resistance.

Conclusion: With the presence of such high prevalence of various β-lactamases in clinical isolates of gram-negative bacilli and also other types of antibiotic resistance, antibiotic policy should be made, and strict adherence should be followed.

Keywords: Extended spectrum β-lactamase, AmpC β-lactamase, Metallo β-lactamase.

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INTRODUCTION

Urinary tract infections (UTIs) are considered among the most common infectious diseases occurring in either the community or health-care setting [1].

Hence, these infections should be treated with appropriate antibiotics to avoid the development of the resistance among the organisms. We are very close to the era when no antibiotic will be effective for treating bacterial infections. Antibiotic resistance is increasing at an alarming speed and is really a matter of serious concern. Inappropriate use of antibiotics in medical and veterinary practice is responsible for the current situation.

Beta-lactam group of antibiotics is commonly used drugs for treating various types of infections including UTIs.

Unfortunately, Gram-negative bacteria have acquired resistance to beta-lactam group of antibiotics and other commonly used antibiotics. Hence, we are left with very limited options. Extended spectrum β-lactamases (ESBLs) were first reported in Europe in 1983 [2]. Plasmid-mediated AmpC β-lactamases (AmpC) was reported for the first time in 1988 [3].

There are many types of bacterial resistance in Gram-negative bacteria, e.g., resistance due to ESBL production [4], AmpCβ production [5], due to porin deficiency [6], and efflux mechanism [7]. Among these, extended spectrum β-lactamase and AmpC β-lactamase found to be the most common [8].

METHODS

The study was conducted in 2016 between March and July on isolates from patients with significant bacteriuria from both in patient department and outpatient department. These clinical isolates were nonrepetitive.

Two 90 mm plates of Mueller-Hinton agar were used. One (Plate 1) for detecting various types of enzymes and the second plate (Plate 2) for susceptibility to various antibiotics depending on the type of organism isolated, lower UTI or complicated UTI, age, pregnancy, and renal function or any other clinical condition of the patient.

Plate 1: It was inoculated with 0.5 McFarland standard of the organism to be tested and incubated at 35±2°C in ambient air. Seven antibiotic discs were placed in a specific order for the expression of various enzymes, e.g., ESBLs, AmpC, inducible AmpC, and MBL (metallo β-lactamases) as shown in Fig. 1. Ceftazidime (CAZ) 30 mg, cefoxitin (CX) 30 mg, imipenem (IPM) 10 mg, IPM + EDTA (IE) 10+30 mg, and cefepime (CPM) 30 mg.

CPM + clavulanic acid (CFC) 30/10 mg, CX + cloxacillin (CXX) 30/10 mg.

Antibiotic susceptibility was done according to Clinical Laboratory Standards Institute (CLSI) guidelines 2016 [9].

All the discs were obtained from HiMedia. Quality control strains American Type Culture Collection (ATCC) *Escherichia coli* 25922 and ATCC *Klebsiella pneumoniae* 700603 were used as ESBL negative and positive control, respectively, and were obtained from HiMedia.

ESBL

Combination disc method was used. A difference of increase in diameter of ≥ 5 mm in the zone diameter of the CPM alone and combination with CFC (CPM + CA) (Fig. 2), was considered as positive for the production of ESBL enzyme [10].

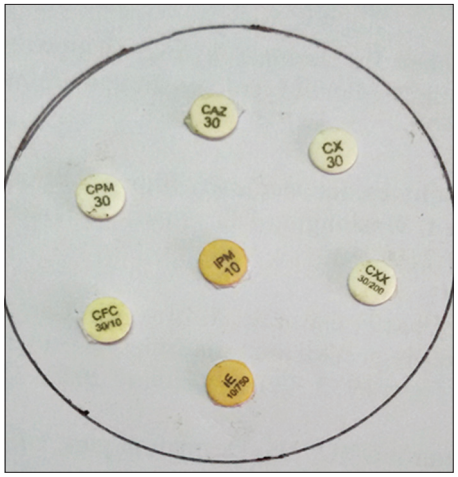

Fig. 1: Plate 1: Placement of discs on Mueller-Hinton agar in a specific order

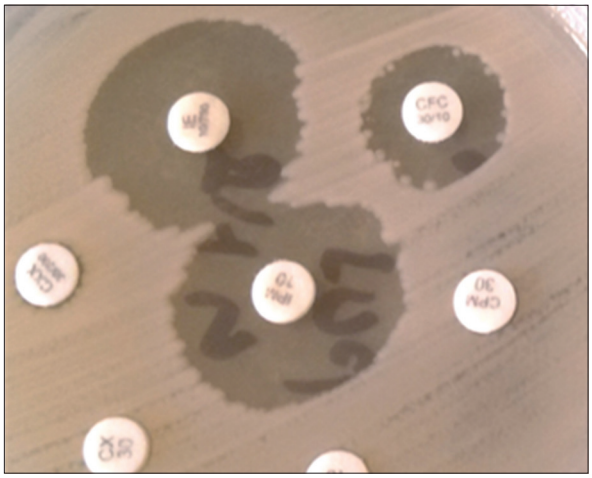

Fig. 2: Plate 2: Extended spectrum β-lactamase

AmpC

a. When there is difference in the zone of inhibition between CX + CXX disc combination and CX alone of ≥4 mm (Fig. 2), indicates the production of AmpC [11].
b. AmpC (Inducible): Blunting of the zone of CAZ (reporter substrate) toward inducing substrate like CX and IPM by 2 mm (Fig. 6). The edge-to-edge distance between CX and CAZ disc should be 15 mm [12].

Metallo β-lactamases (MBL)

Organisms were suspected to be MBL producer when they were found to be resistant to both IPM and meropenem.

MBL production was further confirmed by IE double disk synergy test.

An isolate was considered MBL producer when the zone of inhibition was ≥5 mm with IE disc as compared to the zone of inhibition produced by IPM disc alone [13].

RESULTS

Out of a total 165 isolates, 66 (40%) were positive for ESBL production (Fig. 2), 31 (18.78%) were found to produce AmpC (Fig. 3), 16 (9.69%) showed co-production of ESBL, and AmpC enzymes (Fig. 4). 3 (1.81%) isolates were found positive for MBL production and 2 (1.21%) were found to co-produce ESBL and MBL (Fig. 5). Furthermore, 1 (0.60%) strain of *Citrobacter* sp. was found positive for inducible third generation cephalosporin resistance (Figs. 6 and 7, Table 1).

Fig. 3: AmpC β-lactamases

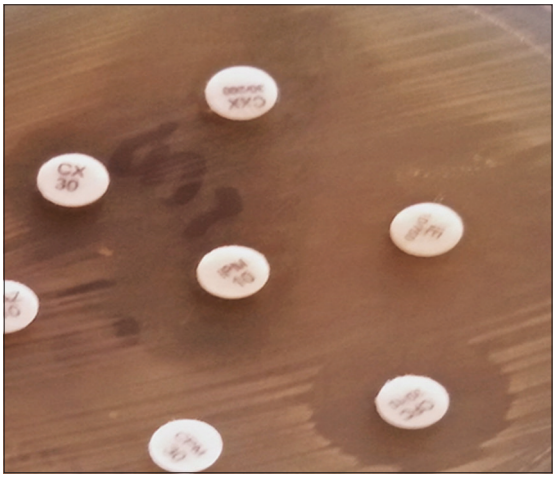

Fig. 4: Extended spectrum β-lactamase and AmpC β-lactamases

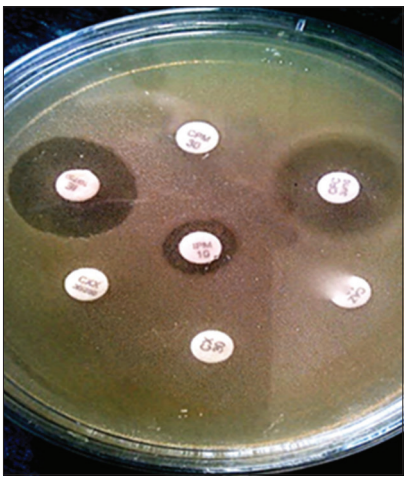

Fig. 5: Extended spectrum β-lactamase and Metallo β-lactamase

DISCUSSION

In this study, we detected β-lactamase enzymes using certain combinations and approximation of antibiotic discs in a particular order

to determine various β-lactamases singly or in various combination if the organism is also producing another enzyme.

Fig. 6: Inducible resistance (Blunting of zone of inhibition around ceftazidime toward cefoxitin disc)

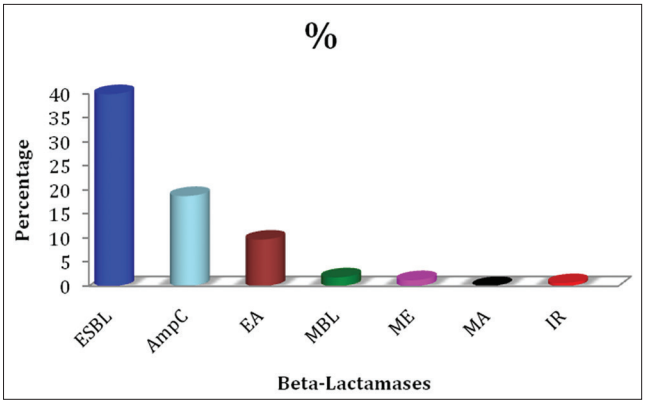

Fig. 7: Percentage of production of various beta-lactamase enzymes extended spectrum β-lactamases, AmpC β-lactamases, Metallo β-lactamase, ESBL + AmpC, MBL + ESBL, MBL + AmpC, inducible third generation cephalosporin resistance

It is very important to look for the occurrence of various β-lactamases in the isolates to avoid the treatment failure, which may lead to serious complications, especially in complicated UTI.

We preferred using CPM (Zwiteronic, also referred to as fourth generation cephalosporin) as ESBL screening agent. High-level AmpC expression has minimal effect on the activity of CPM, which is the reason this drug is considered more reliable for the detection of ESBL in the presence of an AmpC [14].

Sensitivity with CX-CXX combination has been found to be (95%) and specificity (95%) with cut-off an increase in zone diameter of ≥4 mm [11].

Recently, some studies have been carried out looking for the occurrence of various enzymes in the clinical isolates of Gram-negative bacteria (Table 2). In studies carried out by Oberoi *et al.* and Kolhapure *et al.* [16,18] they have looked for the co-production of various types of enzymes. Fortunately, our study shows the lowest prevalence of MBL and MBL + ESBL production.

A previous study conducted by us last year showed good susceptibility results for treating some of the uropathogens with nitrofurantoin (90.66%) [19]. However, this drug is effective only for treating lower UTI because due to rapid elimination, sufficient urinary concentration for treating upper UTI is not achieved.

ESBL producing organisms can be treated with β-lactam group of drug along with β-lactamase inhibitor combination. Furthermore, quinolones and aminoglycosides can be tested for susceptibility. If the organism is co-producing AmpC, inhibitors are not effective. Although AmpC producing isolates are susceptible to four generation cephalosporin, while ESBL producing organisms are variably resistant to four generation cephalosporin [20], CPM may not be effective for treating ESBL, and AmpC producing bacterial infections due to high inoculum effect [21]. The available clinical data have shown that carbapenems are more effective than CPM in treating serious infections due to large numbers of AmpC producing bacteria [22].

Enterobacter cloacae, *Enterobcater aerogenes*, *Citrobacter freundii*, and *Serratia marcescens*, *Providencia* sp., *Morganella morganii*, and *Pseudomonas aeruginosa* may develop resistance during prolonged therapy with third generation cephalosporin (oxyimino-cephalosporin) as a result of derepression of AmpC-lactamase. Therefore, isolates that are found initially susceptible may become resistant within 3-4 days

Table 1: Prevalence of ESBL, AmpC, MBL, inducible resistance and co-existence of these enzymes among Gram-negative bacilli in urinary isolates

Organisms	Total isolates	n (%)						IR
		ESBL	AmpC	EA	MBL	ME	MA	
E. coli	140	53 (37.857)	28 (20)	14 (10)	1 (0.714)	0	0	0
K. pneumoniae	15	9 (60)	2 (13.33)	1 (6.66)	1 (6.66)	1 (6.66)	0	0
Citrobacter spp.	6	2 (33.33)	1 (16.66)	1 (16.66)	1 (16.66)	0	0	1 (16.66)
Proteus mirabilis	2	2 (100)	0	0	0	0	0	0
P. aeruginosa	2	0	0	0	0	1 (50)	0	0
	165	66 (40)	31 (18.78)	16 (9.69)	3 (1.81)	2 (1.21)		1 (0.60)

E. coli: Escherichia coli, K. pneumonia: Klebsiella pneumonia, P. mirabilis: Proteus mirabilis, P. aeruginosa: Pseudomonas aeruginosa, ESBL: Extended spectrum β-lactamase, AmpC: AmpC β-lactamases, EA: ESBL+AmpC, MBL: Metallo β β-lactamase, ME: MBL+ESBL, MA: MBL+AmpC

Table 2: Recent studies showing prevalence of various β-lactamases in different states of India

Author's name	Year and place	ESBL%	AmpC%	EA%	MBL%	ME%	MA%
Nagedo *et al.* [15]	2012, Bhopal	70.38	52.05	-	23.05	-	-
Oberoi *et al.* [16]	2013, Amritsar	35.16	5.49	6.59	10.98	8.79	3.67
Haider *et al.* [17]	2014, Aligarh	54.9	36.6	-	17.9	-	-
Kolhapure *et al.* [18]	2015, Hyderabad	38.52	10.33	9.77	9.20	4.81	6.23
Thakur *et al.* (our)	2016 Muzaffarnagar	40	18.78	9.69	1.81	1.21	0

ESBL: Extended spectrum β-lactamase, AmpC: AmpC β-lactamases, MBL: Metallo β-lactamase, ME: MBL+ESBL, MA: MBL+AmpC

after beginning of therapy. Testing of repeat isolates may be warranted. CLSI guidelines 2016 [9].

CONCLUSION

With such high prevalence of various β-lactamases in clinical isolates of Gram-negative bacilli and also other types of antibiotic resistance, antibiotic policy should be made, and strict adherence should be followed. Staff members involved in antibiotic susceptibility reporting should keep themselves updated with the current knowledge. Restricted use of third and fourth generation cephalosporin. Infection control practice such as proper hand washing, isolation of the patient harboring resistant organism, dealing with outbreaks, and antibiotic policy with the appropriate use of antibiotics should be framed.

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