Research Article

Phenotypic detection of ESBL and MBL in Gram Negative bacilli isolated from clinical specimens

Kamble D[1]

[1]Miss. Deepali Shivajirao Kamble, Tutor, Department of Microbiology, SKNMC, Pune, India

Address for correspondence: Miss. Deepali Shivajirao Kamble, Email: deepali19kamble@yahoo.com

Abstract

Introduction: Antimicrobial resistance is a growing threat worldwide. The prevalence of Extended spectrum beta lactamases (ESBLs) and Metallobetalactamases (MBL) among Gram negative bacilli constitutes a serious threat to current beta-lactam therapy leading to treatment failure. **Material and Methods:** ESBL was detected by double disc diffusion test using ceftazidime alone and in combination with clavulanic acid. MBL detection was done by Imipenem EDTA combined disc diffusion test. **Results:** Out of 549 Gram negative bacilli resistant to 3G cephalosporin, 179(32.60%) were ESBL producers and out of 236 Gram negative bacilli resistant to carbapenem, 47(19.19%) were MBL producers. ESBL production was observed in *E.coli*, *Klebsiella* spp. *Proteus spp* and *Citrobacter* spp, while MBL production was observed in *Pseudomonas aeruginosa*, *Acinetobacter spp, E.coli* and *Klebsiella spp* isolated from various clinical samples. **Conclusion:** Simple disc method can be routinely employed to detect these common resistance mechanisms which will reduce the mortality and also spread of such resistant strains.

Keywords: Extended spectrum –beta lactamases, Metallobetalactamases, Gram Negative Bacilli.

Introduction

Resistant bacteria are emerging worldwide as a threat to the favorable outcome of common infections in community and hospital settings. Hospital acquired infections are most commonly caused by Gram-negative bacilli, particularly by members of Enterobacteriaceae family. These microbes are known to exhibit multidrug resistance. Beta-lactams (mainly extended-spectrum cephalosporins and carbapenems) and fluoroquinolones are used to treat infections caused by these microorganisms [1]. Resistance to third generation Cephalosporins is mediated by Extended Spectrum Beta Lactamase enzymes(ESBL)[2]. ESBLs are enzymes that mediate resistant to Cephalosporins and Aztreonam (but not the cephamycins or carbapenems) by hydrolysis and inhibited by by β-lactamase inhibitors such as clavulanic acid [3]. ESBL producing isolates, in addition to being resistant to ß-lactam antibiotics, often exhibit resistance to other classes of drugs such as aminoglycosides, cotrimoxazole, tetracycline and Fluoroquinolones [4]. ESBLs are often located on plasmids that are transferable from strain to strain [5].

Carbapenems are used as drug of choice to treat infections caused by beta-lactam resistant bacteria. But extensive and sometime unnecessary use emergences carbapenem resistant bacteria. Resistance to carbapenem is predominantly mediated by metallo-betalactamases [6].

Early detection of MBL and ESBL producing organisms is crucial to establish appropriate antimicrobial therapy and to prevent their interhospital and intrahospital dissemination. So the present study was undertaken to detect ESBL and MBL in Gram negative bacilli from clinical isolates.

Material and Methods

The study was conducted in tertiary care hospital, Pune during 2009 to 2011. A total of 1278 Gram negative isolates were isolated from various samples (blood, urine, sputum, pus, fluids). They were processed and

Manuscript received: 4th Aug 2015
Reviewed: 14th Aug 2015
Author Corrected: 27th Aug 2015
Accepted for Publication: 11th Sept 2015

identified by standard Microbiological procedures [7]. The antibiotics susceptibility testing was performed by Kirby- Bauer disc diffusion method using Clinical and Laboratory Standard Institute (CLSI) guidelines (2011). 549 isolates resistant to the third generation cephalosporins were tested for ESBL production and 236 isolates showing resistance to imipenem were tested for MBL production.

Detection of ESBL: This was performed by double disc diffusion method. Test organism were inoculated on Mueller hinton agar. The ceftazidime (30 µg) and ceftazidime-clavulanic acid (30 µg / 10 µg) were placed at a distance of 20 mm apart on the agar. An increase of ≥ 5mm in zone of inhibition of the combination discs in comparison to the ceftazidime disc alone was considered to be ESBL producer

Detection of MBL: This was performed by Imipenem EDTA combined disc test. Two (10 mcg) imipenem discs were placed on a plate inoculated with the test organism on Mueller hinton agar, and 10µl of 0.5 M EDTA solution was added to one disc. A zone diameter difference between the imipenem and imipenem + EDTA of ≥7 mm was interpreted as a positive result for MBL production.

Results

Out of 549 Gram negative bacilli 179 (32.60%) were ESBL producers and from 236 Gram negative bacilli 47(19.91%) were MBL producers. E. coli showed maximum ESBL production (35.23%).Maximum MBL producers was seen in Pseudomonas spp.(23.62%). Majority of ESBLs were isolated from urine followed by pus and sputum, while majority of MBL were from pus followed by urine.

Table 1: ESBL producers among different isolates

Organisms	Total no. of isolates resistant to cephalosporin	Isolates positive by Ceftazidime and Ceftazidime+clavulanic acid double disc diffusion (%)
E. coli	298	105 (35.23)
Klebsiella spp.	188	62 (32.97)
Proteus spp.	54	11 (20.37)
Citrobacter spp.	5	1 (20)
Salmonella spp.	4	0 (00)
Total	**549**	**179 (32.60)**

The majority of the ESBL producers were *E. coli* (35.23%) followed by *Klebsiella* spp. (32.97%). No ESBL producers were found among the *Salmonella* spp. and only one isolate (20%) of *Citrobacter* spp. was found to produce ESBLs

Table 2: MBL producers among different isolates

Organisms	Total no. of isolates resistant to carbapenem	Isolates positive by Imipenem EDTA double diffusion disc method (%)
Pseudomonas aeruginosa	127	30 (23.62)
Acinetobacter spp.	80	15 (18.75)
E. coli	18	01(5.55)
Klebsiella pneumoniae	11	01 (9.09)
Total	**236**	**47 (19.91%)**

The majority of the MBL producers were *Pseudomonas aeruginosa* (23.62%) followed by *Acinetobacter* spp. (18.75%), *Klebsiella* spp. (9.09%) and *E. coli* (5.55%)

Table 3: Distribution of ESBL and MBL producers in various clinical specimens

Specimens	ESBL producers (%)	MBL producers (%)
Blood	4(2.24)	3(6.38)
Pus	71(39.66)	20(42.55)
Urine	78(43.58)	19(40.42)
Sputum	11(6.15)	00(00)
Fluids	08(4.46)	2(4.2)
CSF	00(00)	00(00)
Others	07(3.92)	3(6.38)
Total	**179(100)**	**47(100)**

Maximum number of ESBLs producers were isolated from Urine 78 (43.58%) followed by pus (39.66%) While maximum number of MBLs producers were isolated from Pus 20 (42.55%) followed by urine (40.42%) No ESBLs and MBLs producers were reported from a cerebrospinal fluid (CSF).

Discussion

The emergence of antibiotic resistance is a matter of great concern, particularly in hospitals. Antibiotic resistant bacteria appear to be biologically fit and capable of causing serious life threatening infections. The increase in antibiotic resistance among gram-negative bacilli, such as Enterobacteriaceae group, *Pseudomonas aeruginosa* and others, is a notable example and how bacteria can procure, maintain and express new genetic information that can confer resistance to one or several antibiotics. Resistance in gram-negative bacteria is a serious problem and calls for an effective infection control measures to curb their dissemination [8, 9].

Recent reports show that resistance to various groups of antibiotics particularly to fluoroquinolones and beta lactam antibiotics is increasing in the members of the family Enterobacteriaceae and *P. aeruginosa* making the treatment regimens limited.

In our study out of 549 Gram negative bacilli, 179 were ESBL producers. ESBLs were predominantly present among *E. coli* 105(35.23%) followed by *Klebsiella* spp. 62(32.97%), *Proteus* spp. 11(20.37%) and *Citrobacter* spp. 1(20%).

Our findings of *E. coli* as the most common ESBLs producing Gram-negative bacilli followed by *Klebsiella* spp. is exactly similar to Agrawal et al, Tsering et al, Shiju et al, [10,11,12]. These studies also report *E. coli* as the most common ESBLs producing Gram-negative bacilli and *Klebsiella* spp. as the second most common ESBLs producing Gram-negative bacilli.

Our findings of isolation of maximum number of ESBLs producers from urine followed by pus appears to be similar to majority of the earlier studies, which also reported maximum isolation from urine and pus [10,13,14], These workers have also reported maximum isolation of ESBLs from urine. Our findings totally disagree with Kusum et al. and Kumar et al., who reported maximum isolation of ESBLs producers from sputum and exudates [15, 16].

In our study out of 236 Gram negative bacilli, 47 were MBL producers. MBLs were predominantly present among *Pseudomonas spp* 30(23.62%) followed by *Acinetobacter* spp. 15(18.75%), E.coli1 (5.55%) and Klebsiella spp1(9.09%).

Our findings of *P. aeruginosa* as the most common MBLs producer correlate with Kumar et al.[16] Our findings of isolation of maximum number of MBLs producers from pus fairly correlates with Kumar et al. [17] & Rao et.al [18].

Correct identification of ESBL and MBL positive strains in due time is mandatory not only for optimal patient management but also for immediate institution of appropriate infection control measures to prevent the spread of these organisms. Simple disc method can be routinely employed to detect these resistant strains. Disc diffusion test would screen all ESBL and MBL Gram negative bacilli in the diagnostic laboratory. These methods are technically simple and inexpensive [19,20].

Conclusion

Simple phenotypic screening tests are proved to be rapid and convenient for the detection in the clinical laboratory. To overcome the problem of emergence and the spread of multidrug resistant organisms, a combined interaction and cooperation between the microbiologists, clinicians and the infection control team is needed.

Funding: Nil
Conflict of interest: None.
Permission of IRB: Yes

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How to cite this article?

Kamble D. Phenotypic detection of ESBL and MBL in Gram Negative bacilli isolated from clinical specimens . *Int J Med Res Rev* 2015;3(8):866-870. doi: 10.17511/ijmrr.2015.i8.163.

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