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Screening for ESBL, Amp C and Carbapenemase Production among Enterobacteriaceae Using 12 Disc Method in Blood Culture Isolates

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Abstract: Antibiotic resistance among gram negative bacteraemia is a huge problem in health care settings. Detection of extended spectrum beta lactamases (ESBL), Amp C and carbapenemases would help implementing empirical treatment in bacteremia patients. There is a need of a simple and inexpensive screening test for multidrug resistant isolates. A total of 125 multidrug resistant isolates of Enterobacteriaceae were screened by twelve disc method and confirmed by the modified Hodge test and imipenem EDTA method. Among the 125 multidrug resistant isolates, *Klebsiella pneumoniae* was the most common 58(46.4%) followed by *Escherichia coli* 31(24.8%).The production of ESBL, MBL, KPC and Amp C were 61.6%,22.4%,7.2% and 32% respectively. The presents study indicates the burden of ESBL, Amp C and Carbapenemase production among Enterobacteriaceae using 12 disc method in blood culture isolates. The 12 disc method is a simple, cost effective and easy screening method for multidrug resistant isolates.
Key words: Twelve disc method, blood stream infection, ESBL, MBL, Amp C.

INTRODUCTION

Multidrug resistant gram negative bacteraemia is a significant problem in health care setting for the past one decade [1]. Extended spectrum beta lactamases (ESBL) are important and common resistance mechanism encountered in hospital setup. These are generally transmitted by plasmids. The problem is compounded further as they carry other antibiotic resistance genes along with ESBL [2]. Amp C beta lactamases hydrolyse all beta lactam antibiotics except cefepime and carbapenems. [3]With ESBL and AmpC isolates, carbapenems are used as treatment option.

With the emergence of carbapenemase production the situation is worrisome as we are left with last resort drugs that are tigecycline and colistin [4]. Early initiation of appropriate antibiotic in bacteremic patients would reduce risk of mortality [1]. However, with the emergence of multidrug resistance, empirical antibiotics often fail to decrease mortality. Hence, the present study was conducted to detect the burden of ESBL, Amp C and Carbapenemase production among Enterobacteriaceae using twelve disc method in blood culture isolates. The 12 disc method is a simple, cost effective and easy screening method for ESBL, Amp C, MBL and KPC detection, prompt and appropriate treatment could be initiated at the earliest.

MATERIALS AND METHODS

A cross-sectional study was conducted for one year in a tertiary care teaching hospital. A total of 125 multidrug resistant isolates of Enterobacteriaceae from blood cultures with clinical suspicion of septicaemia were included in the study.

The organisms were identified by standard microbiological techniques. The strains were then subjected to the 12 disc method [5].

Twelve disc method [5].

The Mueller Hinton Agar (MHA) prepared was poured in 150mm sterile plastic petridishes. Approximately 42 ml of the medium was poured per plate. The poured plates were stored at 4°C and used within 7 days of preparation.

A 0.5 Mc Farland standard inoculum of the test strain was prepared and a lawn culture was done on the MHA plate prepared. The twelve antibiotic discs (Himedia, Mumbai, India) were then placed on the MHA plate. The antibiotic discs included are aztreonam (30µg), ceftazidime((30µg), ceftazidime /clavulanate (30/10µg), cefotaxime ((30µg), cefotaxime /clavulanate (30/10µg), cefoxitin (30µg), cefotetan (30µg), ceftriaxone (30µg), cefepime (30µg), ertapenem (10µg), imipenem(10µg) and meropenem(10µg) in a specific

order. The plates were incubated within 15 minutes of disc application and incubated at 37°C in ambient air for 24 hours. The zones of inhibition were measured as sensitive, intermediately sensitive and resistant as per CLSI 2015[6].

Interpretation

ESBL production was positive if zone of inhibition around the disc of ceftazidime and cefotaxime disc with clavulanic acid was ≥ 5mm than that of ceftazidime and cefotaxime disc alone.

AmpC production was positive if the strain was resistant to cefoxitin (Zone diameter ≤14 mm) but susceptible to cefepime (Zone diameter ≥25 mm).

MBL production was suggested if the strain was resistant to all carbapenems (Zone diameter of imipenem ≤19 mm, meropenem ≤19 mm and ertapenem ≤18 mm).This was confirmed by Imipenem EDTA combined disc test as described by Yong *et al.* [7].

Imipenem EDTA combined disc test

The test strain was inoculated on MHA plate as per CLSI guidelines. Two imipenem disc were placed on the inoculated MHA plate. To one of the disc 750 µg of EDTA was added. The inhibition zone of imipenem and imipenem EDTA was compared after 16-18 hours of incubation at 35°C. A positive test was indicated if the zone enhancement with EDTA was ≥7mm than imipenem disc alone [7].

KPC production was suggested if the strain was imipenem sensitive (Zone diameter ≥23 mm) and ertapenem resistant (Zone diameter ≤19 mm). This was confirmed by Modified Hodge test as per CLSI guidelines [6].

Statistical Analysis

All the data were entered in Microsoft excel and analyzed using SPSS 15.0 Version (SPSS Inc. Chicago IL, United States of America). All categorical variables were expressed as number and proportion. Categorical variables were compared between the two groups using Chi-square test. *p* value < 0.05 was considered significant.

RESULTS

The study involved 125 multidrug resistant isolates of Enterobacteriaceae from blood cultures over a period of one year. Among the enrolled patients, 80(64%) were male patients with a male: female ratio of 1.8:1.Most of the patients 52% were in the age group of 19-40 years. A total of 32(25.6%) of the patients were from the intensive care unit and the rest were from wards.

Table 1 depicts the various organisms isolated in blood culture. Among the 125 isolates *Klebsiella pneumonia* constituted 58(46.4%) followed by *Escherichia coli* 31(24.8%).

Susceptibility pattern of isolates

The susceptibility pattern of the isolates to common antibiotics is mentioned in Table 2. Among the 125 isolates, in the carbapenem group of drugs, ertapenem was highly sensitive with 97(77.6%) followed by meropenem 95 (76%) and imipenem76 (60.8%). In the quinolone group, levofloxacin was a highly effective drug with 55.2% sensitivity. However, among the aminoglycosides both the drugs, gentamicin and amikacin had low sensitivity of 26.4% and 14% respectively.

Sensitivity to Ampicillin was more significantly associated with ESBL than Non ESBL isolates However sensitivity to cefoxitin and cefotetan was more significantly associated with non ESBL producers than ESBL producers (P value <0.05)(Table 3).

On comparing MBL with Non MBL, we found that betalactam/betalactamase inhibitor combinations like ampicillin/sulbactam and cefotaxime/clavulanic acid were significantly more sensitive in MBL producers than non MBL producers (Table 3).

Table 4 depicts the coproduction of various enzymes among the blood stream isolates

Coproduction of ESBL and AmpC was 17.6%, while ESBL and MBL was 10.4%.In 7.2% of isolates there was production of ESBL, Amp C and MBL production.

Table-1: The various species of organisms isolated in blood culture

Organism	Number(%)
Klebsiella pneumoniae	58(46.4)
Escherichia coli	31(24.8)
C. fruendii	16(12.8)
C. koseri	9(7.2)
E. aerogenes	9(7.2)
E. cloacae	2(1.6)

Table-2: The antibiotic susceptibility pattern of the isolates to common antibiotics

Antibiotic	Number(%)
Ampicillin	31(24.8)
Piperacillin	7(5.6)
Amoxyclav	57(45.6)
Ampicillin sulbactum	49(39.2)
cefuroxime	22(17.6)
Ceftazidime	24(19.2)
Cetriaxone	50(40)
Cefepime	49(39.2)
Cefoxitin	31(24.8)
Cefotetan	55(44)
Cefotaxime	41(32.8)
Cefotaxime/clavulanic acid	72(57.6)
Ceftazidime/clavulanic acid	82(65.6)
Aztreonam	60(48)
Imipenem	76(60.8)
Meropenem	95(76)
Ertapenem	97(77.6)
Piperacillin/tazobactum	39(31.2)
Ticarcillin/clavulanic acid	46(36.8)
Gentamicin	33(26.4)
Amikacin	30(24)
Cotrimoxazole	33(26.4)
Ciprofloxacin	17(13.6)
Levofloxacin	69(55.2)
Ofloxacin	56(44.8)

DISCUSSION

The current cross sectional study, revealed that *Klebsiella pneumoniae* 46.4% was the most common isolate in blood culture. The highest sensitivity was for carbapenems group of drugs and least sensitivity was for aminoglycoside group of drugs. The production of ESBL, MBL, KPC and Amp C were 61.6%,22.4%,7.2% and 32% respectively.

Blood stream infections are an important cause of mortality and morbidity among patients. Bacteremia may lead to septicemia, a life threatening condition in which the multiplying bacteria release toxins into blood stream and triggers the release of cytokines, causing fever, chills, malaise and lethargy with difficulty in breathing [8]. Every year around 2,50,000 patients are affected by hospital acquired blood stream infection as per US data[9].

Antibiotic resistance is a major problem in hospital setups. The spread of ESBL, Amp C, MBL and recently KPC are increasing rampantly among blood stream infections, therefore, proper infection control and antibiotic policy are required to prevent spread of these infections. With high level of beta lactamase production the treatment options are limited [10].

The previous colonization or infection with ESBL producing Enterobacteriaceae is associated with the ESBL bacteremia. It is also found that exposure to fluroquinolones and first generation cephalosporin increases the risk of bacteremia. Flouroquinolones are known to produce a selective pressure on the patients gut flora which leads to proliferation of ESBL Enterobacteriaceae[1].

In the current study the rate of isolation of *Klebsiella pneumoniae* was 46.4% followed by *Escherichia coli* 24.8%.This is similar to study by Rudesh SM *et al*. where they have shown an isolation rate of *Klebsiella* spp was 33.1% [11]. However a low rate of isolation was shown by Altun *et al*. (19.2%) [10] and Jain A *et al*. (24.6%) [12].

Among the 125 isolates, in the carbapenem group of drugs, ertapenem was highly sensitive with 97(77.6%) followed by meropenem95 (76%) and imipenem76 (60.8%).These results correlate with the study conducted by Gndham *et al.* their rate of isolation was 62% in case of imipenem and meropenem.[13] Aminoglycosides such as gentamicin and amikacin had low sensitivity of 26.4% and 14% respectively in our study. This is similar to findings of 18.75% sensitivity to gentamicin and 24% sensitivity to amikacin observed by Ameriwala *et al*.[14]. Fifteen percent sensitivity to amikacin was shown by Datta *et al*.[15].

Table-3: Comparison of the antibiotic susceptibility pattern of ESBL and Non ESBL, MBL and Non MBL and Amp C and Non AmpC producing Entetrobacteriaceae isolates to various antibiotics

Antibiotic	ESBL			MBL			Amp C		
	ESBL producer (n=86)	Non ESBL producer (n=39)	P value	MBL producer (n=28)	Non MBL producer (n=97)	P value	Amp C producer (n=40)	Non AmpC producer (n=85)	P value
Ampicillin	29 (33.7)	2(5.1)	0.0013	10 (35.7)	21 (21.6)	0.2042	8 (20)	23(27.1)	0.5284
Piperacillin	5 (5.8)	2(5.1)	0.8772	2(7.1)	5(5.2)	0.6869	0(0)	7(8.2)	0.0957
Amoxyclav	36 (41.8)	21(53.8)	0.2925	9(32.1)	48(49.5)	0.1592	18(45)	39(45.9)	1.0000
Ampicillin sulbactum	32(37.2)	17(43.6)	0.6317	16(57.1)	33(34.0)	0.0468	13(32.5)	36(42.4)	0.3306
cefuroxime	14(16.3)	8(20.5)	0.7471	1(3.6)	21(21.6)	0.0535	7(17.5)	15(17.6)	1.0000
Ceftazidime	9(10.5)	15(38.5)	0.0006	5(17.9)	19(19.6)	0.8377	6(15)	18(21.2)	0.4743
Cetriaxone	31(36.0)	19(48.7)	0.2531	10(35.7)	40(41.2)	0.7592	16(40)	34(40)	1.0000
Cefepime	32(37.2)	17(43.6)	0.6317	12(42.6)	37(38.1)	0.8179	40(100)	9(10.6)	0.0001
Cefoxitin	16 (18.6)	15(38.5)	0.0309	3(10.7)	28(28.9)	0.0871	0(0)	31(36.5)	0.0001
Cefotetan	29(33.7)	26(66.7)	0.0012	9(32.1)	46(47.4)	0.2229	18(45)	37(43.5)	1.0000
Cefotaxime	20(23.3)	21(53.8)	0.0015	12(42.6)	29(29.9)	0.2899	15(37.5)	26(30.6)	0.5406
Cefotaxime/clavulanic acid	64(74.4)	8(20.5)	0.0001	22(78.6)	50(51.5)	0.0197	27(67.5)	45(52.9)	0.1742
Ceftazidime/clavulanic acid	72 (83.7)	10(25.6)	0.0001	22(78.6)	60(61.9)	0.1572	28(70)	54(63.5)	0.5479
Aztreonam	39 (45.3)	21(53.8)	0.4916	13(16.4)	47(48.5)	0.8501	21(52.5)	39(45.9)	0.5662
Imipenem	47 (54.7)	29(74.4)	0.0583	0(0)	76(78.3)	0.0001	21(52.5)	55(64.7)	0.2394
Meropenem	64 (74.4)	31(79.5)	0.6975	0(0)	95(97.9)	0.0001	29(72.5)	66(77.6)	0.6539
Ertapenem	68 (79.1)	29(74.4)	0.7235	0(0)	97(100)	0.0001	27(67.5)	70(82.4)	0.0705
Piperacillin/tazobactum	23 (26.7)	16(41.0)	0.1650	12(42.6)	27(27.8)	0.2006	10(25)	29(34.1)	0.4082
Ticarcillin/clavulanic acid	32 (37.2)	14(35.9)	0.8879	7(25)	39(40.2)	0.2123	14(35)	32(37.6)	0.8439
Gentamicin	24(27.9)	9(23.1)	0.7274	8(28.6)	25(25.8)	0.9581	14(35)	19(22.4)	0.1911
Amikacin	20(23.3)	10(25.6)	0.9495	6(21.4)	24(24.7)	0.9120	8(20)	22(25.9)	0.5107
Cotrimoxazole	21 (24.4)	12(30.8)	0.5980	5(17.9)	28(28.9)	0.3571	9(22.5)	24(28.2)	0.6640
Ciprofloxacin	15 (17.4)	2(5.1)	0.1143	4(14.3)	13(13.4)	0.9044	4(10)	13(15.3)	0.5784
Levofloxacin	48 (55.8)	21(53.8)	0.9913	20(71.4)	49(50.5)	0.0811	23(57.5)	46(54.1)	0.8474
Ofloxacin	32(37.2)	24(61.5)	0.0193	12(42.6)	44(45.4)	0.9849	16(40)	40(47.1)	0.5636

Table-4: Coproduction of various beta lactamases

Beta lactamase enzymes	Total no. of isolates	Percentage
ESBL	42	33.6
MBL	3	2.4
KPC	9	7.2
Amp C	6	4.8
ESBL+ Amp C	22	17.6
MBL+ Amp C	3	2.4
ESBL+MBL	13	10.4
Amp C+ESBL+MBL	9	7.2

In our study Ampicillin was more sensitive in ESBL producers compared to nonESBL producers. On the contrary antimicrobials like cefotetan and cefoxitin were more sensitive in non ESBL producers. Betalactam/betalactamase inhibitor combinations like ampicillin/sulbactam and cefotaxime/clavulanic acid was more sensitive in MBL producers than non MBL producers and was found to be statistically significant. These findings could help us in using beta lactam combinations for patient treatment instead of high end drugs like tigecycline and colistin.

Prevalence of ESBL in India ranges from as low as 6.6% to as high as 91% [16]. In our study the rate of ESBL production is 61.6% which is comparable from studies conducted in North India showing a rate of 68% and 91.7% by Mathur *et al.* and Wattal *et al.* [17,18].Amp C production in our study was 32% which is similar to study conducted by Hemalatha *et al.* [19].

MBL and KPC production was 22.4% and 7.2% in our study. These findings correlate with a study by Chauhan *et al* showing 20.72% as carbapenemase producers [20].

CONCLUSION

The prevalence of ESBL, Amp C, MBL and KPC is on a rise since last one decade among blood culture isolates. To complicate situation further is the coproduction of various beta lactamases. Screening of beta lactamases would help in infection control and antibiotic policy. Hence an easy, economical and cost effective method like the twelve disc test could help in screening for beta lactamases.

REFERENCES

1. Freeman JT, McBride SJ, Nisbet MS, Gamble GD, Williamson DA, Taylor SL, Holland DJ. Bloodstream infection with extended-spectrum beta-lactamase-producing Enterobacteriaceae at a tertiary care hospital in New Zealand: risk factors and outcomes. International Journal of Infectious Diseases. 2012 May 1;16(5):e371-4.
2. Tumbarello M, Sali M, Trecarichi EM, Leone F, Rossi M, Fiori B, De Pascale G, D'Inzeo T, Sanguinetti M, Fadda G, Cauda R. Bloodstream infections caused by extended-spectrum-β-lactamase-producing Escherichia coli: risk factors for inadequate initial antimicrobial therapy. Antimicrobial agents and chemotherapy. 2008 Sep 1;52(9):3244-52.
3. Mohd Khari FI, Karunakaran R, Rosli R, Tee Tay S. Genotypic and Phenotypic Detection of AmpC β-lactamases in Enterobacter spp. Isolated from a Teaching Hospital in Malaysia. Galdiero M, editor. PLOS ONE. 2016;11:e0150643.
4. Pandey A, Asthana A, Madan M, Chauhan K. Evaluation of phenotypic tests for detection of Klebsiella pneumoniae carbapenemase and metallo-beta-lactamase in clinical isolates of Escherichia coli and Klebsiella species. Indian Journal of Pathology and Microbiology. 2015;58:31.
5. Schreckenberger P, Rekasius V. Detecting resistance to beta Lactams in Gram-negative Bacilli. Tersedia pada URL: http://hardydiagnostics. com/articles/Antibiotic-Resistance. pdf. Diunduh tanggal. 2012 Nov;2.
6. CLSI. Performance standards for antimicrobial susceptibility test. Approved Standard. CLSI Document M100‑S25. 25th ed. Wayne, Pennysylvani: Clinical and Laboratory Standards Institute. 2015.
7. Yong D, Lee K, Yum JH, Shin HB, Rossolini GM, Chong Y. Imipenem-EDTA disk method for differentiation of metallo-β-lactamase-producing clinical isolates of Pseudomonas spp. and Acinetobacter spp. Journal of clinical microbiology. 2002 Oct 1;40(10):3798-801.
8. Qureshi M, Aziz FA. Prevalence of microbial isolates in blood cultures and their antimicrobial susceptibility profiles. Biomedica. 2011 Jul;27(4).
9. Lukenbill J, Rybicki L, Sekeres MA, Zaman MO, Copelan A, Haddad H, Fraser T, DiGiorgio MJ, Hanna R, Duong H, Hill B. Defining incidence, risk factors, and impact on survival of central line-associated blood stream infections following hematopoietic cell transplantation in acute myeloid leukemia and myelodysplastic syndrome. Biology of Blood and Marrow Transplantation. 2013 May 1;19(5):720-4.
10. Altun S, Tufan ZK, Yağcı S, Önde U, Bulut C, Kiniki S. Extended spectrum beta-lactamases, AmpC and metallo beta-lactamases in emerging multi-drug resistant gram-negative bacteria in intensive care units. Sci Rep. 2013;2(4):707.
11. Rudresh SM, Nagarathnamma T. Extended spectrum β-lactamase producing Enterobacteriaceae & antibiotic co-resistance. The Indian journal of medical research. 2011 Jan;133(1):116.
12. Jain A, Roy I, Gupta MK, Kumar M, Agarwal SK. Prevalence of extended-spectrum β-lactamase-producing Gram-negative bacteria in septicaemic neonates in a tertiary care hospital. Journal of Medical Microbiology. 2003 May 1;52(5):421-5.
13. Gandham P, Amatullah F. Antibiotic susceptibility and resistance patterns of Enterobacteriaceae in a teaching hospital in a rural area. Journal of Microbiology and Biotechnology Research. 2017 Mar 27;5(2):1-4.
14. Amreliwala S, Durgad S, Poojary A. Carbapenem sparing options for the treatment of ESBL and AmpC producing Enterobacteriaceae in hemodynamically stable patients an in vitro study. Int. J. Curr. Microbiol. App. Sci. 2015;4(2):513-21.
15. Datta P, Gupta V, Garg S, Chander J. Phenotypic method for differentiation of carbapenemases in Enterobacteriaceae: Study from north India. Indian Journal of Pathology and Microbiology. 2012 Jul 1;55(3):357.
16. Basavaraj MC, Jyothi P, Peerapur BV. The prevalence of ESBL among Enterobacteriaceae in a tertiary care hospital of North Karnataka, India. J. Clin. Diagn. Res. 2011;5(3):470-5.
17. Mathur P, Kapil A, Das B, Dhawan B. Prevalence of extended spectrumβ- lactamase producing Gram negative bacteria in tertiary care hosptital. *Indian J Med Res* 2002;115:153-157.
18. Wattal C, Sharma A, Oberoi JK, Datta S, Prasad KJ, Raveendr R. ESBL-An emerging threat to antimicrobial therapy. Microbiology Newsletter. 2005:1-8.
19. Hemalatha V, Padma M, Sekar U, Vinodh TM, Arunkumar AS. Detection of Amp C beta lactamases production in Escherichia coli & Klebsiella by an inhibitor based method. Indian journal of medical research. 2007 Sep 1;126(3):220.

20. Chauhan K, Pandey A, Asthana AK, Madan M. Evaluation of phenotypic tests for detection of Klebsiella pneumoniae carbapenemase and metallo-beta-lactamase in clinical isolates of Escherichia coli and Klebsiella species. Indian Journal of Pathology and Microbiology. 2015 Jan 1;58(1):31.