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Prevalence and Detection of AmpC β-Lactamases in Gram Negative Bacilli from BIHS Hospital, Mirpur, Dhaka

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Abstract: *AmpC β lactamases have a broad substrate profile that includes penicillin, cephalosporin and monobactam. In contrast to ESBLs, AmpC β lactamase are active against cephamycines and are not inhibited by β- lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam. The present study was designed to determine the occurrence of AmpC β lactamase enzymes harboring Gram negative bacilli in Bangladesh Institute of Health Science Hospital, Mirpur, Dhaka. Total 212 isolates were isolated from various clinical samples. Screening test was done by using various 3rd generation cephalosporin (3GC) and cefoxitin. Organisms resistant to cefoxitin and reduced susceptibility to 3GC were tested for presence of AmpC β lactamase enzyme by DAT test, AmpC Disk Test and Modified Three Dimensional Test (Confirmatory tests).Out of 212 isolates, 67(31.6%) were found to be resistant to cefoxitin (screening positive) of which 54 (80.5%) were found to be positive for AmpC βlactamase by confirmatory test. Out of 67 screening positive AmpC βlactamase, 27 (40.3%) were only AmpC βlactamase and 40(59.7%) isolates were positive for both ESBL and AmpC βlactamase. Out of 54 AmpC β lactamase 8 (14.8%) were inducible (chromosomal mediated) and 46 (85.2%) were plasmid mediated AmpC β lactamase.Regular monitoring of incidence of AmpC β Lactamase should be done along with routine sensitivity test. As high level expression of AmpC β lactamases may mask recognition of ESBL, detection of AmpC β lactamase should be done along with ESBL.AmpC β lactamase is transmissible through plasmid. So, it is of great public health concern.AllAmpC βlactamase producing organisms were susceptible to imipenem.*

Keywords: *AmpC β-lactamase, ESBL, cefoxitin, third generation cephalosporins(3GC)*

1. INTRODUCTION

The advances in medicine have improved the patient care. However, the infectious diseases are still continuing to be a major cause of morbidity and mortality all over the world.[1]One of the greatest medicaladvances of 20th century was the discovery of penicillin in 1928 by Sir Alexander Flaming. The practical application of penicillin started in 1941. Additional antibioticswere also discovered since that time. Within three years of advent of penicillin bacteria started producing penicillinase which could destroy penicillin. After the penicillin, extended spectrum antibiotics like methicillin, cloxacillin and cephalosporins were developed which could effectively treat infections produced by penicillinase producing bacteria. However with each class of antibiotics, newβ- lactamase enzymes were produced by bacteria that made these new antibiotics ineffective.[2]More than thousands of these β-lactamases enzymes exist in Gram negative bacteria. Many of the most clinically important and recently identified β- lactamases include ESBL, AmpC β lactamases, Klebsiella Pneumonae Carbapenemase (KPC), and metallo beta lactamase (MBLs).[3] Many multidrug resistant bacteria produce multiple β- lactamases including combination of ESBLs, AmpC β lactamase and carbapenemase as well as non-enzyme resistance mechanisms (eg. Porin loss, efflux pump).[3]

Plasmid mediated AmpC β- lactamases were first reported in the late 1988.[4]The number of infections caused by AmpC β- lactamase producing organisms is increasing. Distinguishing between AmpC β lactamase and ESBL producing organisms has epidemiological significance and may have therapeutic importance as well.[5]AmpC β lactamase producing organisms can act as hidden reservoir for ESBL and also high level expression of AmpC β lactamases may mask recognition of ESBL. Enterobacteriaceae producing both AmpC β lactamase and ESBL have been increasingly reported worldwide.[6]

Expression of AmpC β- lactamases is generated by chromosomalor plasmid genes. The chromosomally mediated AmpC β lactamase production is either constitutive or inducible. The organisms like *Citrobacterfreundii, Enterobacter cloacae, Enterobacteraerogenes, Providencia spp, Morganellamorganii, Serratiaspp and pseudomonas spp.* posses chromosomally mediated AmpC β-lactamase.[7]

Plasmid mediatedAmpCβ- lactamaseshave arisen through transfer of chromosomal gene for AmpCβ-lactamases on to the plasmid.[7]Unlike chromosomal, they are uninducible.[8]Plasmid mediated AmpC β-lactamase have been found most frequently from *Klebsiellasp, Proteus sp, Salmonella typhi, E.coli, Citrobacterfreundii et*c.[8,9]

Like ESBLs, plasmid mediated AmpC β lactamases have a broad substrate profile that includes penicillin, cephalosporin and monobactam. In contrast to ESBLs, plasmid mediated AmpC βlactamase are active against cephamycines and are not inhibited by β- lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam.[10, 11]

As ESBL and AmpC β- lactamase are main cause of multi drug resistance in Gram negative bacteria (GNB), so early detection and identification of these organisms is necessary for appropriate antimicrobial therapy, timely introduction of infection control procedures to limit the spread of these MDR organisms in hospital settings as well as in community and for epidemiological survellence.[12,13,14]

With this background this study was undertaken to detect the prevalence of AmpC β lactamases in Gram Negative Bacilli, as not much study is done on this topic, especially in Bangladesh.

2. Materials and Method

This study was carried out in BIHS Hospital from January 2013 to December 2013. Total 212 Gram negative bacilli wereisolated from various clinical samples fromhospitalized patient as well as patients attending outpatientdepartment andidentified by standard biochemical reactions. Modified Karby Bauer disc diffusion method was followed. Ceftazedime, amoxiclave (amoxicillin + clavulanic acid), cefotaxime and cefoxitin were placed 15 - 20 mm apart edge to edge in a straight line. [15]

3. Detection of AmpC β lactamase

Screening Test: This test was done along with routine sensitivity test**:**

Criteria used for suspecting an organism to be AmpC βlactamase producers are:[15.]

1. Decrease zonediameter for various 3GCs: cefotaxime (30µg) ≤27mm, ceftazedime (30µg)≤22mm, ceftriaxone (30µg)≤25mm, aztreonam(30µg)≤27mm.
2. Resistant to cefoxitin.

Confirmatory Test:

It is done by two ways:

1. Detection of Inducible (chromosomal) AmpC β lactamase production
2. Detection of Plasmid- mediated AmpC β lactamase Production

Detection of Inducible (chromosomal) AmpC β lactamaseproduction:

This is also known as **Disk Antagonism Test (DAT):** Isolates that fulfil the above criteria were subjected to Disk Antagonism Test for inducible AmpC β lactamase production by the method of Sanders et.al.[16]Briefly the organisms tested for inducible AmpC β lactamase were inoculated on Muellar Hinton Agar plate and then cefoxitin and cefotaxime discs were placed in 15 mm away edge to edge from each other. Plates were incubated at 37 ^{0}C for 24 hours. After incubation plates were examined for blunting or straightening of zone of inhibition near cefoxitin disc indicated the test positive (Fig. I).

Fig1. *Disc antagonism test (DAT). Blunting of the cephalosporin disk adjacent to cefoxitin disk. The test is Positive.*

Detection of Plasmid- mediated AmpC β lactamase Production: This was done by two ways: i. AmpC Disk Test. ii. Modified Three Dimensional Test.

i. AmpC Disk Test: Screen positive organisms were tested for AmpC β- lactamase production by AmpC disk test. Briefly 0.5 McFarland suspension of ATCC *E.coli* 25922 was inoculated on the surface of Mueller – Hinton Agar Plate. A 30 µg cefoxitin disc was placed on the inoculated surface of the MHA agar plate. A sterile plain disc inoculated with several colonies of the test organisms was placed besides the cefoxitin disc almost touching it, with the inoculated disk face in contact with the agar surface. After overnight incubation at 37 ^{o}C, the plates were examined for either an indention or a flattening of the zone of inhibition, indicating enzymatic inactivation of cefoxitin (positive result), or the absence of a distortion, indicating no significant inactivation of cefoxitin(Fig.II).[17, 18]

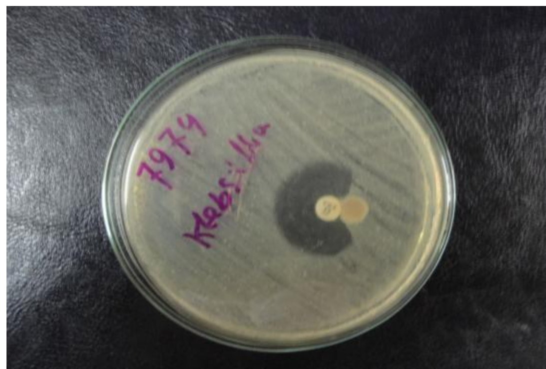

Fig2.*AmpC disk test: presence of indentation towards cefoxitin disk indicates test positive*

ii. Modified Three-Dimensional Test: Fresh overnight growth of test organism(10- 15mg) from Mueller-Hinton Agar plate was transferred to sterile micro centrifuge tube. The growth was suspended in peptone water and was pelleted by centrifugation at 3000 rpm for 15 min. Crude enzyme extract was prepared by repeated freeze thawing in -80^{0}C for 5 - 7 times. A lawn culture of E.coli ATCC 24922 was prepared on MHA plate and cefoxitin (30mg) discs was placed on the plate. Linear slits were cut using a sterile surgical blade 3mm away from the cefoxitin disc and a circular well made at outer edge of the slit. 30 -40 µl of the enzyme was added to the well, without overflowing. The plates were kept upright for 5-10 minutes until the liquid dried and were incubated at 37 ^{0} C for overnight. After incubation plate was examined for Clear distortion of zone of inhibition of cefoxitin disk is taken asAmpC β lactamase producer (Fig.III).[19]

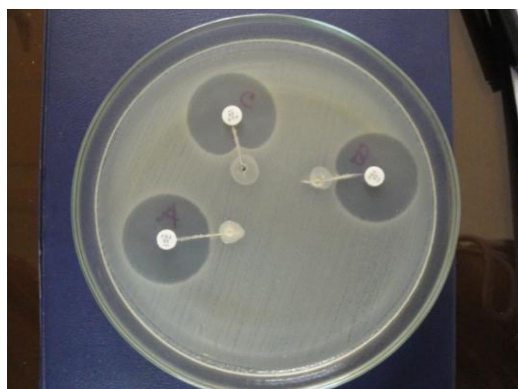

Fig3. ***Modified three-dimensional test:*** *Organism showing clear distortion in the zone of inhibition. Strain B & C are positive for AmpC beta-lactamase production.*

4. Result

This study was conducted from January 2013to December 2013 in the Department of Microbiology, Bangladesh Institute of Health Science Hospital, Mirpur, Dhaka. A total of 212 isolates from different samples like urine, pus, wound swab, sputum and blood were studied. The maximum number of isolates were E.coli (62.3%) followed by Klebsiella (17.4%), Enterobacter (8.0%), Pseudomonas (5.2%), Acinetobacter (3.8%), Proteus (2.80%) and Citrobacter (0.50%) shown inTable I.

TableI. *Prevalence of AmpC β lactamase in Gram negative bacilli.*

Name of organisms	Number (%)	AmpC β lactamase			
		Screening Positive (N)			Confirmatory Positive N(%)
		FOX alone = R	ESBL+ & FOX =R	Total FOX=R	
E.coli	132(62.3%)	03	16	19	15 {11.4}
Klebsiella sp.	37(17.4%)	05	09	14	12 {32.4}
Enterobacter sp.	17(8%)	06	05	11	08 {47.0}
Peudomonas sp.	11(5.2%)	04	05	09	09 {81.8}
Acinetobacter sp.	8(3.8%)	04	02	06	07 {87.5}
Proteus sp.	6(2.8%)	04	03	07	02 {33.3}
Citrobacter sp.	1(0.5%)	01	0	01	01 {100}
Total	**212 (100)**	27	40	67	**54 {25.5 }**

Note: *( ) indicate vertical percentage, { } indicate horizontal percentage*

R= resistant, Fox= Cefoxitin, ESBL= Extended Spectrum β Lactamase

N=Number

Out of 212 isolates, 67 (31.6%.) were found to be resistant to Cefoxitin (positive for screening test for AmpC β lactamase) of which 54 (80.5%) were found to be positive for AmpC β lactamase by confirmatory test by applying Disk Antagonism Test, AmpC Disk Test, and Modified Three Dimensional Test shown in Figure I, II and III.

Out of 67 screening positive AmpC βlactamase, 27(40.3%) wereonlyAmpC β lactamase positiveand

40(59.7%) isolates were positive for both ESBL and AmpC β lactamase. AmpC βlactamase production was predominant in Citrobacter (100%)followed by Acinetobacter (87.5%), Pseudomonas (81.8%), Enterobacter (47.05%), Klebsiella(32.4%), proteus (33.3%) and E.coli (11.4%) respectively.

In our study, out of 54 AmpC β lactamases, 8 (14.8%) were inducible (chromosomal mediated), and 46 (85.2%) were plasmid mediated AmpC β lactamase positive. All β lactamases producing organisms were susceptible to imipenem.

5. Discussion

Antimicrobial drug resistance is emerging worldwide as a major public health problem. Selective pressure by misuse and overuse of antibiotics in the hospitals has resulted in the emergence and dissemination of resistant bacteria in many areas of hospitals.[21]The AmpC βlactamases are cephalosporinases which belongs to the molecular class C, as was classified by Amber and group I under a classification scheme of Bush and Jacobi et.al.[10]These are clinically significant as they may confer resistance to a wide variety of β lactam, narrow spectrum, expanded spectrum and broad spectrum cephalosporin, aztreonam and most significantly, the β-lactam plus β lactamase inhibitor combinations.[20]

Enterobacteriaceae produce AmpC βlactamases alone or in combination with ESBLs. It is also noted that high level expression of the AmpC β lactamases may mask the recognition of the ESBLs leading to false negative result.[6]

In our study, AmpC βlactamases production was seen in 25.5% (N=212) isolates. Incidence of AmpC β lactamases in different studies were 16.0%(Laghawe et al), [21]15.1% (Sanguintti et al),[25]10.6% (Moland et al),[24] 66.43% (Rodrigues et al),[15] and3.3%(Ratna et al).[23]

In our study predominant organism was *E.coli* 132(63.3%) followed by *Klebsiella sp.*27(17.4%).In the study of Mahmuda et al where predominant organism was *E.coli* 65(27.6%) followed by pseudomonas 48(20.4%) and in the study of Laghwae et al[21]where predominating organism was*E.coli* 154(35.6%) followed by Pseudomonas 113(26.2%). It is evident that all authors found *E.coli* as predominant organism followed by *Pseudomonas* but in our study we found *Klebsiella* as a 2nd predominant organism.[21]

In our study,out of 212 isolates, 67 (31.6%) were found to be resistant to cefoxitin (Screening test for AmpC β-lactactamase) of which 54(80.5%) were positive for AmpC β lactamase (confirmatory positive by Disk Antagonism Test, AmpC Disk Test and Modified Three Dimensional Test). In the study of Laghwaeet al[21] 137(31.7%) were cefoxitinresistantof which 69(50.4%) were positive for AmpC βLactamases. Mahmuda et al[18] reported 134(57%) cefoxitin resistant of which 63(47%)were

AmpC βlactamases positive. Whereas Manchnada et al [19]found 26(19.3%) cefoxitin resistant of which 17(65.4 %) were AmpCβ-lactamase producer. So, it is evident that prevalence of Cefoxitin resistance

(Screening test for AmpC β lactamases) is same as ours in the study of Laghwae et al[21], higher in study of Mahmuda et al[18]and lower in the study of Manchanda et al[19]; but prevalence of confirmatory test for AmpC β Lactamases is lower than ours in all studies.

In our study, out of 212 isolates, 67 (31.6%) were AmpC producer in screening test(Cefoxitin resistant)of which 27 (40.3%) were positive for only AmpC β lactamase and 40(59.7%) isolates were positive for both ESBL and AmpC β lactamases. In the study of Laghawe et al [21]out of 432 isolates,69(16.0%)were AmpC βlactamase producer of whichpureAmpC β lactamase producer were 48(69.6%) and 21 (30.4%) were positive for both AmpC β lactamases and ESBL.

It is to be noted that AmpC β lactamases have been associated with False ESBL negative (i.e. false in vitro susceptibility to cephalosporins) due to high level expression of AmpC β lactamase. That is if AmpC β lactamase is positive, the ESBL is also positive even if in vitro susceptibility shows ESBL negative. [18]

In our study,out of 54 AmpC βlactamases, 8(14.8%) were inducible (chromosomal mediated) and 46 (85.2%) plasmid mediated AmpC βlactamases.In the study of Mahmuda et al[18]23 (22.7%) were inducible AmpC βlactamase and 63(47%) were Plasmid mediated AmpC βlactamases positive.Effect of inducible(Chromosomal)AmpC β lactamases and plasmid mediated AmpC β lactamases is same on antibiogram. So, Cefoxitin disk should be placed appropriately(i.e. placing Fox disk 15mm awayfrom Cefotaxime disk) to induce AmpC β lactamase. Importance of AmpC β lactamase also lies in the fact that they are transmissible to other bacteria.Chromosomal mediated i.e.inducibleAmpC β lactamase are also transmissible via plasmid. [22]In this study all (100%) AmpC β lactamase producing organisms were found to be sensitive to imipenem, this correlates with the study of Laghawe et al, [21]Mahmuda et al[18] and Avinash et al. [22]

6. Conclusion

Regular monitoring of the incidence of the AmpC β lactamase production by organisms is necessary. It should be done regularly with routine sensitivity tests. Along with the detection of ESBL, it is necessary to detect AmpC β lactamases as they can act as a hidden reservoir for ESBL and high level expression of AmpC βlactamases may mask recognition of ESBL. Enterobacteriaceae producing both AmpC and ESBL have been increasingly reported worldwide. AmpC β lactamases can lead to transmission of drug resistance to other bacteria through plasmids. All AmpCβlactamase producing organisms were found sensitive to imipenem. So imipenem should be kept as reserve drugs and they should be used only in patient who have infection with multidrug resistant strain especially the strains which produce ESBL and AmpC βlactamase.

References

[1] LL, Lazo JS, Parker KL, McGraw- hill medical publishing division, United States of America Chapter 14: Penicillin, Cephalosporins and other β lactam antibiotics 1127 –54.

[2] Bradford PA. Extended Spectram β lactamase in the 21st century: Characterization, Epediomology and Detection of this important resistance threat. ClinMicrobiol Rev, 2001; 14: 933- 55.

[3] Bush K, et al. Epidemiological expansion and clinical challenges of new β lactamase. Ann Rev Microbiol. 2011; 65: 455 – 478.

[4] Thomson KS. Controversies about extended spectrum &AmpC β lactamases, Emerging Infectious Diseases. 2001; 7: 333 – 36.

[5] Courdon PE, Hanson ND, Climo MW. Occurrence of extended spectram and AmpC β lactamases in bloodstream isolates of Klebsiella pneumonia: Isolates harbor plasmid mediated Fox – 5 & ACT- 1 AmpC β lactamases J Clin Microbiol, 2003;41:772- 77.

[6] Pitout J DD, Reisbig MD, Venter EL, Church DL, Hanson ND, Modification of the double disk test for detection of Enterobacteriaceae producing extended spectram & AmpC β lactamases, J ClinMicrobiol, 2003; 41 : 3933- 35.

[7] Livermore DM. β lactamases in laboratory and clinical resistance, ClinMicrobiol Rev. 1995; 8: 557 – 84.

[8] Thomson KS. Controversies about extended spectram&AmpC β lactamases. Journal of emerging infectious diseases. 2001; 7: 333 – 36.

[9] Philippon A, Arlet G, Jacoby GA. Plasmid determined AmpC type β lactamases, Antimicrob Agents Chemother. 2002; 46: 01 11.

[10] Bush K; Jacoby GA; Medeiros AA. (1995). A functional classification scheme for β lactamases and its correlation with molecular structure. Antimicrob Agents chemother. 39, 1211- 33.

[11] Clinical laboratory Standard Institute (CLSI).(2003).Performance standards for antimicrobial disk susceptibility test. 8thed.Approved standards, M2 – A8, Wayne, Pa (USA).

[12] Canton R, Coque TM (2006) The CTX- M beta lactamase pandemic. CurrOpinMicrobiol 9(5):466-475.

[13] Pfaller MA, Segreti J (2006). Overview of the epidemiological profile and laboratory detection of extended spectrambetalactamase. Clin Infect Dis 42(Suppl 4): S153-S163.

[14] Ramphal R, Ambrose PG (2006) Extended spectrum beta lactamase and clinical outcomes: current data. Clin. Infect Dis 42 (suppl 4):S164-S172).

[15] Rodrigues C, Joshi P, Jani SH, Alphonse, Radhakrishnan,R. Mehta A. Detection of β lactamase in nosocomial Gram negative clinical isolates, Indian J of Med Microbiol. 2004; 22: 247- 50.

[16] Sanders, C.C; Sandesr, W.E; Goering, H.V.(1982). In vitro antagonism of β lactam antibiotics by cefoxitin. J Antimicrob Chemother. 21.968- 75.

[17] Black, J.A; Moland, E.S; Thomson, K.S(2005). AmpC disk test for detection of Plasmid mediated AmpC β lactamases in Enterobacteriaceae lacking Chromosomal AmpC β lactamase. J. Clin. Microbiol. 43 (7). 3110- 3113.

[18] MahmudaParveen R., Harish B.N., Parija S.C. AmpC β lactamases among Gram Negative Clinical isolates from a tertiary hospital, South India. Brazilian Journal of Microbiology (2010) 41: 596-602.

[19] Manchanda, V.; Sing N.P.(2003). Occurrence and detection of AmpC β lactamases among Gram Negative clinical isolates using a modified three dimensional test at Guru Teg Bahadur Hospital, Delhi, India.JAntimicrobChemother. 51, 415-8.

[20] Arora S, Bal M. AmpC β lactamase producing bacterial isolates from aKolkata hospital. Indian J Med Res. 2005; 122:224- 33.

[21] LaghaweAvinash R, MS.JaitlyNeelam K, ThombareVilasR. Prevalance of AmpC beta lactamase in Gram negative bacilli. Journal of Pharmaceutical and biomedical sciences, 2012, 20 (07).

[22] Avinash L, Neelan J, Vilash T. The simultaneous detection of the ESBL and AmpC β lactamases in Gram Negative Bacilli. Jurnal of clinical and Diastonic Research. 2012 May (Suppl-2), Vol(6):660-663.

[23] Ratna AK, Menon I, Kapur I, Kulkarni R. The occurance and the detection of the AmpC β lactamases at the referral hospital in Karnataka. Indian J Med. Res. 2003; 118: 29-32.

[24] Moland ES, Sanders CC, Thomson KS.Can results obtained with commercially available microscan microdilution panels serve as an indicator of β lactamase production among Escherichia coli and Klebsiella isolates with hidden resistance to expanded spectram cephalosporin and Aztreonam? J ClinMicrobiol. 1998: 36: 2575-79

[25] Sanguinetti M, Posterralo B, Spanu T, Ciccaglion D, Romano L, Fiori ET al. Chracterization of clinical iolates of Enterobacteriaceae from Italy by the BD Phonix extended spectram β lactamase detection method, J Clin M crobiol. 2003; 41: 1463 68.