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Prevalence and detection of co-existence of multiple β lactamases in clinical isolates: A diagnosticchallenge and a dangerous trend

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Abstract

Introduction: The greatest threat that diagnosticians and physicians face today is from the multi drug resistant bacteria. The multi-drug resistance associated with extended spectrum β lactamases (ESBL), Amp-C and metallo β lactamase (MBL) producers poses a considerable therapeutic challenge leading to treatment failure. So the present study was conducted to detect the occurrence of various β Lactamases in the clinical isolates. *Materials and Methods:* We included a total of consecutive 206 isolates of Escherichia coli and Klebsiella pneumonia in our study, over a period of one year. Antibiotic susceptibility testing was performed in accordance with guidelines by Clinical and Laboratory Standards institute (CLSI). ESBL production was confirmed by using the double disc diffusion test recommended by CLSI. AmpC disc test was performed for confirmation of AmpC production and MBL producers were detected using EDTA disc potentiation test. *Result:* Of the total 206 isolates, 46 (22.33%) were found to be pure ESBL producers, 64 (31.06) were AmpC producers out of which only one isolate produced pure AmpC. MBL production was confirmed in 11 (5.33%) isolates. The significant finding of this study was the co production of ESBL and AmpC which was detected in 53 (25.72%), taking the total number of ESBL producers to 99 (48.05%). AmpC and MBL were co produced by 10 (4.85%) isolates. Conclusion: Routine laboratory detection of multiple beta lactamases has become the need of the hour and is recommended, since the occurrence and co-existence of beta lactamases in Gram negative bacilli is on the rise.

Keywords: Extended Spectrum Beta Lactamases (ESBL); AmpC Beta Lactamase; Metallo Beta Lactamase (MBL); Co-Producers.

Introduction

β-lactam antibiotics are among the most frequently prescribed antibiotics worldwide [1]. Among the variety of mechanisms that can provide resistance to β-lactam antibiotics in Gram-negative bacilli, the production of β-lactamase is by far the single most important factor [1]. Extended spectrum β-lactamases (ESBLs), AmpC β-lactamases and metallo β-lactamases (MBLs) are the major causes of β-lactam resistance in Gram negative bacteria [2].

The ESBL enzymes are beta-lactamases capable of conferring bacterial resistance to the penicillins; first-, second- and third-generation cephalosporins; and aztreonam (but not the cephamycins or

carbapenems), and which are inhibited by beta-lactamase inhibitors such as clavulanic acid [3]. AmpC beta-lactamases confer resistance to a wide variety of β-lactam drugs, including α-methoxy-β-lactams, such as cefoxitin, narrow, expanded-, and broad-spectrum cephalosporins, β-lactam-beta lactamase inhibitor combinations, and aztreonam. Metallo beta-lactamases (MBLs) are the Ambler class B beta-lactamases which have the capacity to hydrolyze with the exception of aztreonam, all beta-lactams including carbapenems [4].

To detect resistance to various ß-lactam group drugs via ESBLs, AmpC, and MBL, various phenotypic tests can be used. However, phenotypic testing may be misleading, especially when both ESBL and AmpC ß-lactamases co-exist and mask each other, which results in misreporting and failure in clinical treatment of patients, while the presence of both MBLs and AmpC ß-lactamases in a isolate confers carbapenem resistance [5]. In view of the increasing significance of coexistence of β-lactamases, the present study was undertaken to know the prevalence of coexistence of ESBLs, AmpC β-lactamases and MBL in clinical isolates of *Escherichia coli* and *Klebsiellapneumoniae* by using different phenotypic methods.

Materials and Methods

The present study which aimed at detecting the prevalence of Co-producers of beta lactamases, was carried out in Department of Microbiology, at a tertiary care hospital in Karnataka over a period of one year from December 2013 to December 2014. A total of 206 consecutive isolates of *Escherichia coli* and *Klebsiellapneumoniae* together which were isolated from various clinical samples like pus, blood, body fluids, urine etc were included in this study. Standard microbiological procedures were followed in identification of the isolates and their Antibiotic susceptibility testing was carried out by Kirby Baeur method as per the Clinical and Laboratory StandardInstitute (CLSI) criteria [6].

ESBL detection [6,3]

A zone diameter of <22mm for Ceftazidime 30µg disk was taken as Screening test positive for ESBL. All *E.coli* and *Klebsiellapneumoniae* which were screening positive were subjected to phenotypic confirmatory tests for ESBL detection.

Phenotypic confirmation test for ESBL detection [6,3]: Lawn culture of the isolate was made. Ceftazidime disks (30µg) alone and in combination with clavulanate (10 µg) were placed at a distance of 20 mm and incubated for 24 hours at 37°C. A difference of ≥5 mm between the zone diameters of ceftazidime disk and the ceftazidime-clavulanate combination disk was taken to be confirmatory for ESBL production.

Amp-C production [7]: Isolates were screened for Amp-C production by using Cefoxitin disk (30µg). Isolates with cefoxitin zone of <18 mm were considered as screen positives for Amp-C beta-lactamase production.

Phenotypic confirmatory test: AmpC disk test [7]: A lawn culture of Escherichia coli ATCC 25922 was prepared on MHA plate. Sterile disks (6mm) moistened with sterile saline (20 µl) were taken and inoculated with several colonies of the test organism. The inoculated disk was then placed beside a Cefoxitin disk (almost touching) on the inoculated plate. The plates were incubated overnight at 37^0 C.A positive test appears as a flattening or indentation of the cefoxitininhibition zone in the vicinity of the test disk. An undistorted zone of inhibition is considered as a negative test.

MBL production: The isolates exhibiting resistance to imipenem were suspected of MBL production. MBL production among them was confirmed byImipenem-EDTA disc test. Two 10 µgimipenem discs were placed on the lawn cultured MHA plate of test organism. 5µL of sterile 0.5 ml solution of EDTA was added on one of the disc.The inhibition zones of imipenem-EDTA discs were compared after 16-18 hours incubation at 35°C. An increase in the zone size of atleast 7mm around the imipenem-EDTA disc compared to plain imipenem disc was recorded as an MBL- positive strain [8].

Results

99 (48.05%) Gram negative clinical isolates were screened positive for ESBL production out of the total 206 clinical isolates processed. Confirmatory test confirmed the production of pureESBL in 46 (22.33%) isolates. Among the 206 isolates, 76 (36.89%) isolates were found to be resistant to cefoxitin (Screening test positive for AmpC). AmpC disk test confirmed the production of AmpC in only 64 (31.06%) isolates.16 (7.76%) isolates were screening test positive for MBL and MBL production was confirmed in 11 (5.33%) isolates using EDTA disc potentiation test. Co-production

of ESBL and AmpC was found in 53 (25.72%) clinical isolates.AmpC and MBL co-occurred in 10 (4.85%) organisms. The remaining 94 (45.63%) isolates were not found to harbour any of the three enzymes. Table 1 shows the distribution of different beta lactamases in the isolated organisms. Chart 1 depicts the antibiotic resistance patterns of beta lactamase produers and non producers.

Discussion

The incidence of infections due to organisms resistant to β lactam agents has increased sharply in recent years [9]. This increasing incidence of multiple β lactamase producing organisms all over the world holds even more significance because organisms frequently also possess resistance factors to other classes of antibiotics, notably the aminoglycosides and fluoroquinolones [10]. Most of the laboratories do not consistently test for β lactamases [11], and this can be detrimental to the patient who is treated with a cephalosporin in the mistaken belief that the isolate is susceptible [12]. The present study was conducted to detect the prevalence of Co-producers of beta lactamases and the challenges faced in detecting them using phenotypic tests.

In the present study, 46 (22.33%) isolates were confirmed to produce pure ESBL, of which 26 (12.62%) were E.coli and 20 (9.70%) were Klebsiellapneumoniae. In the western studies conducted by Lautenbach et al. [9], Ozgunes et al. [13], Schwaber et al. [14] and Lee Young et al. [15], *K.pneumoniae*was the commonest organism found. Thus our study performed on Indian population did not correlate with the above four western studies. In another Indian study conducted by Shanthi M and Uma Sekar [16] at Chennai, *E.coli* was found to be the predominant ESBL producing isolate, accounting for 72.02%, followed by Klebsiella. The predominance of ESBL positive *E.coli*in the study by Shanthi M et al. [16] and our study points

Table 1: Organism wise distribution of ESBL, AmpC, MBL and co-production.

Organism/ β-lactamase	*Escherichia coli*	*Klebsiellapneumoniae*	*Total*
Pure ESBL	26 (12.62%)	20 (9.70%)	46 (22.33%)
Pure AmpC	01 (0.48%)	0	01 (0.48%)
Pure MBL	0	01 (0.48%)	01 (0.48%)
ESBL + AmpC	32 (15.53%)	21 (10.19%)	53 (25.72%)
AmpC + MBL	05 (2.42%)	05 (2.42%)	10 (4.85%)
ESBL + MBL	0	0	0
Non producers	54 (26.21%)	41 (19.90%)	95 (46.11%)
Total	118 (57.28%)	88 (42.71%)	206 (100%)

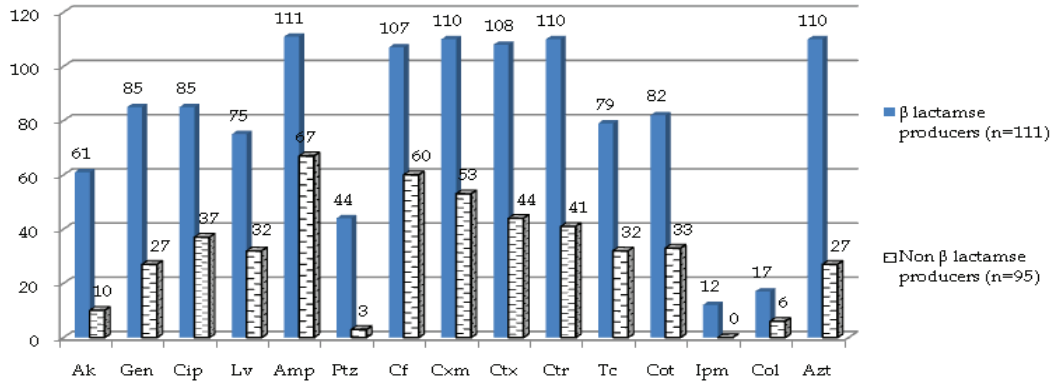

Chart 1: Antibiotic resistance patterns of beta lactamase producers and non producers

Index: Ak=amikacin, Gen=gentamicin, Cip=ciprofloxacin, Lv=levofloxacin, Amp=amoicillin, Ptz=piperacillin-tazobactam, Cf=cephalothin, Cxm=cefuroxime, ctx=cefotaxime, ctr=ceftriaxone, Tc=tetracycline, Cot=cotrimoxazole, Ipm=imipenem, Col=colistin,Azt=aztreonam.

towards the predominance of the isolate in this geographical location as compared to the western reports. The present study detected presence of AmpC β lactamase in 64 (31.06%) isolates. Other studies have shown AmpC occurrence varying from 14.8% - 52.1% [17-20]

Since the incidence of co producers of ESBL and AmpC is rising through out the world, we faced certain challenges in detection of these co producers. The inhibitor-based confirmatory test approach for ESBL detection is most reliable for isolates that do not coproduce an inhibitor resistant beta-lactamase, such as AmpC. High level expression of AmpC may prevent recognition of an ESBL. This problem is more common in tests with species or strains that produce a chromosomally encoded inducible AmpC beta-lactamase. With these organisms, clavulanate may act as an inducer of high level AmpC production and increase the resistance of the isolate to other screening drugs, producing a false negative result in the ESBL detection test [21]. Since the co-producers produced both ESBL and AmpC beta lactamase enzymes, the confirmatory test for ESBL detection by Cephalosporin/clavulanate combination disk test could not be interpreted correctly. However studies have indicated that AmpC has minimal effect on activity of cefepime, making it a more reliable indicator of ESBL production in presence of AmpC [22]. So the susceptibility pattern of all AmpC producers to cefepime was noted down in this study and it was found that, out of total 64 Amp C producers, 53 (25.72%) isolates were resistant to cefepime. This indicated 53 isolates were co producers of ESBL and Amp C, making the total number of ESBL producers 99 (48.05%). Other confirmatory tests using Tazobactam and sulbactam as inhibitors instead of clavulanate,are proven to be more reliable in ESBL detection in presence of AmpC [21].

In our study, only one isolate was found to produce pure AmpC β lactamase, while one isolate was detected to produce pure MBL. 10 (4.85%) isolates were found to co produce AmpC and MBL. Various studies have documented the incidence of multiple beta lactamase production ranging from 1.33% to 42.75% [17-20]. The reason for wide range in incidences could be factors like different antibiotic usage pattern leading to gene mutations or difference in normal flora depending upon cultural, nutritional or ethnic variations in different populations. The major cause of high incidence of ESBL and Amp C coproduction is inappropriate use of extended spectrum cephalosporins [10].

The majority of ESBL and AmpC producing organisms were found to exhibit high rates of resistance to commonly used antibiotics as shown in chart 1. Specifically, high rates of resistance observed to fluoroquinolones (76.57% resistance to ciprofloxacin, 67.56% resistance to levofloxacin) and cotrimoxazole (73.87% resistance) are most worrisome, as these cost effective drugs are most widely used. The results of present study were correlating with studies done by Baral P et al [23], Tumbarello M et al. [24] and Ozgunes I et al [13]. All the above studies showed that ESBL and AmpC producing organisms had a multidrug resistance potential. This might be due to the fact that ESBL genes are usually found in large plasmids that also contain other antimicrobial resistance genes, and hence ESBL-producing organisms may also show resistance to aminoglycosides, tetracyclines, chloramphenicol and/or aminoglycosides [14]. Another reason for such multidrug resistance potential of ESBL producers might be due to selective antibiotic pressure in the environment.

Conclusion

High degree of antibiotic co-resistance among beta lactamase producers emphasizes the judicious use of antimicrobials. Imipenem still remains most effective drug against ESBL and AmpC producing organisms followed by piperacillin-tazobactam [25]. Multiple beta lactamase detection and drug susceptibility can be routinely undertaken to enable the clinicians to select appropriate antibiotics [26]. Besides, formulating and adhering to an antibiotic policy is imperative to prevent selection pressure & spread of multidrug resistance strains.

References

1. Pitout JDD, Sanders CC, Sanders Jr WE. Antimicrobial Resistance with Focus on β-Lactam Resistance in Gram-Negative Bacilli. The American Journal of Medicine 1997 July;103(1):51-59.
2. Gupta V. An update on newer ß-lactamases. Indian J MedRes 2007;126:417-27.
3. Paterson DL, Bonomo RA. Extended spectrum betalactamase: a clinical update. Clin. Microbiol.rev. 2005;18(4):657-86.
4. Bradford PA. Extended spectrum beta-lactamases in 21st century: Characterization, Epidemiology and Detection of this important resistance threat. ClinMicrobiol Rev 2001;14(4):933-51.

5. Thomson KS: Extended-spectrum-beta-lactamase, AmpC, and carbapenemaseissues. J ClinMicrobiol, 2010;48:1019-25.
6. Clinical and laboratory Standards Institute. Performance standards for antimicrobials susceptibility testing. Twenty-second informational Supplement 2012:50.
7. Singhal S, Mathur T, Khan S, Upadhyay DJ, Chugh S, Gainnd R, Rattan A. Evaluation of Methods for Amp-C beta lactamases in gram negative clinical isolates from tertiary care hospitals. Indian J Med Microbiol 2005;23:120-24.
8. Varaiya A, Kulkarni M, Bhalekar P, Dogra J. Incidence of metallo-beta-lactamase-producing Pseudomonas aeruginosa in diabetes and cancer patients. Indian J PatholMicrobiol 2008;51:200-3.
9. Lautenbach E, Patel JB, Bilker WB, Edelstein PH, Fishman NO. Extended-Spectrum β-Lactamase-Producing *Escherichia coli* and *Klebsiella pneumonia*: Risk Factors for Infection and Impact of Resistance on Outcomes. Clinical Infectious Diseases 2001 April;32:1162-71.
10. Turner PJ. Extended-Spectrum β-Lactamases. Clinical Infectious Diseases 2005;41(4):S273-5.
11. Goossens H, Malhotra-Kumar S, Eraksoy H, et al. Results of two world-wide surveys into physician awareness and perceptions of extended-spectrum β-lactamases. ClinMicrobiol Infect 2004;10:760-2.
12. Paterson DL, Wen-Chien K, Von Gotteborg A, et. Al. Outcome of cephalosporin treatment for serious infection due to apparently susceptible organisms producing extended-spectrum β-lactamases: implications for the clinical microbiology laboratory. J ClinMicrobiol 2001;39:2206-12.
13. Ozgunes II, Erben NN, Kiremitci AA, Elif D ED Kartal, Durmaz GG, Colak HH et al. The prevalence of extended spectrum beta lactamase producing *Escherichia coli* and *Klebsiellapneumoniae* in clinical isolates and risk factors. Saudi Med J 2006; 27(5): 608-12.
14. Schwaber MJ, Venezia SN, Kaye KS, Ami RB, Schwartz D, Carmeli Y. Clinical and Economic Impact of Bacteremia with Extended-Spectrum β-Lactamase-Producing Enterobacteriaceae. Antimicrobial Agents and Chemotherapy 2006 Apr;50(4):1257-62.
15. Lee SY, Kotapati S, Kuti JL, Nightingale CH, Nicolau DP. Impact of Extended-Spectrum β-Lactamase_ producingEscherichia coli and Klebsiella species on Clinical Outcomes and Hospital Costs: A Matched Cohort Study. Infect Control HospEpidemiol 2006;27:1226-32.
16. Shanthi M, Sekar U. Extended spectrum beta lactamase producing Escherichia coli and Klebsiella pneumonia: Risk factors for infection and impact of resistance on outcomes. *J* assoc physicians India 2010;58:41-44.
17. Haider M, Rizvi M, Fatima N, Shukla I, Malik A.Necessity of detection of extended spectrum betalactamase, AmpC and metallo-beta-lactamases in Gramnegative bacteria isolated from clinical specimens. Muller J Med Sci Res 2014;5:23-8.
18. Grover N, Sahni BAK, Bhattacharya S. Therapeuticchallenges of ESBLs and AmpC beta-lactamase producersin a tertiary care center. Med J Armed forces India 2013;69:4-10.
19. Rawat V, Singhai M, Verma PK. Detection of different βlactamases and their co-existence by using various disccombination methods in clinical isolates ofEnterobacteriaceae and Pseudomonas spp. J Lab Physicians 2013;5:21-5.
20. Chatterjee SS, Karmacharya R, Madhup SK, Gautham V, Das A, Ray P. High Prevalence of Co-expression of newerβ-lactamases (ESBLs, AmpC β-lactamases and metallo βlactamases) in Gram negative bacilli. Indian J Med Microbiol 2010;28:267-8.
21. Thomson KS. Controversies about Extended-Spectrum and AmpC Beta-Lactamases. Emerging Infectious Diseases 2001 March;7(2):333-36.
22. Khan MKR, Thukral SS, Gaind R. Evaluation of a modified double disc synergy test for detection ofExtended spectrum β lactamases in AmpC β-lactamaseproducing Proteus mirabilis. Indian J Med Microbiol 2008;26(1):58-61.
23. Baral P, Neupane S, Shrestha B, Ghimire KR, Marasini BP, Lekhak B. Clinical and microbiological observational study on AmpC β-lactamse-producing *Enterobacteriaceae* in a hospital of Nepal. Brazilian J Infect Dis 2013;17(2):256-59.
24. Tumbarello M, Spanu T, Sanguinetti M, Citton R, Montuori E et al. Bloodstream Infections Caused by Extended-Spectrum-β-Lactamase-Producing *Klebsiella pneumonia*: Risk Factors, Molecular Epidemiology and Clinical Outcome. Antimicrobial Agents and Chemotherapy 2206 Feb; 50(2):498-504.
25. Rupp ME, Fey PD. Extended spectrum beta lactamase producing Enterobacteriaceae. Drugs 2003;63(4): 353-65.
26. Rudresh SM &Nagarathnamma T.Extended spectrum β-lactamase producing Enterobacteriaceae& antibiotic co-resistance.Indian J Med Res 2011 Jan;133:116-18.