INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com Volume-5, Issue-1, Jan-Mar-2014 Coden : IJABPT Copyrights@ 2014

ISSN : 0976-4550

Received: 09th Nov-2013 *Revised: 17th Nov-2013* *Accepted: 20th Nov-2013*

Research article

PHENOTYPIC DETECTION OF CARBAPENEM RESISTANCE IN CLINICAL ISOLATES OF ACINETOBACTER BAUMANII IN KANCHIPURAM

Sivasankari S, Senthamarai, Anitha.C, Apurba Sastry, Sandhya bhatt, Kumudhavathi M.S, Amshavadhani S.K

Department of Microbiology, Meenakshi Medical College & RI, Enathur, Kanchipuram

ABSTRACT: Acinctobacter species are common non fermentative gram negative bacilli isolated in clinical laboratory most frequently encountered species. Acinetobacter resistance is develop due to acquired resistance. Because of frequent multidrug resistance isolates carbapenems have become important for treating resistant strains. There is a need for rapid screening & detection of MBL in Acinetobacter to modify the treatment. The present study was aim to determine the resistance of A.baumanii complese to various classes of drugs and to carbapenems and MBL production. Samples such as urine, blood, sputum, pus & body fluids. All samples were processed as per CLSI guidelines. Meropenem resistant strains were screened for carbapenemase and MBL production. Out of 92 Acinetobacter 85 (92.39%) were Acinetobacter baumanii. More than 80% resistance is seen in 3rd generation Cephalosporins. Out of 21 meropenem resistant strains 14 were carbapenemase positive and 3 were MBL producers. Our study shows raising trend of multidrug resistance and carbapenem. This will help in early detection and better treatment modalities.

Key words: Phenotype, Carbapenem, Clinical isolates, Acinetobacter

Abbreviations: MRSA–Methicillin resistant Staphylococcus aureus, ESBL – Extended sputum β - lactamases GNB – Gram negative bacilli, MDR – Multi drug resistance

INTRODUCTION

Acinetobacter *spp*. are considered common oxidase negative non fermentative gram negative bacilli isolated in clinical laboratory (Anil Kumar V et al., 2011).During the last decade, multidrug resistant Acinetobacter baumanii have been reported due to cross contamination by colonized hands of hospital persons (A Amjad et al., 2011). Acinetobacter baumanii, a member of Acinetobacter Calcoaceticus – A member of baumanii complex makes up to 73% of all Acinetobacter *spp*. and it is commonly isolated from clinical samples (Antony et al., 2008). Most frequently encountered *spp.* are Acinetobacter baumanii and it is commonly associated with infections such as bacteremia, urinary tract infections, meningitis, skin & soft tissue infections & pneumonia (Bouvet PJM, et al., 1986). After MRSA & ESBL, another β lactamase causing resistance, among GNB is Carbapenemase resistance is increasing (Clare Franklin et al., 2006). Carbapenemase antibiotics play a crucial role in treatment of serious nosocomial infections due to Acinetobacter baumanii. Acinetobacter spp. can cause frequent resistance to commonly used antibiotics because of the acquired resistance mechanisms like antibiotic inactivating enzymes, effuse pumps ribosomal binding site mutations and down regulation of porin channels on the cell membrane giving rise to multidrug resistant isolates (E Bergogne – Berezin et al., 1996). Because of the frequent MDR isolates carbapenems have become important for treating the resistant strain. Carbapenemases especially transferrable mettalo – β – lactamases (MBLS) are most feared because of their ability to hydrolyze virtually all drugs (Franklin C et al., 2006). Acquired carbapenem can be either mettalo β lactamases (MBL) such as VIM, IMP & non MBL. MBL genes have propensity to disseminate quickly to other species of gram negative bacilli (Gaur A et al., 2008). Detection of carbapenemase is difficult. It can be detected both phenotypically and genotypically. Modified Hodge test (MHT) is simple and easy test to detect phenotypically in laboratory. Through OXA is the predominant carbapenemase which is responsible for Carbapenem resistance, reports in IMP or VIM class mettalo β lactamase producing Acinetobacter spp. are increasing (Gomty Mahajan et al., 2011).
Hence, there is a need for rapid screening and detection of MBL in Acinetobacter, so as to modify the treatment. Hence this study was done in our hospital to determine the resistance of A. baumanii complex (Acb complex) to various class of drugs and to carbapenems and to detect the prevalence of carbapenemase and MBL production phenotypically, so as to modify therapy and initiate effective infection control measures.

MATERIALS AND METHODS

This study was done from May 2012 to May 2013 at MMCH & RI, Kanchipuram. The clinical specimens comprising of urine, blood, sputum, pus and body fluids were routinely cultured. Colonies suspected of Acinetobacter were identified by gram staining, colony morphology, negative oxidase reaction, motility. There after identified as Acinetobacter *spp.* by standard protocol (Gupta V et al., 2006). Antibiotic sensitivity testing was done by Muller Hinton agar using Kirby bauer disc diffusion method with commercially available discs (HI – media) and by following CLSI guidelines. The following antibiotics were used in the study were Amikacin, Gentamycin, ciprofloxacin, cefotaxime, ceftazidime, cefaperazone, pipercillin – tazobactum, Meropenem. Meropenem resistance was used as an indicator for carbapenemase production. Meropenem resistance was detected using MIC by agar dilution method as recommended by CLSI guidelines (< 4 µg / ml – sensitive, 8 µg/ml – intermediate, > 16 µg/ ml resistant). ATCC 27853 Pseudomonas aeruginosa were used as control strain. All the strains which showed resistance to Meropenem were further screened for Carbapenemase and MBL production by modified Hodge test (MHT) and EDTA disc synergy (EDS) test, respectively.

Modified Hodge test

All the meropenem resistant strains were subjected to MHT for detection of Carbapenemases. A 0.5 Mc farland detection of E.coli ATCC 25922 in 5ml of BHI broth was prepared. Lawn culture was made on MHA and 10 µg meropenem disc was placed on the centre of plate. Test organism was streaked in a straight line from the edge of the disk to the edge of the plate. Meropenem resistance was detected after incubation overnight at 35°C. The positive test is interpreted by the presence of clover leaf shaped indendation of E.coli growing along the test organism growth streak within the disc diffusion zone (Hemalatha V et al., 2011).

MBL detection: Combined disc test: (CDT)

The test strain was inoculated on plates with MHA plates as per CLSI guidelines for antibiotic sensitivity testing. The presence of MBL was determined by placing two imipenem discs on the inoculated plate, in which 10µl of 0.1 M EDTA (292 µg) was added to one of the imipenem disks. After overnight incubation at 37°C, the inhibition zones of imipenem and imipenem with EDTA were compared. A zone difference of > 4 mm between the imipenem and the imipenem EDTA inhibition zones were confirmed as MBL positive (Jeong SH et al., 2006)

RESULTS

A total of 92 Acinetobacter spp. were isolated from various clinical samples and were identified upto species level as A.baumanii 85(92.39%) and 7 (7.60%) were A.lwoffi

Table-1 showing antimicrobial resistance pattern (n = 92)

Antibiotics	N	%
Amikacin	72	78.26
Gentamycin	70	76.08
Ciprofloxacin	74	80.45
Cefotaxime	76	82.60
Ceftazidime	75	81.52
Cefaperazone	77	83.69
Cefepime	22	23.91
Piperacillin – Tazobactam	12	13.04
Meropenem	21	22.82

Resistance pattern of Acinetobacter revealed that more than 80% resistance seen in 3rd generation cephalosporins followed by Aminoglycosides and Quinolone, indicating the high prevalence of multidrug resistance. Maximum number of Acinetobacter isolates were from pus (32) 34.78% followed by blood 22 (23.91%) & urine 13 (14.13%). A total of 21 (22.82%) meropenem resistant acinetobacter were detected by disc diffusion. MIC for Meropenem isolates ranged from 8 and 64 µg/ml. Out of 21 Meropenem resistant strains 14/21 (66.66%) were found to be carbapenemase positive by MHT. These positive isolates were further tested by combined Disc test and 21.42% (3/14) were found to be MBL producers phenotypically.

DISCUSSION

In the present study, Acinetobacter baumanii was the predominant species (92.39%), followed by Acinetobacter lwoffi (7.60%). This is concordant with Sinha et al., in his study done in North India that A.baumanii were (92.14%) and A. lwoffi were (6.42%) (E Bergogne–Berezin et al., 1996).

In our present study, maximum number of Acinetobacter spp was isolated from pus 34.78% followed by blood 23.91%. Sinha et al 2013 have also isolated maximum Acinetobacter isolates from pus (37.14%) followed by blood (22.85%) & urine. Antimicrobial susceptibility of Acinetobacter seems to vary considerably among various centers even among different wards of the same hospital (Joshi SG et al., 2003). Gomty mahajan et al showed that 70% of isolates were resistant to 3rd generation Cephlosporins, aminoglycosides & quinolones. In our study also Acinetobacter *spp* showed more than 80% resistance to 3rd generation Cephalosporins & aminoglycosides. In our study pipercillin – Tazobactum showed the least resistance (13.04%) which is concordant with a study done by Anil kumar et al 2011 (19.25%) in his resistance pattern. In India, carbapenems are used as a last resort in infections due to multidrug resistant Acinetobacter infections in any nosocomial setting (Konemann et al., 2010). Carbapenems have broad spectrum activity and they are stable to hydrolysis by β-lactamases including ESBL's and Amp C β-lactamases. In recent years, Meropenem resistance is found to emerge from various parts of the world and even from different regions of the country (Kumar AV et al., 2011, Lee K et al., 2001).

In our study 22 – 82% of isolates were resistant to Meropenem. A similar percentage (20.0%) of resistance to Meropenem was observed by other researchers. A study done in Puducherry shows 89% of resistance to meropenem (Whereas Taneja et al & Srinivasan et al have been reported moderate resistance. Out of 21 carbapenem resistant isolates in our study 14/21 (66.66%) isolates were found to produce carbapenemase enzyme by MHT and remaining 7 isolates were negative by MHT. In a study done by Gomaty Mahjan et al, isolated 47.6% of Carbapenemase by MHT.

Various Indian studies which have used MHT to detect Carbapenemase production in the Acb complex, reported a wide prevalence range of Carbapenemase producing A.baumanii varying from 2.2% to 71% (Manikal VM et al., 2003).only one study in India used MHT to detect Carbapenemase production of Acb with low prevalence of 2.2% (N Sinha J et al., 2013). MHT was used as a screening method to detect carbapenemase producers hence it gives a positive result with strains that producers carbapenamases like MBL, Klebsiella pneumonia carbapenemase and OXA – type β lactamases. Carbapenem resistance can occur due to decreased expression on outer membrane proteins, combined with Amp c lactamases & the Modification of penicillin binding proteins & the efflux mechanisms (Anil Kumar V et al., 2011). In our study of the 21 MHT positive strains, only 4 (21.42%) were found to be MBL producers, which was tested with combined disk test method. This is concordant with Anil Kumar et al who have also observed 21% of MBL producers in their study. Similar results were also obtained by Franklin et al by using CDT (16%) and by Lee et al 2003 by DSST (14%). Uma et al., have reported high percentage of MBL producing Acinetobacter (71%) by DSST and Gupta et al have reported 7.5% where as sinha et al found that none of their isolates were MBL producers.

The ability of the MBL's isolates to participate in horizontal MBL gene transfer with other GNB contribute for MBL out breaks. Hence detection of MBL and Carbapenemases is very important in laboratory detection to prevent the ongoing infection spread (Qualej et al., 2003).

CONCLUSION

In our study shows raising trend of multidrug resistance and carbapenem poses a threat to treat infection.

CDT which is easy to carry out with less cost effective, this can be adopted routinely for detecting MBL phenotypes. This will help in early detection and better treatment modalities & regular monitoring to control infections due to Acinetobacter infections.

REFERENCES

Anil V.Kumar, Vishnu S.pillai (2011). The Phenotypic Detection Of Carbapenemase In Meropenem Resistant Acinetobacter Calcoaceticus–Baumannii Complex In A Tertiary Care Hospital In South India, Journal of clinical & Diagnositic research April, Vol – 5(2); 223 – 226

A., Amjad; I. A., Mirza; S. A., Abbasi; U., Farwa; N., Malik; F., Zia (2011). Modified Hodge test: A simple and effective test for detection of carbapenemase production .Iranian Journal of Microbiology; Vol. 3 Issue 4, p189.

Anton Y. Harald scifert and David L (2008). Acinetobacter spp. emergence of a successful pathogen. Clin Microbial Rev; 21; 538 – 582.

Bouvet, P J M, Grimont, P A D (1986). Taxonomy of the genus Acinetobacter with the recognition of Acinetobacter baumannii sp. Int J Syst Bacteriol 36, 228-240.

Clare Franklin, Lisa Liolios, and Anton Y (2006). Peleg Phenotypic Detection of Carbapenem-Susceptible Metallo-β-Lactamase-Producing Gram-Negative Bacilli in the Clinical Laboratory J Clin Microbiol. September; 44(9): 3139–3144.

E Bergogne-Bérézin and K J Towner (1996) Acinetobacter spp. as nosocomial pathogens: Microbiological, clinical, and epidemiological features. Clin Microbiol Rev. April; 9(2): 148–165.

Franklin C, Liolios L, Peleg AY (2006). Phenotypic detection of carbapenem-susceptible metallo-beta-lactamase-producing gram-negative bacilli in the clinical laboratory. J Clin Microbiol. Sep; 44(9):3139-44.

Gaur A, Garg A (2008). Observations on carbapenem resistance by minimum inhibitory concentration in nosocomial isolates of Acinetobacter species: An experience at a tertiary care hospital in North India. J Health Popul Nutr. Jun;26(2):183-8.

Gomty Mahajan, Sheevani Sheemar, Shashi Chopra (2011) Carbapenem resistance and phenotypic detection of carbapenemases in clinical isolates of *acinetobacter baumanii Indian Journal of Medical Sciences, Volume 65, Issue 1 [p. 18-25].*

Gupta V, Datta P, Chander J (2006). Prevalence of metallo-beta lactamase (MBL) producing Pseudomonas spp. and Acinetobacter spp. in a tertiary care hospital in India. J Infect. May;52 (5):311-4. Epub 2005 Oct 6.

Hemalatha V, Sekar V, Kamat V (2011). Detection of MBL producing pseudomonas aeuroginosa in hospitalized patients. Indian J.Med., Res 122; 148 – 52.

Joshi SG, Litabe GM, Ghole VS (2003). Multidrug resistant Acinetobacter baumanni isolates from a teaching hospital. J.infect chemoth; 9; 187 – 90.

Jeong SH, Bae IK, Park KO, Sobn SG, Jang SJ (2006). Outbreaks of imipenem resistant Acinetobacter banumanii producing Carbapenemases J.Microbiol; 44;423 – 31

Konemann (2010) – Color Atlas and Tentborh of Diagnostic Microbiology – 5th Edition,pg 253 – 309.

Kumar AV, Pillai VS, Dinesh KR (2011). The Phenotypic detection of Carbapenem in Meropenem resistant Acinetobacter baun complex in a Tertiary care hospital in South India J.Clinical Diagnosis res; 2; 223 – 6.

Lee K, Chong Y, Shin HB, Kim YA, Yong D, Yum JH (2001). Modified Hodge and EDTA-disk synergy tests to screen metallo-beta-lactamase-producing strains of Pseudomonas and Acinetobacter species. Clin Microbiol Infect. Feb;7(2):88-91.

Manikal VM, Landman D, Saurina G, Oydna E, Lal H (2003). Endemic carbapenem-resistant Acinetobacter species in Brooklyn J.Microl; 24; 224 – 28.

Maharwal S, Srinivasa M, Taneja N (2003). Imipenem resistance in non fermenters causing nosocomial urinary tract infections Indian J Med Sci; 57; 294 – 9.

N Sinha, J Agarwal, S Srivastava, M Singh (2013). Analysis of carbapenem-resistant *Acinetobacter* from a tertiary care setting in North India Year. Indian journal of medical microbiology, 31 (1); Pp: 60-63.

Neeraj Goel, Chand Wattal, Jaswinder Kaur Oberoi, Reena Raveendran, Sanghamitra Datta and Kamal Jeet Prasad (2011). Trend analysis of antimicrobial consumption and development of resistance in non-fermenters in a tertiary care hospital in Delhi, India J.Antimicrob.Chmother; 66; 1625 – 30.

Neelam Taneja, Gagandeep Singh, Meenakshi Singh, Meera Sharma (2011). Emergence of tigecycline & colistin resistant *Acinetobacter baumanii* in patients with complicated urinary tract infections in north India J.Med Res ; 133: 681-684

Noyal MJ, Menezes GA, Harish BN, Sujatha S, Parija SC (2009). Indian J Med Res. Simple screening tests for detection of carbapenemases in clinical isolates of nonfermentative Gram-negative bacteria. Jun;129(6):707-12.

Quale J, Bratu S, Landman D (2003). Molecular epidemiology and mechanisms of carbapenem resistance in Acinetobacter baumannii endemic in New York City. Clin Infect Dis. 15; 37 (2):214-20. 2003 Jul 9.

Uma Karthika R, Srinivasa Rao R, Sahoo S, Shashikala P, Kanungo R (2009). Phenotypic and genotypic assays for detecting the prevalence of metallo-beta-lactamases in clinical isolates of Acinetobacter baumannii from a South Indian tertiary care hospital. J Med Microbiol. ; Apr; 58 (Pt 4):430-5. 1099/jmm.0.002105-0.