ISID Small Grant Program Report

Clinico epidemiologic and Molecular Characterization of Metallo beta lactamases (MBLs) producing *Pseudomonas aeruginosa* causing Nosocomial Infections

Small Grants Awardee: Ms.Saradiya Chatterjee MSc, Associate Research Officer
Study Supervisor: Dr.Anand Manoharan PhD MPH, Scientist
Institution: Prof. Benjamin M Pulimood Laboratories for Infection, Immunity and Inflammation, Department of Medicine Unit I and Infectious Diseases, Christian Medical College and Hospital, Vellore 632004, India.
Period of Study: One year (January–December 2009)
Source of Funding: International Society for Infectious Diseases, Small Grant, Fall 2008.

Background:

The emergence of Metallo-Beta-Lactamases (MBLs) in major clinical pathogens was first described in the early 1990s in Japan, and is now a problem of global magnitude. MBLs producing *P.aeruginosa* (PSA) isolates have been responsible for several nosocomial outbreaks causing serious infections in compromised patients including those with cancer, burns, and cystic fibrosis. The high prevalence of co-resistance to betalactam, aminoglycoside and quinolone against PSA has necessitated increased use of carbapenems. MBL production among PSA is one of the several mechanisms causing carbapenem resistance (CARB-R) transferable by integrons. Lack of sufficient reports from India in this area indicated the need for this study. Moreover limited studies that exist are single institution and retrospective in nature. There have been no prospective studies looking into the risk factors for acquisition of MBLs PSA.

Methods:

During March-September 2009 BMPLIII received 105 consecutively collected Pseudomonas *spp*. causing infections of (skin and soft tissue-58, respiratory tract-19, blood-14, urine-10, and other sites-4) from four Indian medical centers sited at diverse geographical regions. Antimicrobial susceptibility testing by Kirby Bauer method against ceftazidime (CZD), cefepime (FEP), piperacillin/tazobactam (TZP), ticarcillin /clavulanic acid (TIM), gentamicin (GEN), amikacin(AK), ciprofloxacin (CIP), imipenem (IMP), meropenem (MEM), aztreonam (ATM) and colistin (CL) was done. MBL was screened by the Combined Disk Diffusion Test (CDDT) method using 0.5M EDTA (930µg) as the inhibitor with IMP, positives (inhibition ≥7mm) confirmed by IMP+EDTA Etest and PCR (VIM and IMP genes).

Results:

Of the 100 PSA confirmed isolates, 21(21%) were MBL producers. Overall resistance to CIP 56 %> CZD 43% >AK 45 %> FEP 40%. Resistance pattern among MBL/NMBL was CZD(95.2%/29.1%), FEP(90.5%/26.6%), TZP(85.7%/15.2%), TIM(100%/30.4%), GEN(100%/32.9), AK(100%/30.4%), CIP(100%/44.3%), IMP(100%/12.7%), MEM(90.5%/15.2%), ATM(76.2%/44.3%). Overall resistance to IMP and MEM was 31% and 30% respectively. All isolates were susceptible to CL. Multi Drug Resistance (MDR) (resistant to ≥2 classes of antimicrobials) was 55(55%).Overall 23(23%) were CDDT positive. IMP+EDTA Etest and PCR confirmed 21 to be MBL positives. Among the MBL isolates all were VIM positive, one MBL PSA (SSTI) was positive for IMP gene & VIM which on sequencing was confirmed to be IMP 7 & VIM 2 [First time report from India].

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Saradiya Chatterjee

Ms. Saradiya Chatterjee is currently pursuing her PhD in Immunology at the University of Pierre and Marie Curie, Paris, France. She completed her Masters in Biotechnology from Vellore Institute of Technology, Vellore, India and was thereafter a Research Associate in the Department of Medicine Unit I and Infectious Diseases at the Prof Benjamin M Pulimood Laboratories for Infection, Immunity and Inflammation, Christian Medical College and Hospital, Vellore, India. Her previous work includes microbiological and molecular characterization of nosocomial pathogens and in vitro *evaluation of newer antimicrobials like tigecycline. She was awarded the Senior Research Fellowship by the Council for Scientific and Industrial Research, India and an international travel grant from the Department of Science and Technology, India to attend the 14th International Congress on Infectious Diseases held at Miami, Florida. She was awarded an ISID Small Grant in Fall 2008.*

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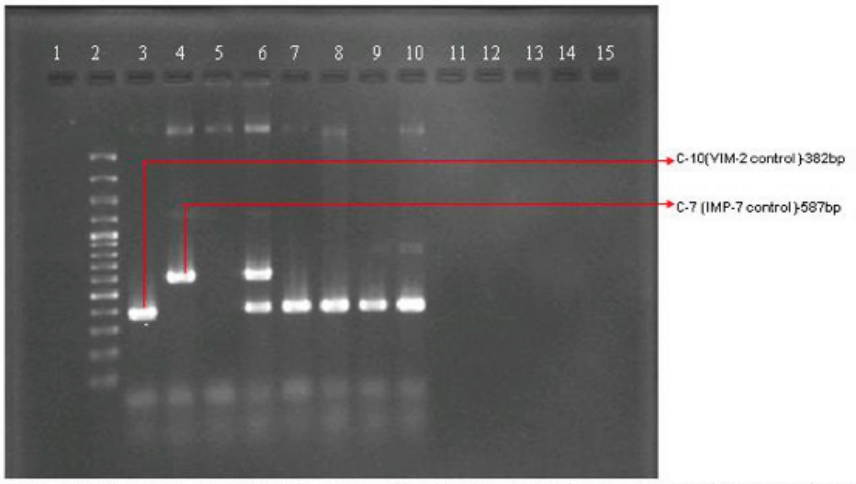
Sensitivity and specificity of CDDT was 100% and 97.5% respectively when compared to PCR. Diabetes mellitus was found to be the major risk factor in PSA infections. History of prior antibiotic usage of aminoglycosides (40%), 3rd generation cephalosporins (35%), quinolones (35%), carbapenems (30%), penicillins (15%), betalactam+betalactamase inhibitors (5%), polymixin(30%), colistin (15%) was noted for the patients with MBL infection. Clinical improvement or cure with modification of initial antibiotics was found in 95% (19/20) patients with MBL PSA.

PCR Gel Picture for VIM and IMP genes



Legend: Lane 1- Blank, Lane 2- 100bp Ladder, Lane 3- C-10(VIM-2 control), Lane 4- C-7 (IMP-7 control), Lane5 ATCC 27853 *P.aeruginosa* (negative control), Lane 6-MS 53(VIM and IMP positive), Lane 7-2ADU13(VIM positive), Lane 8- IPA 13(VIM positive), Lane 9- IPA 26(VIM positive), Lane 10- IPA30 (VIM positive), Lane 11- Water Blank

Fig 3: PCR Gel Picture

Conclusions:

MBL production is an important mechanism of carbapenem resistance among PSA at multiple centers. History of prior antibiotic usage was found to be a risk factor for MBL PSA in the study. No MBL PSA nosocomial outbreak recorded among study centers. Development of MBL PSA can be minimized by using appropriate choice of antibiotics. CDDT is a useful and cost effective method for screening MBL, can be introduced in routine clinical microbiology laboratory.

Study Presentation:

14th International Congress on Infectious Diseases, Miami, Florida.

S. Chatterjee, A. Kumar, K. N. Prasad, D. Mathai, A. Manoharan. Clinico epidemiologic and molecular characterization of metallo beta lactamases (MBLs) producing nosocomial *Pseudomonas aeruginosa* (PSA). Int J Infect Dis 2010;14S1:23.001.

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