

Fecal carriage of Metallo Beta Lactamase Producing Organisms Isolated from Indoor Patients

Ritu Aggarwal¹, Nidhi Goel¹ and Uma Chaudhary²

¹4/8 FM, Medical Campus, PGIMS, Rohtak-124 001, Haryana, India.

²Department of Microbiology, Pt. B.D. Sharma Institute of Medical Sciences, Rohtak, Haryana, India.

ABSTRACT

Incidence of MBL producing isolates has been increasing distinctly over last few years. Discovery of NDM-1 in *Enterobacteriaceae* has further complexed the picture. Treatment of these patients pose a serious challenge, as these pathogens are resistant to all commonly used antibiotics. Plasmidic location of the gene has facilitated their spread to the normal human flora. The emergence of these strains is therefore a major concern and the one that microbiologists cannot afford to ignore. So an observational prospective study was conducted in a tertiary care teaching hospital to detect the presence of MBL producing bacteria among the stool of indoor patients. A total of 62 isolates from 130 stool samples were studied for MBL production using imipenem and MPA discs. Any increase in zone of inhibition was considered positive. Antimicrobial sensitivity profile was determined against commonly used antimicrobials. MBL production was found in 20(32.25%) isolates. Out of 62 isolates, 12 (29.26) *E coli* and 8 (38.09%) *Klebsiella spp* were found to be positive for MBL production. Maximum sensitivity (100%) was seen with polymixin B and colistin. These isolates were highly resistant ($\geq 60\%$) to the rest of the antimicrobials tested. Our data reports that MBL enzyme exists among stool isolates in our institute. These MBL producing strains in the stool may contribute to both nosocomial and community transmission of these highly resistant strains. Good infection control practices and antibiotic management interventions are instrumental in containing these strains.

Keywords: Community acquired infection, drug resistance, fecal carriage, MBL, NDM-1.

INTRODUCTION

Antimicrobial resistance is a serious problem that threatens the continued effectiveness of antimicrobials. Currently, metallo beta lactamase enzymes (MBLs) are clinically most threatening enzymes that can hydrolyse all β lactams except aztreonam. This MBL mediated resistance cannot be inhibited by conventional β -lactamase inhibitors. MBL producers are also frequently resistant to fluoroquinolones and aminoglycosides resulting in tremendous therapeutic consequences. A recent report of superbug New Delhi Metallo- β - Lactamase (NDM-1) drives the concern of rapidly increasing antibiotic resistance among hospital isolates.^{1,2,3}

Gene for the MBL enzyme may be part of chromosome or the plasmidic DNA. This plasmid location has facilitated the ready dissemination of the MBL producing Gram negative bacteria. The problem gets aggravated when the gene is acquired by normal human flora and manifest as the source of endogenous infections. These MBL producing strains in the stool may contribute to nosocomial transmission of antibiotic resistant

gram negative pathogens due to fecal shedding on to patient's skin and environmental surfaces. Moreover, patients may carry MBL producers in their gut while getting discharged from the hospital and can be source of community onset MBL infection. Such patients also act as bacterial reservoir that increases dispersal of resistance in healthy population. As we are several years away from the development of a safe therapeutic inhibitor; their continued spread would be a clinical calamity.^{1,2,4}

Detection of gastrointestinal colonization among patients is a part of infection control interventions in hospital. So, clinical microbiologists have significant role to play in the detection of MBL carriers and controlling the spread of these multidrug resistant bacteria.¹ This preliminary study was designed to detect the MBL producing organism in the stool of indoor patients of our hospital.

MATERIAL AND METHODS

This prospective study was conducted in the Department of Microbiology. A total of 62 isolates from 130 consecutive stool samples from indoor patients were studied for MBL

production over a period of one month. All the stool samples were processed and isolates were identified using standard microbiological techniques.⁵ For the detection of MBL, double disc synergy test method was used. A lawn Culture of the test isolate was prepared on MHA plate using broth suspension of turbidity matched with 0.5 mc Farland standard as per CLSI guidelines. Test was carried out by using 2-mercaptopyruvic acid (MPA 3µl, undiluted) (Hi Media Mumbai India) as MBL inhibitor against imipenem (Imp) disk. A sterile blank filter paper disk was placed on the inoculated agar plate on which MPA was poured. An antibiotic disc of Imp was placed at edge to edge distance of 10mm from chelating agent disc. After overnight incubation the presence of even a small synergistic inhibition zone was interpreted as positive.⁶

Antimicrobial sensitivity profile was determined against commonly used antimicrobials, using Kirby Bauer disc diffusion test and results were interpreted according to the CLSI guidelines. Antimicrobials tested were cotrimoxazole (25µg), doxycycline (30µg), ciprofloxacin (5µg), amikacin (30µg), amoxyclav (30µg), ceftazidime (30µg), meropenem (10µg), piperacillin/tazobactam (100/10µg), colistin (10 µg), polymixin B (300 units). Mueller Hinton agar and susceptibility discs used in the study were procured commercially from Hi, Media (Mumbai, INDIA).^[7] *Escherichia coli* ATCC 25922 was used as control strain.

RESULTS

Out of 62 stool isolates, 41 were *E.coli* and 21 were *Klebsiella* spp. MBL production was found in 20(32.25%) isolates. Out of 41 *E.coli* tested, 12 (29.26%) were found be MBL producer phenotypically. Eight (38.09%) *Klebsiella* spp out of total 21 isolates were found to be positive for MBL production.(FIG-1) These isolates were highly resistant (>75%) to most of the antimicrobials tested viz cotrimoxazole, doxycycline, ciprofloxacin, amoxyclav, ceftazidime and meropenem. Resistance to amikacin, piperacillin and imipenem ranged from 60% to 65%. Maximum sensitivity (100%) was seen with polymixin B and colistin. (FIG-2).

DISCUSSION

Gastrointestinal tract acts as a reservoir for drug resistant bacteria. It provides the site for the transfer of drug resistance genes. It has been observed that infections are often preceded by intestinal colonization by the resistant bacteria. Intestinal tract colonization facilitates both health care associated and

community spread of the resistant strain. In contrast to fecal carriage with ESBL producing bacteria, carriage with MBL producing bacteria has been rarely discussed.^{2,4,8}

In the present study, 62 stool isolates were studied for MBL production. It was observed that 20(32.25%) were producing MBL phenotypically. Both the *E.coli* and *Klebsiella* spp isolated from the stool were found to be MBL positive in this study. Fecal carriage of MBL producing Enterobacteria has been reported by various authors worldwide.^{1,8,9,10}

In our study, a very high rate of antimicrobial resistance has been observed against the antimicrobials tested except colistin and polymixin B. Similar findings have also been depicted by other investigators in their study.^{1,9,11,12,13,14} Knowledge of the susceptibility profile of these isolates helps in selective decontamination of the digestive tract.^{4,15} But acquisition of such high drug resistance by these colonizing strains makes this regimen ineffective.

Fecal carriage of such highly drug resistant MBL positive bacteria would be of great therapeutic and epidemiological challenge for both health care settings and the community. There are well documented reports of intercontinental spread of NDM-1 strains. Moreover, secondary dissemination with these highly resistant strains has been recognized in United Kingdom. D'Andrea et al have reported cross transmission of NDM-1 among three patients who have attended same day hospital clinic, shared the same room, restrooms, nurses and physicians. Index case in this report had visited NDM-1 endemic region.¹⁰ First documentation of community spread of MBL strain was given by Poulou et al.² Management of this challenging situation is difficult and requires early identification and reporting of such highly drug resistant strains, so that infection control practices can aid in containing the spread of the strains.

One of the potential limitations of this study is that molecular epidemiologic analysis and characterization of MBL types was not carried out. Still, our study reveals that MBL producing organisms exists in the stool of the indoor patients in our health care settings. This fecal carriage of MBL producing organisms may result in both intrahospital and community spread of the strains. To combat this problem, efforts should be made to isolate and characterize plasmids and to screen for the liable MBL. Furthermore, this is an important alarming signal for the implementation of most stringent infection control practices and good antibiotic stewardship.

REFERENCES

1. Birgy A, Doit C, Kurkdjian PM, Genel N, Faye A and Arlet G. Early detection of colonization by VIM-1 producing *E.coli* in two children returning to France. *J Clin Microbiol.* 2011;49(8): 3085-7.
2. Poulou A, Spanakis N, Pournara S, Pitiriga V, Ranellou K and Markou F. Recurrent healthcare-associated community-onset infections due to *Klebsiella pneumoniae* producing VIM-1 metallo- β -lactamase. *J Antimicrob Chemother.* 2010;65:2538-42.
3. Tato M, Coque TM, Ruiz-Garbajosa P, Pintado P, Cobo J and Sader HS. Complex clonal and plasmid epidemiology in the first outbreak of Enterobacteriaceae infection involving VIM-1 Metallo- β -Lactamase in Spain: Toward Endemicity. *Clin Infect Dis.* 2007;45:1171-8.
4. Donskey JC. Antibiotic regimens and intestinal colonisation with antibiotic resistant gram-negative bacilli. *Clin Infect Dis.* 2006;43(2):62-9.
5. Duguid JP, Collee JG and Fraser AG. Laboratory strategy in the diagnosis of infective syndromes. In: Collee JG, Duguid JP, Fraser AG, Marmion BP, editors. *Mackie and MacCartney: Practical Medical Microbiology.* 13th ed. Singapore: Longman Singapore Publishers; 1989;600-49.
6. Arakawa Y, Shibata N, Shibayama K, Kurokawa K, Yagi T and Fujiwara H. Convenient test for screening Metallo- β -Lactamase producing gram-negative bacteria by using thiol compounds. *J Clin Microbiol.* 2000;38(1):40-3.
7. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; 21st informational supplement. CLSI/NCCLS M100-S21 Wayne (PA): Clinical and Laboratory Standards Institute, 2011.
8. Gijón D, Curiao T, Baquero F, Coque TM and Cantón R. Fecal carriage of carbapenemase producing Enterobacteriaceae: a hidden reservoir in hospitalised and non-hospitalised patients. *J Clin Microbiol.* 2012;50(5):1558-63.
9. Perry JD, Naqvi SH, Mirza I A, Alizai SA, Hussain A and Ghirardi S. Prevalence of fecal carriage of Enterobacteriaceae with NDM-1 carbapenemase at military hospitals in Pakistan, and evaluation of two chromogenic media. *J Antimicrob Chemother.* 2011;66:2288-94.
10. D'Andrea MM, Venturelli C, Giani T, Arena F, Conte V and Bresciani P. Persistent carriage and infection by multidrug-resistant *Escherichia coli* ST405 producing NDM-1 Carbapenemase: Report on the First Italian Cases. *J Clin Microbiol.* 2011;49(7):2755-8.
11. Arora S, Saha S and Bal M. Imipenem-resistance among multi-drug resistant clinical strains in urinary infections from Kolkata. *Ind J Med Res.* 2007;125:689-91.
12. Varaiya A, Kulkarni N, Kulkarni M, Bhalekar P and Dogra J. Incidence of metallo β lactamase producing *Pseudomonas aeruginosa* in ICU patients. *Indian J Med Res.* 2008;127:398-402.
13. Toraman ZA, Yakupoqlari Y and Kirzigil A. Detection of metallo β -lactamase production and antibiotic resistance with E-test method in *pseudomonas*, *acinetobacter* and *klebsiella* strains, in Turkey. *J Infect Chemother.* 2004;10(5):257-61.
14. Bhongle NN, Nagdeo NV and Thombare VR. The prevalence of Metallo β -Lactamases in the clinical isolates of *Pseudomonas aeruginosa* in a tertiary care hospital: An alarming threat. *J of Clinical and Diagnostic Research.* 2012;6(7):1200-2.
15. Chaudhary U and Aggarwal R. Intestinal Colonisation with ESBL-producing Bacteria. *Indian Journal of Clinical Practice.* 2009;19(10):22-3.