Original Research Article

Detection of Metallo Beta Lactamase Production in Gram-Negative Bacilli Isolated in A Tertiary Care Hospital of Bihar

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Received: 06-11-2021 / Revised: 27-12-2021 / Accepted: 12-01-2022

Abstract

Introduction: This study was designed to generate updated information on the burden of metallo-beta-lactamase producing Gram negative bacteria from a tertiary care hospital of Bihar so that an effective antimicrobial stewardship policy can be formulated and implemented to circumvent the rising threat of antimicrobial resistance. **Methodology:** The prospective study was conducted at Jawahar Lal Nehru Medical College, Bhagalpur, Bihar from August 2020 January 2021 to assess the prevalence of metallo-beta-lactamase production. A total of 500 different clinical samples from patients of all age groups received in the microbiology laboratory for routine examination and culture, during the study period of six months were included in the study. All the sample specimens were processed by standard microbiological operating procedure for isolation and identification of microorganisms. Antibiotic susceptibility test of all the clinical isolates to antibiotics from various categories (supplier: Hi media Laboratory Limited, Mumbai, India) was performed by Kirby-Bauer disk diffusion method and interpretation of the results was made in compliance with CLSI guidelines. We considered the isolates resistant to at least three classes of first-line antimicrobial agents as the MDR strains. **Result:** Out of 500 samples ,15.2% of the samples showed growth. Out of these 76 growth positive samples, a little more than half, 56.5% were Gram-negative isolates with *E. coli* being the most prevalent. Out of the total 43 Gram negative isolates, 26 (60.5%) samples were from the outdoor patients (OPD) and the remaining samples were from the indoor (hospital admitted) patients. MBL was detected in 3 (6.9%) of the culture positive Gram-negative bacteria. Out of the 43 Gram negative bacterial isolates tested, majority of the *Enterobacteriaceae* members were found to be resistant to ceftazidime. Among the ceftazidime resistant *Enterobacteriaceae* members, the most effective antibiotics was nitrofurantoin followed by amikacin. All the ceftazidime resistant *Enterobacteriaceae* members were also resistant to ampicillin. **Conclusion:** antimicrobial resistance is a growing threat worldwide with increasing resistance to third generation cephalosporins becoming a cause of concern among *Enterobacteriaceae*; early detection and infection control practices are the best defense against these organisms.

Keywords: Metallo Beta Lactamase, Gram-Negative Bacilli.

Introduction

Though primarily regarded as contaminants or incidental organisms, gram negative non fermenting bacilli (NFGNB) are becoming increasingly important as opportunistic pathogens in immunocompromised patients[1]. They can also cause infection by gaining access to normally sterile body sites through trauma or a foreign body. Non-fermenters are emerging with increasing frequency as agents of opportunistic and often serious infections as well as nosocomial infections[2]. They are most commonly isolated from patients with serious underlying disease who had abuse of wide spectrum antimicrobials agents, surgical procedures, prolonged hospital stay, prolonged mechanical instrumentation or tracheostomy, genitourinary instrumentation, in burns patients and low birth weight babies[3]. They are also frequently isolated from cases such as septicaemia, meningitis, pneumonia, urinary tract infection and surgical wound infection.3 These infections have the potential to spread from patient to patient via fomites or hands of the medical personnel[4]. Non-fermenting gram negative bacilli are innately resistant to many antibiotics and are known to produce Extended Spectrum Beta lactamases and Metallo Beta lactamases[5].

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Acquired Metallo-β-lactamases (MBL) have recently emerged as one of the most worrisome resistance mechanisms owing to their capacity to hydrolyze all β-lactams, including carbapenems. Metallo-β-lactamase-producing clinical isolates within institutions and their ability to participate in horizontal MBL gene transfer with other pathogens in the hospital is posing great risk. Five different types of MBLs whose prevalence are increasing rapidly are IMP, VIM, SPM, GIM and SIM. Among these, IMP and VIM are the most predominant. With the global increase in the occurrence and types of MBLs, early detection is crucial, the benefits of which include timely implementation of strictinfection control practices and treatment with alternative antimicrobials[5]. Molecular techniques are available to detect MBL producers. But such techniques are not available at tertiary centers. Among the simple and cheaper methods available for testing MBL production, the imipenem (IMP)- EDTA combined disc test is sensitive and specific one. Antimicrobial treatment of the infections caused by these agents is difficult due to its multidrug resistance (MDR) and rapid selection of high level MDR to various groups of antibiotics like Beta-lactams, Aminoglycosides and Fluoroquinolones, posing problem for both treatment and infection control[6-10].Since, the knowledge about the types of enzymes that may be present can serve to guide the infectious disease physician toward choosing appropriate therapy without the need for extensive secondary testing, detection of MBL producing isolates is of paramount importance in clinical setting. Given the background, this epidemiological study was designed to generate updated information

on the burden of metallo-beta-lactamase producing Gram negative bacteria from a tertiary care hospital of Bihar so that an effective antimicrobial stewardship policy can be formulated and implemented to circumvent the rising threat of antimicrobial resistance.

Methodology

The prospective study was conducted at Jawahar Lal Nehru Medical College, Bhagalpur, Bihar from August 2020 January 2021 to assess the prevalence of metallo-beta-lactamase production among the ceftazidime-resistant Gram-negative rods (GNRs) isolated from different clinical samples. A total of 500 different clinical samples (sputum, pus, tracheal secretion, bronchial secretion, urine, and body fluids like CSF and peritoneal fluid) from patients of all age groups received in the microbiology laboratory for routine examination and culture, during the study period of six months were included in the study.All the sample specimens were processed by standard microbiological operating procedure for isolation and identification of microorganisms following the Manual of clinical microbiology[11]. Briefly, the samples were inoculated in routine culture media (blood agar, MacConkey agar, chocolate agar), subjected for microscopic examination as Gram's-stained preparation and inoculums from culture plates tested in biochemical media for identification of the bacteria based upon their morphology, cultural characteristics and biochemical properties, in compliance with Manual of Clinical Microbiology[11].Antibiotic susceptibility test of all the clinical isolates to antibiotics from various categories (supplier: Hi media Laboratory Limited, Mumbai, India) was performed by Kirby-Bauer disk diffusion method and interpretation of the results was made in compliance with CLSI guidelines[12]. Control strains of *P. aeruginosa* ATCC 27853, and *E. coli* ATCC 25922 were used in parallel as a part of quality control as well as for validation of the test performed. We considered the isolates resistant to at least three classes of first-line antimicrobial agents as the MDR strains[13].In this study phenotypic detection method as described below was followed for the detection of MBL isolates.The isolates were subjected for MBL detection when the zone of inhibition (ZOI) for ceftazidime (CAZ) (30 µg) was <18 mm. The sensitivity or resistance pattern to imipenem (IPM) (10 µg) and/or meropenem (MEM) (10 µg) were not considered for MBL detection as bacteria might harbor "hidden MBL". Thus, to ascertain not a single isolate carrying hidden MBL is missed, we used ceftazidime resistance as the screening tool. A suspension of bacteria equivalent to 1:10 dilution of 0.5 McFarland were used to prepare a lawn culture in Muller Hinton agar and subsequent application of the antibiotic discs was carried out[14, 15].Two IPM disks (10 µg), one containing 10 µl of 0.5 M (750 µg) anhydrous ethylenediamine-tetraacetic acid (EDTA) and the other without EDTA were placed 25 mm apart (center to center). An increase in zone diameter of ≥7 mm around the IPM-EDTA disk compared to that of the IPM disk alone was considered positive for MBL[16].

Results

A total of 500 different clinical samples (urine, pus, body fluids and sputum) were included from patients admitted or attending outdoor patient (OPD) during the study period. Out of them,15.2% of the samples showed growth. Out of these 76 growth positive samples, a little more than half, 56.5% wereGram-negative isolates with *E. coli* being the most prevalent. Out of the total 43 Gram negative isolates, 26 (60.5%) samples were from the outdoor patients (OPD) and the remaining samples were from the indoor (hospital admitted) patients. MBL was detected in 3 (6.9%) of the culture positive Gram-negative bacteria.Distribution of isolates in different clinical samples has been shown in figure 1. Out of the 43 Gram negative bacterial isolates tested, majority of the *Enterobacteriaceae* members were found to be resistant to ceftazidime. Among the ceftazidime resistant *Enterobacteriaceae* members, the most effective antibiotics was nitrofurantoin followed by amikacin. All the ceftazidime resistant *Enterobacteriaceae* members were also resistant to ampicillin. Among the 43 Gram negative bacteria isolated, 3 (6.9%) were metallo-beta-lactamase producers. Among the 3 MBL positive bacteria, 2 were A. calcoaectius baumanii complex and the third was P. aeruginosa. None of the other Gram-negative isolates were found to be metallo-beta-lactamase producers during the study (Table 1). Both the MBL positive A. calcoaectiusbaumanii complex were isolated from pus and the MBL positive P. aeruginosa was isolated from urine.

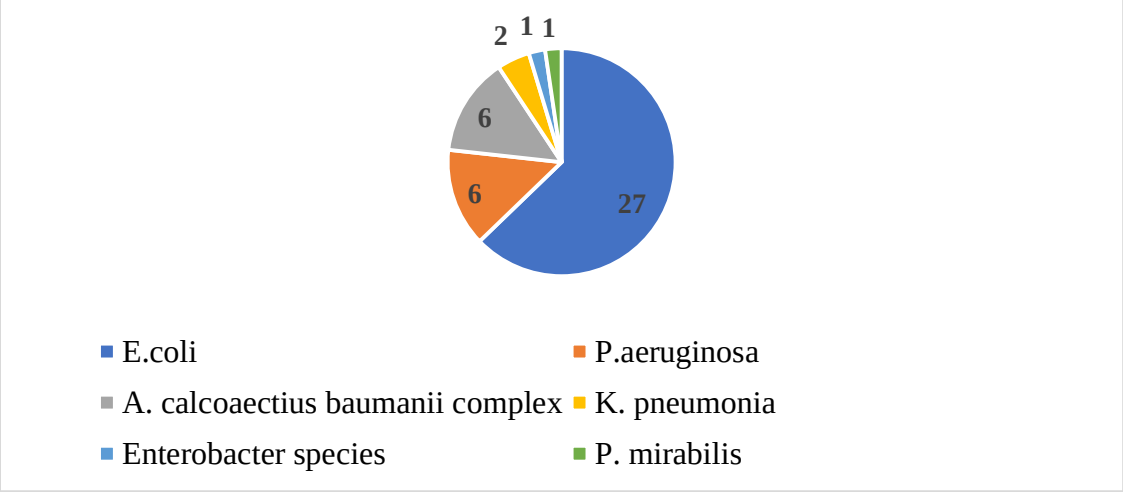

Fig 1: Pie distribution of various gram-negative isolates

Table 1: Table showing distribution of various organisms and their characteristics

Bacteria	MDR positive	MBL positive
E. coli	16	-
P. aeruginosa	2	1
A. calcoaectiusbaumanii complex	4	2
K. pneumonia	1	-
Enterobacter species	-	-
P. mirabilis	-	-

Discussion

Metallo-beta-lactamases are a large and diverse group of beta-lactamases that are now disseminating on mobile genetic elements among clinically important Gram-negative pathogens, limiting treatment options for life-threatening infections[17, 18]. Infection with the metallo-beta-lactamase (MBLs) producing organisms are associated with higher rates of mortality, morbidity, and health care costs[19]. In any nosocomial setting, carbapenems are used as the last resort for treatment of MDR Gram-negative bacterial infection. However, since last 15 years, acquired resistance to this life saving antimicrobial has been increasingly reported not only in *P. aeruginosa* and *Acinetobacter* spp. but also among members of *Enterobacteriaceae* which is mainly mediated by *Klebsiella pneumoniae* carbapenemases (KPC)[20].

A PCR based method is usually considered to be the best method for detecting MBL-producing isolates. However, the increasing number of types of MBLs is creating difficulties in detection of MBLs, since primers used for PCR are usually designed to detect a single gene type[14]. Furthermore, for a developing country like India, use of PCR based technique in surveillance process would be expensive and undesirable from financial aspect to test every single suspected isolate. To circumvent this problem, we have used phenotypic detection technique using imipenem-EDTA combined disk method, which in one hand has sensitivity and specificity of 100 and 98%, respectively and on the other hand is cost effective as well[16].

The highest rate of MDR in our study was seen among *E. coli* strains. Among the total *P. aeruginosa* isolates 44.4% were found to be MDR which is similar to 49.8% MDR reported by Strateva et al.[21]. However, some other studies have reported slightly higher MDR cases i.e. 51.3% and 65.3%, respectively[15, 22]. Likewise, 95% of *Acinetobacter* spp. isolated were reported to be MDR[15]. The current findings is an alarming sign, since almost half of the MBL producers have been found to be MDR strains leaving the medical practisoners with limited therapeutic options to combat such pathogens.The armamentarium against MDR Gram-negative microorganisms has almost been exhausted especially after the advent of carbapenem resistance among them. Until last year, parenteral colistin available as colistin methanesulfonate (CMS) showing potent activity in vitro against MDR nosocomial P.aeruginosa, Acinetobacter spp., Stenotrophomonas maltophilia, Enterobacter spp. and Klebsiella spp., including ESBL and carbapenemase-producers[23, 24] was the ultimate treatment option. However, recent reporting of plasmid-mediated colistin resistance in Escherichia coli isolated from animals, food, and patients in China by Liu et al. in November, 2016[25], has left us with no option. Hence, proper implementation of infection control strategy, active antimicrobial stewardship approach, improved laboratory detection, judicious use of antimicrobial agents, along with regular national level surveillance can be some of the arbitration measures to control as well as aiding formulation of strategy in tackling drug resistance issues like the current one under discussion.

Conclusion

The findings of our study demonstrated a higher prevalence of MDR and MBL positive *P. aeruginosa* and *Acinetobacter* spp., which have been globally incriminated with adverse clinical outcome including a higher morbidity and mortality rate. The study results demonstrate the serious therapeutic and epidemiological threat of the spread of metallo-beta-lactamase producers. Since, antimicrobial resistance is a growing threat worldwide with increasing resistance to third generation cephalosporins becoming a cause of concern among *Enterobacteriaceae*; early detection and infection control practices are the best defense against these organisms.

References

1. Solanki S, Sharma A, Saileela K. Evaluate the Distribution of Gram-Negative Non-Fermenting Bacteria and their Resistant Pattern in Clinical Isolates among the Rural Population in South India. Int.J.Curr. Microbiol.App.Sci 2017;6: 461-468.
2. Koneman EW, Alen SD, Janda WM, Schreckenberger PC, Winn WC. The Non fermenting gram-negative Bacilli. In wood Gail (ed). Color Atlas and text book of diagnostic microbiology. 6th edition. USA, Lippincott Williams and Wilkins Company, 2006;305-391.
3. Steinberg JP, Rio DC. Gram Negative and Gram variable bacilli. In, Mandell GL, Bennett JE, Dolin R (ed). Principles and Practice of Infectious diseases. 6th edition. Philadelphia, USA, Elsevier Publication, 2010; 2751-2768.
4. Murray PR, Baron EJ, Jorgensen JH, Landry ML. In, Manual of Clinical Microbiology, Vol I, 8th edition, American Society of Microbiology, 2003; 734- 834.
5. Rubin SJ, Granato PA, Wasilauskas BL. In, Glucose Nonfermenting Gram Negative Bacteria. Lennette EH, Balows A, Hausler WT Jr, Shadomy HT (ed). Manual of clinical Microbiology, 4th edition. WASHINGTON, DC, American Society for Microbiology,1980; 330-349.
6. Yashodhara P, Shyamala S. Identification and characterisation of non-fermenters from clinical specimens. Indian J Med Microbiol 1977;15:195-197.
7. Wisplinghoff H, Perbix W, Seifert H. Risk factors for nosocomial blood stream infections due to Acinetobacter baumanii: A Case control study of adult burn patients. Clin Infect Dis 1999;28:59-66.
8. Levin MH, Olson B, Nathan C, Kabins SA, Weinstein RA. Pseudomonas in the sinks in an intensive care unit: relation to patients. J Clin Pathol 1984; 37:424-427.
9. Clinical Laboratory Standards Institute. CLSI DocumentM100-S20. Wayne, PA: CLSI; 2010. Performance Standards for Antimicrobial Susceptibility Testing. Twentieth Informational Supplement.
10. Gupta R, Malik A,RizviM,Ahmed M. Presence of Metallo-beta-lactamases (MBL), extended-spectrum beta-lactamase (ESBL) &AmpC positive nonfermenting Gram-negative bacilli among Intensive Care Unit patients with special reference to molecular detection of blaCTX-M &blaAmpC genes. Indian J Med Res. 2016; 144: 271–275.
11. Versalovic J, et al. Manual of clinical microbiology. 10th ed. Washington, DC: American Society of Microbiology; 2011.
12. Clinical and Laboratory Standards Institute (CLSI), Performance standards for antimicrobial susceptibility testing, in twenty-third informational supplement. Wayne: Clinical and Laboratory Standards Institute 2013.
13. Adams-Haduch JM, et al. Genetic basis of multidrug resistance in Acinetobacter baumannii clinical isolates at a tertiary medical center in Pennsylvania. Antimicrob Agents Chemother. 2008;52(11):3837–43.
14. Yong D, et al. Imipenem-EDTA disk method for differentiation of metallo-beta-lactamase-producing clinical isolates of Pseudomonas spp. and Acinetobacter spp. J Clin Microbiol. 2002;40(10):3798–801.
15. Mishra SK, et al. Metallo-beta-lactamase producing gram-negative bacterial isolates. J Nepal Health Res Counc. 2012;10(22):208–13.
16. Franklin C, Liolios L, Peleg AY. Phenotypic detection of carbapenem-susceptible metallo-beta-lactamase-producing gram-negative bacilli in the clinical laboratory. J Clin Microbiol. 2006;44(9):3139–44.
17. Cornaglia G, et al. Metallo-beta-lactamases as emerging resistance determinants in Gram-negative pathogens: open issues. Int J Antimicrob Agents. 2007;29(4):380–8.
18. Cornaglia G, Giamarellou H, Rossolini GM. Metallo-beta-lactamases: a last frontier for beta-lactams? Lancet Infect Dis. 2011;11(5):381–93.
19. Kaleem F, et al. Frequency and susceptibility pattern of metallo-beta-lactamase producers in a hospital in Pakistan. J Infect Dev. Ctries. 2010;4(12):810–3.
20. Nordmann P, Cuzon G, Naas T. The real threat of Klebsiella pneumoniae carbapenemase-producing bacteria. Lancet Infect Dis. 2009;9(4):228–36.

21. Strateva T, et al. Problematic clinical isolates of Pseudomonas aeruginosa from the university hospitals in Sofia, Bulgaria: current status of antimicrobial resistance and prevailing resistance mechanisms. J Med Microbiol. 2007;56(7):956–63.
22. Khanal S, et al. Beta-lactamase-producing multidrug-resistant bacterial pathogens from tracheal aspirates of intensive care unit patients at National Institute of Neurological and Allied Sciences, Nepal. ISRN Microbiol. 2013;5.
23. Li J, et al. Colistin: the re-emerging antibiotic for multidrug-resistant gram-negative bacterial infections. Lancet Infect Dis. 2019;6(9):589–601.
24. Giamarellou H. Treatment options for multidrug-resistant bacteria. Expert Rev Anti Infect Ther. 2020;4(4):601–18.
25. Liu YY, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis. 2021;16(2):161–8.

Conflict of Interest: Nil
Source of support: Nil