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Emerging resistance to carbapenems in a tertiary care hospital in north India

Ekta Gupta, Srujana Mohanty, Seema Sood, Benu Dhawan, Bimal K. Das & Arti Kapil

Department of Microbiology, All India Institute of Medical Sciences, New Delhi, India

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***Background & objectives*: Carbapenems are beta-lactam antibiotics, presently considered as most potent agents for treating multi-drug resistant Gram-negative bacilli infections. In India carbapenems available for use are meropenem and imipenem, introduced recently. Resistance to these has been reported in a few bacteria especially *Pseudomonas* spp. We therefore retrospectively evaluated the antibiotic susceptibility pattern to these agents amongst various clinical isolates in a tertiary care hospital in north India.**

***Methods*: In this study Gram-negative bacterial pathogens isolated from clinical samples were tested for extended spectrum beta lactamase (ESBL) production. All ESBL positive bacteria were tested for meropenem and imipenem activity pattern using NCCLS guidelines. A total of 2626 consecutively isolated Gram-negative bacteria, which tested positive for ESBL production by the double disk diffusion method, were included.**

***Results*: The different bacteria isolated were *Pseudomonas* spp. 759, *Acinetobacter* spp. 676, *Escherichia coli* 569, *Klebsiella* spp. 343, *Enterobacter* spp. 150, *Citrobacter* spp. 57 and *Proteus* spp. 72. Overall resistance to meropenem was more (22.16%) than imipenem (17.32%). Maximum resistance was seen in *Pseudomonas* spp. M_R 37.6 per cent, I_R 30 per cent. In isolates from intensive care units (ICU) resistance to carbapenems was significantly higher than non-ICU patients.**

***Interpretation & conclusion*: Resistance to meropenem and imipenem was seen in various clinical isolates of Gram-negative ESBL-positive bacteria. There is a need to alarm our clinicians for judicious use of antibiotics.**

Key words Carbapenems - Gram-negative bacilli - resistance

Members of the family Enterobacteriaceae are among the most important bacterial human pathogens accounting for the majority of bacteria isolated from clinical samples[1]. That these Gram-negative bacilli (GNB's) are rapidly acquiring resistance to one or more antimicrobial agents traditionally used for treatment is a matter of concern.

Till now, extended spectrum β lactamase (ESBL) production by GNB was considered as the most important threat to clinical therapeutics[2,3]. Increasing prevalence (66.8 to 71.5%) of infections due to ESβL positive bacteria has been observed in various studies from our institute[3-5]. This has led to a parallel increase in the usage of β-lactamase inhibitor/β-lactam combinations, monobactams and carbapenems. However, last few years have witnessed resistance to these drugs as well[6,7].

Carbapenems are β-lactam antibiotics, presently considered as the most potent agents for treatment of multidrug resistant Gram-negative infections due to the stability of these agents against the majority of β-lactamases and their high rate of permeation through bacterial outer membranes. However, there have been reports of resistance to carbapenems[8,9]. This is of great concern as presently to combat infections by multidrug resistant bacteria, carbapenems are considered the last resort especially in intensive care units (ICU's) and high risk wards.

The carbapenems available for use in India are meropenem and imipenem. Information on the prevalence of resistance to carbapenems in clinical isolates from our country is limited[6]. Therefore, we conducted this retrospective analysis to look for the antimicrobial activity of meropenem and imipenem in Gram-negative bacilli isolated from clinical specimens in a tertiary care hospital in north India.

Material & Methods

The study was conducted in the clinical bacteriology laboratory, Department of Microbiology of the All India Institute of Medical Sciences, New Delhi. The samples (blood, urine, tracheal aspirates/ bronchoalveolar lavage, soft tissue samples and sterile body fluids), received from patients admitted to the hospital during August to December 2004 were processed for isolation and identification of bacterial pathogens according to standard microbiological techniques. Antimicrobial sensitivity testing was performed on Mueller Hinton Agar (Hi-media, Mumbai) plates by disk diffusion method according to National Committee for Clinical Laboratory Standards (NCCLS) guidelines[10]. The diameter of the zones of inhibition of growth was recorded and interpreted as sensitive, intermediate resistant or resistant based on NCCLS guidelines[10]. Organisms with intermediate levels of resistance to the antibiotics were included in the percentage of resistant organisms for final analysis. *Escherichia coli* ATCC 25922 (β-lactamase negative), *Pseudomonas aeruginosa* ATCC 27853 (β-lactamase negative) and *Klebsiella pneumoniae* ATCC 700603 (ESBL positive) strains were used as control organisms.

All the Gram-negative bacteria isolated from these clinical samples were tested for ESBL production by using four disks (concentration in μg) ceftazidime (30), ceftazidime/clavulanic acid (30/ 10), cefotaxime (30), and cefotaxime/clavulanic acid (30/10) and interpreted as per NCCLS guidelines[10].

In all ESBL positive bacteria antibiotic susceptibility pattern to meropenem (Hi-media, Mumbai) (10), and imipenem (Becton Dickinson, USA) (10) was recorded for this study. By Kirby-Bauer method[11] as per NCCLS guidelines, isolates were considered as resistant to meropenem and imipenem if the zone of inhibition was ≤13 mm, intermediate 14-15 mm and sensitive ≥16 mm.

Chi-square test was used to analyse the data statistically.

Results & Discussion

Of the 34,275 samples received in the bacteriology laboratory during the study period, there were 10,300 blood samples, 9565 urine samples, 5010 soft tissue samples, 4900 tracheal aspirates/broncho alveolar lavage (BAL) and 4500 sterile body fluids.

Table. *In vitro* activity of meropenem and imipenem against ESBL positive organisms

Organisms	ESBL	Number (%) resistant to	
	positive	Meropenem (M_R)	Imipenem (I_R)
Pseudomonas spp.	759	286 (37.6)	228 (30)*
Acinetobacter spp.	676	235 (34.7)	184 (27.2)*
Escherichia coli	569	20 (3.5)	12 (2.1)
Klebsiella spp.	343	24 (6.9)	15 (4.3)
Enterobacter spp.	150	9 (6)	9 (6)
Proteus spp.	72	6 (8.3)	5 (6.9)
Citrobacter spp.	57	2 (3.5)	2 (3.5)
Total	2626	582 (22.16)	455 (17.32)**

*P<0.05; **<0.001 for difference between meropenem and imipenem (Chi square test)

A total of 2,626 consecutive isolated ESBL positive GNB's were included in this study; of these, 469 were from ICU patients. The most prevalent were *Pseudomonas* spp. (759, 29%) and *Acinetobacter* spp. (676, 26%). (Table). Overall, resistance to meropenem was 22.16 per cent as compared to imipenem 17.32 per cent (P<0.001). Maximum resistance was seen in *Pseudomonas* spp. (M_R =37.6% I_R =30%) and minimum in *Escherichia coli* (M_R=3.5% I_R = 2.1%).

In ICU patients, *Pseudomonas* spp. and *Acinetobacter* spp. were the most common isolates. Overall resistance to meropenem and imipenem was significantly higher than non-ICU patient's (M_R=37.3%, I_R=31.9%) (P<0.001). Maximum resistance was seen in *Pseudomonas* spp. (M_R =54.5%, I_R =50%).

The resistance to carbapenems especially in *Pseudomonas* spp. results from reduced levels of drug accumulation or increased expression of pump efflux[12]. The resistance may also be due to the production of metallo-β-lactamases (MBL) which can be chromosomally encoded or plasmid mediated[6,12]. Most of these MBL confer resistance to not only carbapenems but also to other β-lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam[13]. There have been increasing reports especially from Japan, of Gram-negative bacteria carrying transferable carbapenems resistance gene bla_{imp}, including isolates of *P. aeruginosa*[8,9]. Therefore, serious medical problems may occur in a hospital due to therapeutic failure, once such strains spread especially in ICUs and high risk wards.

In our institute, meropenem came in to use in 2002 about two years before the use of imipenem. Both these agents are frequently used to treat infections by multiresistant bacteria in ICUs and high risk wards. Within a short span high resistance to both meropenem (22.16%) and imipenem (17.32%) was seen in various clinical isolates. Resistance to these life saving drugs in ICU was very high especially in *Pseudomonas* spp. Overall, imipenem showed better activity than meropenem. These results correlate with the findings in other studies[13].

Limited literature is available regarding the prevalence of resistance to carbapenems in various clinical isolates from our country. Earlier resistance of 12 per cent each was noted to imipenem and meropenem respectively in *P. aeruginosa* in hospitalized patients[6]. In various studies, across the world varying resistance (4-60%) has been seen towards these drugs[14,15].

In conclusion, our study highlights the increasing resistance in Gram-negative bacteria towards meropenem and imipenem in our country. As our study was limited to multi-resistant bacteria (ESBL positive), isolated from admitted patients in a tertiary care hospital, true extent of resistance to these agents among bacterial isolates from community acquired infections may be considerably low. But there is a need to emphasize on the rational use of antimicrobials and strictly adhere to the concept of "reserve drugs" to minimize the misuse of available antimicrobials. In addition, regular antimicrobial susceptibility surveillance is essential.

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Reprint requests: Dr Arti Kapil, Additional Professor, Department of Microbiology
All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029, India
e-mail: akapil_micro@yahoo.com