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Ertapenem susceptibility of extended spectrum β-lactamase-producing Enterobacteriaceae at a tertiary care centre in India

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ABSTRACT

Introduction: Infections caused by multidrug-resistant organisms such as extended spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae have come to assume widespread proportions. Carbapenems (imipenem and meropenem) are the drugs of choice for the treatment of infections caused by ESBL-producing organisms. There is limited clinical data regarding the efficacy of the latest carbapenem, called ertapenem, against these organisms in the Indian subcontinent. In this study, ertapenem susceptibility in ESBL-producing clinical isolates was evaluated. The *in vitro* activities of the three carbapenems were compared in ertapenem-resistant isolates.

Methods: A total of 205 ESBL-producing Enterobacteriaceae collected from inpatients and outpatients at the All India Institute of Medical Sciences, were identified and tested for antimicrobial susceptibility by the disc diffusion method and Vitek 2 advanced expert system. Ertapenem susceptibility was performed by disc diffusion and Vitek 2 in all the isolates and by E-test in 100 isolates.

Results: 191 (93 percent) of the ESBL-producing isolates tested were susceptible to ertapenem. All ertapenem-susceptible isolates were also susceptible to imipenem and meropenem. Isolates with low-level ertapenem resistance retained their susceptibility to imipenem and meropenem, whereas those with high-level ertapenem resistance were resistant to both imipenem and meropenem.

Conclusion: Our results suggest that ertapenem may be a viable alternative to other carbapenems for the treatment of infections caused by ESBL-producing clinical isolates. Clinical outcome studies are required to determine if ertapenem is effective for the treatment of infections caused by these organisms.

Keywords: Enterobacteriaceae, ertapenem, extended spectrum β-lactamase, multidrug-resistant organisms

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INTRODUCTION

Extended spectrum β-lactamase (ESBL)-producing strains of Enterobacteriaceae have now emerged as a major problem in hospitalised as well as community-based patients. These organisms are responsible for a variety of infections, such as urinary tract infection, septicaemia, hospital-acquired pneumonia, intra-abdominal abscess, brain abscess and device-related infections.[1] Carbapenems (imipenem [IPM] and meropenem [MEM]) are the drugs of choice for the treatment of infections caused by ESBL-producing organisms. Nevertheless, there is concern that the extensive use of these two drugs will place selective pressure on IPM and MEM, which are the last good defences against multiresistant *Pseudomonas* and *Acinetobacter* spp. Moreover, the multiple daily dosage required for these antibiotics makes them an onerous treatment regimen.

Ertapenem (ETP) (formerly MK-0826; Merck & Co, Inc) is a parenteral carbapenem that was licenced for once daily use in November 2001 in the USA and in April 2002 in Europe.[2] ETP is active against both Gram-positive and Gram-negative bacteria, including Enterobacteriaceae, *Streptococcus pneumoniae* and most species of anaerobic bacteria. Isolates from a variety of infections (intra-abdominal infections, skin/soft tissue infections, community-acquired pneumonia, pelvic infections and urinary tract infections) are inhibited by ETP. It is not active against *Enterococci*, methicillin-resistant *Staphylococcus aureus*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa*.[2] At our institute, ETP came

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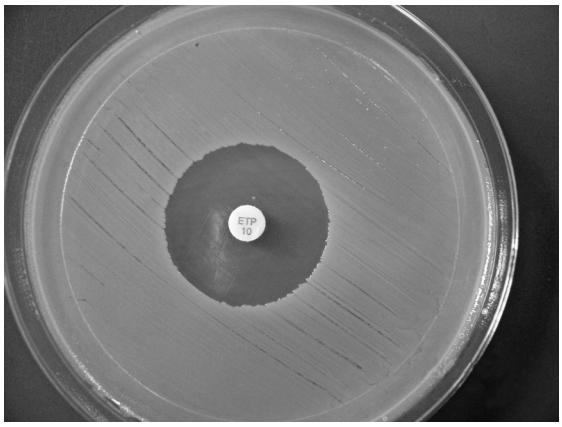

Fig. 1 Photograph shows the result of an ertapenem disc diffusion test, where the isolate is sensitive to ertapenem (zone diameter > 19 mm).

Fig. 2 Photograph shows a sample ertapenem E-test where the MIC for ertapenem < 2 µg/ml (sensitive).

into use recently and constitutes less than one percent of carbapenem consumption. In this context, we designed the present study to evaluate the ETP susceptibility of ESBL-producing Enterobacteriaceae by the minimum inhibitory concentration (MIC)-based method and disc diffusion. A secondary aim was to compare the *in vitro* activity of IPM, MEM and ETP in ETP-resistant isolates.

METHODS

The study was conducted at the Department of Microbiology, All India Institute of Medical Sciences (AIIMS) and the Department of Laboratory Medicine, Jai Prakash Narayam Apex Trauma Centre, AIIMS. AIIMS is a 2,500-bed, tertiary care, referral and teaching hospital, where patients are referred from all over India. A total of 205 ESBL-producing members of the Enterobacteriaceae family, obtained from various clinical samples of admitted and outpatients at the AIIMS Hospital and its Trauma Centre over a period of three months (October–December 2007), were included in the study. All isolates were consecutive, but non-duplicate. Only one isolate per patient was included. The antibiotic susceptibility of the isolates was compared to exclude the clonal origins of the isolates. The isolates were identified according to standard microbiological techniques as well as by the VITEK 2 system (bioMérieux Vitek Systems Inc, Hazelwood, MO, USA) using identity cards for Gram-negative bacilli.

The antimicrobial susceptibility testing of all the 205 isolates was performed by the disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines[3] as well as by the VITEK 2 advanced expert system (AES) using AST-GN13 cards.[4] ESBL screening was done by the disc potentiation test using ceftazidime (CAZ) and ceftazidime and clavulanic acid (CAZ+CLAV) disc (Becton Dickinson, Sparks, MD, USA) according to the CLSI guidelines.[3] The ESBL E-test (CAZ/CAZ+CLAV) (AB Biodisk, Solna, Sweden) was also performed according to the manufacturer's instructions to confirm the presence of clavulanic acid inhibitable ESBLs in selected isolates.[5] *Escherichia (E.) coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as negative and positive controls, respectively, for ESBL testing.

For all isolates, the antibiotic susceptibility testing results for IPM (10 µg), MEM (10 µg) and ETP (10 µg) (Becton Dickinson, Sparks, MD, USA) were performed by the disc diffusion method, as per CLSI guidelines.[3] Isolates were considered as being resistant to MEM and IPM if the zone of inhibition was ≤ 13 mm, intermediate when the zone was 14–15 mm, and sensitive when the zone was ≥ 16 mm, as per CLSI guidelines.[3] For ETP, isolates with a zone diameter ≤ 15 mm were considered to be resistant, those having a zone diameter of 16–18 mm were intermediate, and those with a zone diameter of ≥ 19 mm were sensitive (Fig. 1). The MIC of ETP and IPM was also recorded for all the isolates by the Vitek 2 AES. In addition, the MIC of ETP was determined using the E-test (AB Biodisk, Solna, Sweden) in one hundred isolates as per the manufacturer's instructions.[6] Each ETP E-test strip consists of a predefined gradient of antibiotic, allowing for MIC measurements in the range of 0.002–32 ug/ml.[6] The MIC breakpoints for ETP were taken as per CLSI guidelines.[3] Thus, isolates were considered to be sensitive if the MIC was ≤ 2 µg/ml, intermediate if the MIC was 4 µg/ml and resistant if the MIC was ≥ 8 µg/ml (Fig. 2).

In ETP-resistant isolates, the MICs of IPM and MEM were also determined by the E-test, to assess and compare the *in vitro* activity of the three carbapenems in these isolates. The production of metallo-β-lactamase (MBL) was determined in all carbapenem-resistant isolates by the IPM-ethylenediaminetetraacetic acid (EDTA) combined disc test method.[7]

Table I. Clinical sources of extended-spectrum beta-lactamase L-producing isolates included in the study.

Isolates	No. (%) of isolates	Urine	Blood	Clinical samples*	Pus	Sterile body fluids†
Klebsiella pneumoniae subsp. pneumoniae	109 (53)	15 (14)	24 (22)	46 (42)	20 (18)	4 (4)
Escherichia coli	63 (31)	32 (51)	2 (3)	9 (14)	13 (21)	7 (11)
Proteus mirabilis	10 (5)	6 (60)	–	–	4 (40)	–
Citrobacter koseri	6 (3)	5 (83)	–	–	1 (17)	–
Enterobacter cloacae	11 (5)	–	5 (45)	1 (9)	3 (27)	2 (18)
Pantoea agglomerans	2 (1)	–	1 (50)	1 (50)	–	–
Providencia spp.	2 (1)	–	–	–	2 (100)	–
Morganella morganii subsp. morganii	2 (1)	–	–	–	2 (100)	–
Total	205	58 (28)	32 (16)	57 (28)	45 (22)	13 (6)

* include bronchoalveolar lavage, tracheal aspirate and sputum.
† include cerebrospinal, pleural, peritoneal and synovial fluids.

RESULTS

A total of 205 consecutive, non-duplicate, ESBL-producing members of the Enterobacteriaceae family isolated from clinical samples were included in the study. Of these, 100 isolates were obtained from the AIIMS Hospital and 105 from its Trauma Centre. Of the 205 isolates, 92 (45%) were obtained from patients admitted to intensive care units (ICUs), 72 (35%) from various wards and 41 (20%) from various outpatient departments. The organisms were isolated from various clinical samples, such as blood, respiratory tract (bronchoalveolar lavage, tracheal aspirate, sputum), pus and sterile body fluids (cerebrospinal/pleural/peritoneal/synovial fluid). Of the 205 isolates, 109 (53%) were *Klebsiella pneumoniae subsp. pneumoniae*, 63 (31%) were *E. coli*, 11 (5%) were *Enterobacter cloacae*, 10 (5%) were *Proteus mirabilis*, 6 (3%) were *Citrobacter koseri*, and two each (1%) were *Pantoea agglomerans*, *Providencia* spp. and *Morganella morganii subsp. morganii*. The details of the isolates in the study are shown in Table I. Of the 205 isolates in our study, 196 (96%) were sensitive to IPM, 194 (95%) were sensitive to MEM and 191 (93%) were sensitive to ETP by the disc diffusion and Vitek 2 methods. All the carbapenem-resistant isolates were from the ICUs.

The overall mean ETP MICs for ETP-susceptible isolates in our study was 0.32 ± 0.5 μg/ml (the mean MIC was 0.37 ± 0.13 μg/ml for *Klebsiella* spp and 0.30 ± 0.09 μg/ml for *E. coli*). ETP resistance was noted in 13 isolates (6 *Klebsiella* spp., 5 *Enterobacter* spp. and 2 *E. coli*). There was no discordance between the disc diffusion and Vitek susceptibilities. The E-Test (ETP) MICs also corresponded to the disc diffusion and Vitek susceptibility results. High-level ETP resistance (MIC > 16 μg/ml) was noted in three isolates (2 *Klebsiella pneumoniae* and 1 *Enterobacter cloacae*). The IPM and MEM MICs in ETP-resistant isolates are shown in Table II. The IPM and MEM MICs were raised for ETP-resistant isolates, and this was more pronounced for isolates with high-level ETP resistance (MIC > 16 μg/ml). Isolates with low-level ETP resistance (MIC 4–8 μg/ml) retained their susceptibility to both IPM and MEM at the breakpoint. None of the isolates with high-level ETP resistance were susceptible to either IPM or MEM.

DISCUSSION

A very high prevalence of ESBL-producing Enterobacteriaceae has been reported in our institute.[1] Carbapenems are regarded as reserve agents for treating multidrug-resistant Gram-negative bacterial infections. In our institute, MEM came into use in 2002, about two years before the use of IPM. Both these agents are frequently used to treat infections caused by multiresistant bacteria in ICUs and high-risk wards. Within a short time, a high prevalence of resistance to both MEM and IPM was seen in various clinical isolates at our hospital. Currently, the highest prevalence of carbapenem resistance was seen in *Pseudomonas* spp. (69%) (unpublished data), whereas resistance in ESBL-producing members of Enterobacteriaceae remained low (3%–6%).[8] In the present study, 93% of ESBL-producing clinical isolates were sensitive to ETP, with the MIC from 0.30 ± 0.09 μg/ml for *E. coli* and from 0.37 ± 0.13 μg/ml for *Klebsiella* spp. Similar findings have been reported in a few previous studies.[9-11] All three carbapenems were highly active against members of the Enterobacteriaceae family. The overall susceptibility to IPM, MEM and ETP was 96%, 95% and 93%, respectively.

We found 7% of ESBL-producing clinical isolates to be resistant to ETP. In our institute, ETP constitutes less than 1% of the treatment with carbapenem. Considering its very limited use in our institute, the level of resistance

Table II. Imipenem and meropenem minimum inhibitory concentrations of ertapenem intermediate and resistant isolates.

Isolates	Ertapenem MIC (μg/ml)	Imipenem MIC (μg/ml)	Meropenem MIC (μg/ml)
Klebsiella pneumoniae	> 32	24	> 32
Klebsiella pneumoniae	> 32	24	> 32
Enterobacter cloaceae	> 32	> 32	> 32
Klebsiella pneumoniae	8	0.094	0.19
Klebsiella pneumoniae	8	0.047	0.125
Enterobacter cloaceae	8	0.023	0.125
Escherichia coli	8	0.023	0.19
Klebsiella pneumoniae	4	0.094	0.19
Klebsiella pneumoniae	4	0.032	0.19
Enterobacter cloaceae	4	0.023	0.125
Enterobacter cloaceae	4	0.125	0.125
Enterobacter cloaceae	4	0.032	1
Escherichia coli	4	0.064	0.19

MIC: minimum inhibitory concentrations; indicate E-Test MICs

recorded in this study probably represents a baseline level resistance to ETP. ETP resistance in Enterobacteriaceae remains uncommon. In these genera, resistance is rarely mediated by true carbapenemases. Combined mechanisms of outer membrane permeability defect and ESBLs (often a CTX-M type) or an AmpC enzyme are attributed to ETP resistance.[12,13] In our study, three of the 12 (25%) ETP-resistant isolates produced MBL; these isolates demonstrated high-level resistance to ETP and were also resistant to both IPM and MEM. A history of treatment with IPM was present in four of the nine ETP-resistant isolates which had ETP MIC in the intermediate (4–8 μg/ml) range, while retaining susceptibility to IPM. In a recent report by Lartigue et al,[14] ETP-resistant *E. coli* (MIC > 256 μg/ml) was recovered from the peritoneal fluid of a patient who had been treated with IPM-cilastin for ten days. The isolate was intermediately susceptible to IPM and MEM (MIC 8 μg/L). Hence, an IPM-cilastin containing regimen is likely able to select for ETP resistance and is a cause for concern.

Cross-resistance between ETP and IPM/MEM was not uniform, as noted previously.[15] As expected, isolates producing Group 3 MBLs will be concomitantly resistant to all three carbapenems. ETP is theoretically the carbapenem least likely to permeate Gram-negative bacteria rapidly. We believe that IPM susceptibility can be used as a surrogate marker for ETP susceptibility in IPM-resistant isolates.[15]

To our knowledge, this is the first report of ETP susceptibility of ESBL-producing organisms in India. In conclusion, ETP may be a viable alternative to other carbapenems for the treatment of infections caused by ESBL-producing Enterobacteriaceae. Clinical outcome studies are required to determine if ETP is effective for the treatment of infections caused by these organisms. However, given the possibility of ETP resistance in *Klebsiella* spp., *Enterobacter* spp. and *E. coli*, its *in vitro* activity should be proven by laboratory testing before clinical use. With the introduction of ETP, and an expected increase in the carbapenem use due to an increased prevalence of strains with ESBLs, continuous surveillance of carbapenem resistance appears to be warranted.

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