Escherichia coli: Development of Carbapenem Resistance During Therapy

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A 76-year-old woman had recurrent urosepsis due to extended-spectrum β-lactamase–positive Escherichia coli. Imipenem resistance was detected after long-term imipenem-meropenem therapy. The carbapenem-hydrolyzing enzyme gene was identified as blaKPC-3. To our knowledge, this is the first documented case in which carbapenem-resistant E. coli emerged during therapy with imipenem and meropenem, and the first identification of the carbapenem-hydrolyzing enzyme in E. coli isolates.

Escherichia coli is one of the major etiologic agents for urinary tract infection, sepsis, and meningitis. Therapeutic options have become somewhat limited because of the emergence of organisms carrying extended-spectrum β-lactamases (ESBL) and plasmid-mediated AmpC β-lactamases. The carbapenems (imipenem, meropenem, and ertapenem) are sometimes the only effective agents for treatment of severe infection caused by ESBL–positive E. coli.

Carbapenem-hydrolyzing enzymes are β-lactamases that significantly hydrolyze imipenem, meropenem, and ertapenem and, usually, a wide range of other β-lactam antibiotics. Carbapenem resistance has been rarely reported in E. coli. The occurrence of an outer-membrane porin deficiency and the expression of a plasmid-mediated class C β-lactamase were reported to be responsible for carbapenem resistance in E. coli [1]. There are no previous reports of carbapenem-hydrolyzing enzymes in clinical E. coli isolates. Here, we report what is, to our knowledge, the first strain of E. coli with carbapenem-resistance identified on basis of the production of a carbapenem-hydrolyzing enzyme KPC-3 and the laboratory analysis of the 13 sequential isolates collected from a patient that demonstrated the development of carbapenem resistance during therapy.

A 76-year-old female nursing home resident who had a history of colon cancer, was status post hemicolec-tomy with a colostomy, and had an ileal conduit was hospitalized multiple times at Hackensack University Medical Center (Hackensack, NJ) for recurrent urinary tract infection. On her first hospitalization, the patient had evidence of bilateral hydronephrosis, and percu-taneous stents were placed. At that time, blood and urine cultures grew ESBL–positive, imipenem-susceptible E. coli. Over the next 5 months, the patient was repeatedly hospitalized for urosepsis and received imipenem intermittently (total dose, 38.75 g), after which she was treated with meropenem (5.5 g over 5 days) because she developed progressive renal insufficiency. On her last hospitalization, multiple urine cultures were positive for imipenem-resistant E. coli, even though the nephrostomy tubes were changed. During the 19-day period of this hospitalization, the patient received 12 g of meropenem, after which she received 9.75 g of imipenem and 650 mg of amikacin. No other antibiotics were used. Urine cultures continued to yield imipenem-resistant E. coli. The patient was subsequently returned to the nursing home, where she died.

Thirteen E. coli isolates were available for investigation. Identification of the isolates was initially determined by Vitek GN card (bioMérieux Vitek) and confirmed by 16S rRNA gene nucleotide sequence analysis (Microseq 500, Applied Biosystems).

PFGE analysis separated the isolates into 7 PFGE
patterns (figure 1). Six isolates were susceptible to imipenem (figure 1, lanes 1-6). All others were imipenem-resistant. Although the isolates appeared to be multi-clonal, according to the criteria for bacterial strain typing [2], 10 isolates were more or less closely related (figure 1, lanes 1-10), whereas 3 isolates represented significantly different strains (figure 1, lanes 11-13).

Seven *E. coli* isolates had imipenem MICs ranging from 3 to 16 µg/mL (E-test, AB Biodisk). All isolates were phenotypically ESBL-positive by disk testing (confirmatory test, NCCLS). The isolate with the highest imipenem MIC (16 µg/mL) (isolate 272–1265 A1) was chosen for further study (in addition to being resistant to all β-lactams, the isolate was also resistant to tobramycin, gentamicin, TMP-SMX, ciprofloxacin, levofloxacin, and nitrofuratoin and had intermediate resistance to amikacin). Bioassays with a crude cell sonicate prepared from the isolate demonstrated hydrolysis of imipenem and ertapenem. Isoelectric focusing indicated that this isolate produced 3 clavulanate-susceptible β-lactamases with pI values of 5.4, 6.7, and 7.6 (figure 2, lane 8). Further isoelectric focusing analysis of 4 imipenem-susceptible isolates and the 7 isolates with reduced susceptibility to imipenem is shown in figure 2. The β-lactamase with a pI of 6.7, identical to that of KPC-1 [3], was present in all isolates with an increased imipenem MIC (3–16 µg/mL) and was absent in all isolates susceptible to imipenem.

PCR amplified products were detected using primers directed toward the sequence of the *bla*KPC-2 gene. The complete sequence of the gene *bla*KPC-3 was determined for 1 of the isolates (272–1265 A1). Sequence alignments and analyses with the Basic Local Alignment and Search Tool program of the National Center for Biotechnology Information indicated that the sequence of the gene was 100% identical to the nucleotide sequence of KPC-3 gene (GenBank accession number AF 395881).

Plasmid-mediated, KPC-type carbapenem-hydrolyzing enzymes (KPC-1 and KPC-2) were reported initially in a few strains of *Klebsiella pneumoniae* [2, 4]. The sequence of the *bla*KPC-3 gene, also from *K. pneumoniae*, was recently submitted to Genbank by Tysall et al. [5]. (GenBank accession number AF395881). The *bla*KPC-1-encoding plasmid could not be conjugated into *E. coli* [4] but the KPC-2-encoding plasmid pST4707 [6] was self-transferable. Therefore, it is possible that the transfer of plasmids carrying a KPC gene between members of *Enterobacteriaceae* may escalate the spread of carbapenem resistance. Recently, KPC-2 was identified from *Salmonella enterica* [6] and *Enterobacter cloacae*. In this investigation, we have demonstrated that the production of KPC-3 by *E. coli* strains causes urinary tract infection.

The fact that most *E. coli* isolates from the patient were genetically related and a few later carbapenem-resistant isolates were genetically unrelated strains (according to PFGE analysis [figure 1]) has 2 implications: the original carbapenem-resistant strains were multi-clonal; the selective pressure of antibiotics has made the carbapenem-resistant isolates predominant; and/
or the resistant gene (blaKPC-3) was capable of transferring between genetically related and unrelated strains. We do not know whether the use of antibiotics may facilitate the transfer of antibiotic-resistant genes.

To our knowledge, this is the first documented case of imipenem and meropenem resistance emerging in *E. coli* during treatment of urinary tract infection and the first case of clinical failure of therapy with imipenem and meropenem due to KPC-3-producing *E. coli*. The emergence of carbapenem-resistant strains occurred after long-term treatment with imipenem and meropenem for recurrent urinary tract infection. The occurrence of a carbapenem-hydrolyzing enzyme in *E. coli* is disturbing, because carbapenems are often considered to be drugs of last resort for severe ESBL-positive *E. coli* infection. With the continuing spread of ESBL-positive organisms and the increasing use of carbapenems, it is possible that carbapenem resistance due to KPC-type carbapenemases will occur more frequently among Enterobacteriaceae species.

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**References**