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Impact of carbapenem heteroresistance among clinical isolates of invasive *Escherichia coli* in Chongqing, southwestern China

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Abstract

Although heteroresistance is common in a wide range of microorganisms, carbapenem heteroresistance among invasive *Escherichia coli* infections has not been reported. The objective of this study was to evaluate the clinical significance of carbapenem heteroresistance and to identify risk factors for its acquisition. A case–control study was conducted at a 3200-bed teaching hospital in Chongqing, southwestern China. Successive and non-duplicate nosocomial *E. coli* isolates (n = 332) were obtained from July 2011 to June 2013. Bloodstream isolates made up 50.6% of the strains collected. The rates of heteroresistance were 25.0% to imipenem, 17.2% to ertapenem, and 3.9% to meropenem. The population analysis profile revealed the presence of subpopulations with higher carbapenem resistance, showing MICs ranging from 2.0–128.0 mg/L. Male gender, invasive intervention, antibiotic use and bacterial extended-spectrum β-lactamase (ESBL) production contributed to invasive infections by carbapenem-heteroresistant *E. coli* (CHEC). The production of ESBL was identified as the common independent risk factor for heteroresistance to both ertapenem and imipenem. Pulsed-field gel electrophoresis revealed clonal diversity among the CHEC isolates. Most importantly, characterization of two successive *E. coli* strains isolated from the same patient indicated that carbapenem resistance evolved from heteroresistance. In conclusion, the high prevalence of heteroresistance to carbapenem among invasive *E. coli* merits great attention. Routine detection of ESBLs and the prudent use of imipenem and ertapenem are advocated. The early targeted intervention should be formulated to reduce CHEC infection and carbapenem resistance of *E. coli*.

Keywords: Carbapenem heteroresistance, ESBLs, *Escherichia coli*, invasive infections, risk factors
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Introduction

Recent data have revealed a high incidence of community and nosocomial infections by Gram-negative bacilli [1–4]. *Enterobacteriaceae*, most of which colonize humans, are dominant causes of these infections worldwide, owing to microbial evolution and selection in the presence of broad-spectrum antimicrobial agents. The accumulation of insusceptibility or resistance factors (hydrolytic enzyme production, efflux pumps, and porin deficits, or their combinations) has certainly resulted in the emergence and dissemination of multidrug-resistant *Enterobacteriaceae* [5–7]. Extended-spectrum β-lactamase (ESBL) producers have been frequently identified in community-onset urinary tract infections, intra-abdominal infections, and healthcare-related infections, and have led to the treatment failure of third-generation and fourth-generation cephalosporins, aminoglycosides, and fluoroquinolones, leaving carbapenems as 'the last resort' and 'the best choice'. However, the extensive consumption of carbapenems [8] and the unexpected and worrying prevalence of carbapenemases

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[9] in *Enterobacteriaceae* indicate that the utility of carbapenems is limited, causing fears about the availability of ideal antibiotics to overcome carbapenem-resistant *Enterobacteriaceae* (CRE) and necessitating a better understanding of the evolution of CRE.

As anticipated, local and worldwide surveillance of CRE is ongoing [10]. However, the emergence of phenotypically heteroresistant *Enterobacteriaceae* and the poor performance of automated susceptibility systems in detecting these microorganisms have potentially resulted in an underestimation of the extent of carbapenem-resistant epidemiology and evolutionary processes, resulting in treatment failure. Moreover, whereas *Enterobacter cloacae* [11], *Acinetobacter baumannii* [11–13] and *Klebsiella pneumoniae* [14] have been well documented in terms of heteroresistance traits, *Escherichia coli*, which is more frequently identified in bacteraemia (especially in healthcare settings), has not been well studied.

Accordingly, the clinical surveillance of carbapenem-heteroresistant *E. coli* and predisposing factors for its acquisition are urgently needed. To the best of our knowledge, this is the first study to report non-homogeneous heteroresistance to carbapenems among invasive *E. coli* isolates and their clinical evolution from carbapenem heteroresistance to resistance. This is also the first study to describe the prevalence of non-homogeneous carbapenem-heteroresistant *E. coli* (CHEC) and risk factors for its acquisition.

Materials and methods

Study design and data collection

This retrospective case–control study of nosocomial *E. coli* infections was conducted from July 2011 to June 2013 in the First Affiliated Hospital of Chongqing Medical University, a 3200-bed teaching hospital in Chongqing, southwestern China. Data were retrieved from the hospital information system and the laboratory information system. Isolates were collected from sterile body fluids of patients with culture evidence of nosocomial infection with *E. coli*. Antibiotic susceptibility tests were first completed with the Vitek2 compact (bioMerieux, Hazelwood, MO, USA), and the MICs were later confirmed with the broth microdilution method in the presence of carbapenems. Carbapenem heteroresistance was tested with the disk diffusion method on Mueller–Hinton agar (MHA) (Pangtong Medical Devices, Chongqing, China) with bacterial suspensions (0.5 McFarland standard) and paper disks containing 10 μg of imipenem, ertapenem or meropenem (Thermo Fisher Oxoid, Basingstoke, UK) and MIC gradient strips (bioMerieux). Following incubation at 35°C for 24 h, colonies inside the inhibition zone were considered to be CHEC, and isolates lacking colonies inside the inhibition zone were considered to be non-CHEC. Isolates without an inhibition zone were defined as carbapenem fully resistant isolates. With this strategy, the *E. coli* isolates were divided into three groups. Because carbapenem fully resistant isolates were rare in this hospital setting, only CHEC and non-CHEC cases were enrolled, as the case and control cohorts, respectively. CHEC cases were further followed to determine the potential clinical evolution of heteroresistance in response to the antibiotic pressure of carbapenems. Two clonally related *E. coli* strains, No. 1021 and No. 88, were further studied, owing to their apparent evolution from heteroresistance to resistance.

Demographic and epidemiological data were age, gender, chronic underlying diseases, previous hospital stay, hospital wards, previous antibiotic agent use and combined administration, and ESBL production.

Definitions

Sterile body fluids were defined as specimens that were collected from sterile sites with sterile procedures, such as blood, peritoneal fluid, cerebrospinal fluid, pleural fluid, joint fluid, and tissue biopsy from internal body sites, among other sites.

Previous antibiotic agent use was considered to have occurred if the antibiotics had been used for more than three consecutive days within 30 days before the isolation of invasive *E. coli*.

Heteroresistance to two or more antimicrobial agents was considered to be co-heteroresistance.

Population analysis profiling (PAP) of *E. coli* isolates

Carbapenem heteroresistance was determined by PAP for strains that showed different carbapenem MICs and for the standard carbapenem-susceptible *E. coli* control isolate ATCC 25922. To achieve this goal, one colony from a culture grown overnight on Columbia agar (Pangtong Medical Devices) was inoculated into 5 mL of Luria–Bertani broth (Pangtong Medical Devices). After 24 h of incubation, 0.1 mL of the starting bacterial suspension with a density corresponding to 0.5 McFarland was serially diluted and spread onto MHA containing two-fold serial dilutions of imipenem, ertapenem, or meropenem (Merck & Co., Whitehouse Station, NJ, USA), with concentrations ranging from 0.25 to 256 mg/L. One hundred microlitres of ten-fold serial dilution(s) of a 0.5 McFarland suspension was spread on drug-free MHA and incubated for 48 h at 37°C to determine the CFUs/mL. Colony counts from three replicate plates were performed for all of the isolates, and mean values were estimated. The number of resistant cells in 0.1 mL of starting bacterial suspension was calculated, and the log CFUs/mL was plotted against the antibiotic concentration. The

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