CONCISE REPORT

Phenotypic and genotypic characteristics of community-acquired and hospital-acquired carbapenem-resistant *Enterobacteriaceae* in patients with liver cirrhosis at the National Liver Institute of Egypt

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ABSTRACT

Background: Carbapenem-resistant *Enterobacteriaceae* (CRE) is considered one of the most urgent public health problems worldwide with associated high morbidity and mortality rates. CRE has both community-acquired (CA) and hospital-acquired (HA) danger because of the transmissible nature of plasmids.

Objectives: We aimed to compare the phenotypic and genotypic characteristics of carbapenemase genes in CRE isolates causing CA and HA infections in cirrhotic patients and the distribution of carbapenemase genes in both settings.

Method: CRE isolates were taken from 38 recruited cirrhotic patients at the National Liver Institute at Menoufia University in Egypt between January 2017 and January 2018 with *Enterobacteriaceae* isolates resistant to at least one carbapenem. Isolates were identified and described by conventional techniques and confirmed by the VITEK 2 system, which was also used for antimicrobial susceptibility and the detection of extended-spectrum β-lactamase production. We then phenotypically and genotypically characterized all isolates for the presence of the most prevalent carbapenemase enzymes (*Klebsiella pneumoniae* carbapenemase [KPC], Verona integron metallo-beta-lactamases [VIM], New Delhi metallo-beta lactamase [NDM], and oxacillinase-48 [OXA-48]) and genes using multiplex polymerase chain reaction confirmed results.

Results: All CRE isolates included in this study were resistant to all carbapenems tested and susceptible to colistin, while 20 of the 38 isolates were sensitive to tigecycline. Among the 24 HA CRE isolates, nine isolates (37.5%) contained OXA-48, three (12.5%) contained both OXA-48 and NDM-1, two contained KPC (8.3%), one carried NDM-1 (4.2%), and one included VIM (4.2%). The OXA-48 gene was the most frequent gene in both groups, and no statistically significant difference was found between the two groups in regards to prevalence.

Conclusion: OXA-48 CRE is the most prevalent carbapenemase gene in Egyptian cirrhotic patients with similar phenotypic and genotypic characteristics to CA cases. This indicates the equal prevalence of CRE in community and hospital settings.

KEYWORDS

Carbapenem-resistant *Enterobacteriaceae*; cirrhotic patients

INTRODUCTION

Rapidly emerging antimicrobial-resistant *Enterobacteriaceae* have been noted frequently with decompensated liver cirrhosis patients due to recurrent hospitalizations and repeated exposure to antibiotics either for treatment or prophylactic purposes. In addition, although carbapenem-resistant *Enterobacteriaceae* (CRE) are considered hospital-acquired (HA) pathogens, community-acquired (CA) CRE are also a threat and the knowledge about community-acquired CRE is limited [1, 2].

CRE are capable of inactivating carbapenem via different mechanisms, such as the overproduction of ampC enzymes, extended-spectrum beta-lactamase (ESBLs), carbapenemase enzymes that inactivate the β-lactam antibiotics, including carbapenems, efflux pumps, and deletion of porins [3]. Although CRE are initially considered HA pathogens, CA CRE are also noted [4].The most clinically important carbapenmases are *Klebsiella pneumoniae* carbapenemase (KPC) in the Ambler class A category, Verona integron metallo-beta-lactamases types (VIM), imipenemase, New Delhi metallo-betalactamase-1 (NDM-1) in the class B category, and oxacillinase-48 (OXA-48) in the class D category [5]. The dissemination of KPC, VIM,

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NDM, and OXA-48 among *K. pneumoniae* and *Escherichia coli* has been emerging in different countries [6].

NDM and OXA-48 producers are both HA and CA pathogens, whereas KPC producers are mainly HA isolates [7]. The activity of carbapenemase enzymes is identified by phenotypic assays, while carbapenemase encoding genes are identified by molecular assays [8, 9].

In the current study, we investigated the phenotypic and genotypic characteristics of CA and HA CRE isolates from cirrhotic patients admitted to the National Liver Institute (NLI) at Menoufia University in Egypt.

METHODS

Design

The study was performed from January 2017 to January 2018 at the NLI (Menoufia University, Egypt). NLI is a university hospital with a capacity of 320 beds that provides medical services for 107,450 hepatic patients annually.

The study was approved by the NLI Research Ethics Committee and the Research Ethics Committee of Menoufia University's Faculty of Medicine. Informed consent was obtained from all participants before their enrollment in the study.

Patients

A total of 38 *Enterobacteriaceae* isolates resistant to at least one carbapenem were isolated from different clinical specimens (e.g., blood, urine, sputum, wound, stool, and swabs from central lines and urinary catheters). Patient consent was ensured.

Diagnostic criteria

Inclusion criteria

HA CRE were isolated from patients who were hospitalized for > 48 hours. CRE is considered CA if the infection was present on admission or developed less than 48 hours after hospitalization. The definition of infection or colonization was followed by the guidelines published by the Centers for Disease Control and Prevention.

Exclusion criteria

Enterobacteriaceae isolates that were sensitive to carbapenems or associated with asymptomatic colonization were excluded. Duplicate isolates from the same patient were also excluded, unless they were isolated from different specimens with a distinguishable susceptibility pattern.

Bacterial cultures and antimicrobial susceptibility

Isolates were plated on blood agar and MacConkey agar (Oxoid, UK), depending on the type of clinical specimens. Cultures were then examined macroscopically for colonial morphology and a Gram stain was performed on suspected colonies. All *Enterobacteriaceae* isolates were selected then subcultured at 37° C overnight on MacConkey agar media for purity and further identification tests. Further, confirmation of the isolates was performed using the automated VITEK 2 Compact system (BioMérieux, France) and Gram-negative (GN) cards following the manufacturer's instructions.

Antimicrobial susceptibility and production of ESBL were determined using the VITEK 2 Compact system and AST-GN73 cards following the manufacturer's instructions. Confirmed isolates were stored in nutrient broth supplemented with 16% glycerol at -80° C until used for phenotypic and genotypic characterization [10]. All CRE isolates were then tested for the presence of the most prevalent carbapenemase enzymes (KPC, VIM, NDM, and OXA-48) and genes by phenotypic (Modified Hodge Test) [11] and genotypic methods (multiplex polymerase chain reaction [PCR]) [12].

Statistical method

Data was collected and entered to the computer using the SPSS program for statistical analysis (v. 18, Chicago, IL). Data were entered as numerical or categorical. Numerical data were shown as mean and standard deviation (SD). Student's t-test was done to compare means and SD of two sets of numerical data. Categorical data were expressed as frequency and percent (%) and a chi-squared test (x^2) was used to study association. Whenever any of the expected cells were less than five, Fischer's exact test was used. *P*-value was considered statistically significant when it was less than 0.05.

RESULTS

All CRE isolates included in this study were resistant to all carbapenems tested and susceptible to colistin, while 20 out of 38 isolates were sensitive to tigecycline

Of the 38 CRE isolates, 24 patients had HA infection (63.2%) and 14 patients (36.8%) had CA infection. The mean age of patients with HA infection and CA infection was 49.60± 8.28 years and 45.56± 10.25 years, respectively. There was no significant difference between the two median ages ($P = 0.06$).

Infection, bacterial species, and carbapenemase gene distribution for HA and CA isolates are shown in Table 1. There was no statistically significant difference between the two groups.

The OXA-48 gene was the most frequent gene in CA and HA CRE. Among the 24 HA CRE isolates, nine isolates (37.5%) contained OXA-48, three (12.5%) contained both OXA-48 and NDM-1, two contained KPC (8.3%), one contained NDM-1 (4.2%), and one contained VIM (4.2%). The prevalence of carbapenemase genes in CA isolates was as follows: 28.7% contained OXA-48, 14.3% contained NDM-1, and 7.1% contained both OXA-48 and NDM-1. Our study revealed that the OXA-48 gene was the most frequent gene in both groups and no statistically significant difference was found.

DISCUSSION

Phenotypic and genotypic characteristics in CA and HA CRE isolates causing infections in patients with liver cirrhosis were compared, and the role of carbapenemase genes and their distributions in both CA and HA infections were investigated. Exposure to antibiotics (such as carbapenem and quinolones), healthcare-associated interactions, the presence of indwelling devices, the use of mechanical ventilators, and comorbidities are

TABLE 1: Distribution of carbapenemase-resistant *Enterobacteriaceae* isolates according to site of infection, bacterial species, and carbapenemase genes.

	Hospital-acquired infections (n = 24)	Community-acquired infections (n = 14)	*P*-value
Site of infection			
Pneumonia	3 (12.5%)	4 (28.6%)	0.21
Urinary tract infection	5 (20.8%)	3 (21.4%)	0.96
Bacteremia	4 (16.7%)	3 (21.4%)	0.71
Wound infection	4 (16.7%	4 (28.6%)	0.38
Bacterial species			
Klebsiella pneumoniae	19 (79.2%)	9 (64.3%)	0.31
Escherichia coli	3 (12.5%)	5 (35.7%)	0.09
Morganella morgannii	2 (8.3%)	0 (0.0%)	0.95
Carbapenemase gene			
Negative for all	8 (33.3%)	6 (42.8%)	
OXA-48	9 (37.5%)	4 (28.7%)	0.77
NDM-1	1 (4.2%)	2 (14.3%)	
KPC	2 (8.4%)	0 (0.0%)	
VIM	1 (4.2%)	0 (0.0%)	
OXA-48 and NDM-1	3 (12.5%)	1 (7.1%)	
KPC and NDM-1	0 (0.0%)	1 (7.1%)	

all risk factors responsible for the higher incidence of CRE in these patients. Moreover, the acquisition and transfer of drug-resistant genes through plasmids and transposons and its spread to the community via the fecal-oral route may be responsible for the appearance of CA infections by CRE among such patients [13].

Previous studies reported nearly similar findings: Tang et al. (2016) found that 29.5% of 78 CRE cases were CA, but the study included colonization [4]. Sheng et al. (2016) reported that 21.3% of CRE cases were CA [14]. In contrast to our study, Miller & Johnson (2015) and Thaden et al. (2014) reported lower incidence of CA CRE in comparison to HA (9.8% and 5.6%, respectively) [15, 16].

HA CRE was most frequently associated with urinary tract infections (UTI) (20.8%), while in CA, pneumonia was the most frequent infection (28.6%).This was consistent with other studies showing that UTIs were the most common HA infection, accounting for almost 40% of all nosocomial infections [17], while for CA, pneumonia is the most frequent infectious disease worldwide [18]. Also, Salerno et al. (2016) reported that UTIs, spontaneous bacterial peritonitis, and bacteremia were the most frequent HA infections in cirrhotic patients, while pneumonia was the most frequent CA infection (33%) [19]. On the other hand, Tang et al. (2016) reported that pneumonia was the most common HA CRE infection in cirrhotic patients, followed by UTIs [4].

In regard to the type of bacteria, *K. pneumoniae* was the most common organism (73.7%), followed by *E. coli* (21.1%). Similar results were reported in many studies testing the presence of CRE among hospital and community samples [3, 16, 20]. However, others found that *E. coli* was the most common organism overall, followed by *K. pneumoniae* or *Enterobacter cloacae* (21-23).

The spread of CRE isolates into the community from healthcare settings or vice versa via the fecal-oral route and the highly transmissible nature of plasmid-borne carbapenemases may have contributed to the wide spread of CRE with comparable phenotypic characteristics in both settings.

Although no significant difference was found between the two CRE groups in regard to the genotypic characteristics and the prevalence of carbapenemase genes, OXA-48 was the most predominant gene among the 24 HA CRE isolates (37.5%) and the CA CRE isolates (28.7%). Our observation was consistent with other studies that identified the OXA-48 gene as the most predominant gene [24].

The KPC and VIM genes were only detected in HA CRE, which could be due to the limited number of CA CRE cases.

OXA-48 was also reported to be commonly distributed in the Mediterranean region of Africa and Europe [25] and Saudi Arabia [26], which supports our findings. In addition to OXA-48-like and NDM-1 genes, VIM was detected in one CRE isolate. The low detection rate of this gene may be attributed to the higher prevalence of this gene in Europe than Africa [25]. Moreover, the only *Morganella morganii* isolate detected in our study expressed the OXA-48 gene.

Interestingly, five out of the 38 CRE were found to co-express two carbapenemase genes. NDM-1 genes co-existed with OXA-48 genes in four isolates (three HA and one CA isolate) and co-existed with the KPC gene in one isolate, which confirms the high coexistence rate of different carbapenemases among *Enterobacteriaceae* isolates.

In conclusion, CRE have a wide distribution in the community with comparable phenotypic and genotypic characteristics to those in hospital settings, highlighting the overuse of antibiotics, adequate antibiotic empirical control, and the need for implementation of strict infection control guidelines in healthcare facilities. Further research involving more patients is needed in order to confirm our findings and highlight the need for antimicrobial stewardship. Coordination between infection control teams and healthcare workers is also crucial to prevent the spread of CRE.

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