Molecular epidemiology of carbapenem-resistant Enterobacteriaceae isolated from patients in COVID-19 wards and ICUs in a Bulgarian University Hospital

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ABSTRACT

Many studies report an increase in antimicrobial resistance of Gram – negative bacteria during the COVID-19 pandemic. Our aim was to evaluate the epidemiological relationship between carbapenem-resistant (CR) Enterobacteriaceae isolates from patients in COVID-19 wards and to investigate the main mechanisms of carbapenem resistance in these isolates during the period April 2020 – July 2021. A total of 45 isolates were studied: Klebsiella pneumoniae (n = 37), Klebsiella oxytoca (n = 2), Enterobacter cloacae complex (n = 4) and Escherichia coli (n = 2). Multiplex PCR was used for detection of genes encoding carbapenemases from different classes (blaKPC, blaIMP, blaVIM, blaNDM, blaOXA-48). For epidemiological typing and analysis, ERIC PCR was performed. Two clinical isolates of E. cloacae, previously identified as representatives of two dominant hospital clones from the period 2014 – 2017, were included in the study for comparison. In the CR K. pneumoniae group, 23 (62.2%) carried blaKPC, 13 (35.1%) blaNDM, 10 (27.0%) blaVIM, and 9 (24.3%) were positive for both blaKPC and blaVIM. The blaKPC was identified also in the two isolates of K. oxytoca and blaVIM in all E. cloacae complex isolates. The two CR isolates of E. coli possessed blaKPC and blaOXA-48 genes. Epidemiological typing identified 18 ERIC profiles among K. pneumoniae, some presented as clusters of identical and/or closely related isolates. The carbapenem resistance in the studied collection of isolates is mediated mainly by blaKPC. During the COVID-19 pandemic intrahospital dissemination of CR K. pneumoniae, producing carbapenemases of different molecular classes, as well as continuing circulation of dominant hospital clones of multidrug-resistant E. cloacae complex was documented.

KEYWORDS

COVID-19 wards, carbapenem-resistant Enterobacteriaceae, intrahospital dissemination

INTRODUCTION

Since its emergence in 2020, the SARS-CoV-2 pandemic had a significant impact on each aspect of the public healthcare worldwide, raising long-term issues such as a constantly increasing antimicrobial resistance [1]. Patients with severe COVID-19 in critical conditions usually need prolonged hospitalization, which is often complicated by nosocomial bacterial infections. Different authors report that more than 70% of patients admitted to hospitals have been treated with antibacterial agents, with 33% self-prescribing therapy before hospital admission [2]. The overall rate of bacterial infections among COVID-19 patients was estimated to be 6.9%, varying by patient population, ranging from 5.9% in hospitalized patients to 8.1% in critically ill patients [3]. Some authors report even higher incidence of bacterial infections in intensive care unit (ICU) patients (13.5%–44%), mainly associated with multidrug-resistant (MDR) Gram - negative bacteria [4, 5]. These patients were associated...
with increased risk for severe infections, caused by carba-
penemase-producing *Enterobacteriaceae* and with high
mortality rate [6]. Many studies documented an increase in
antimicrobial resistance during the COVID-19 pandemic in
Gram – negative bacteria, a phenomenon associated with the
excessive and unnecessary antimicrobial therapy, non-
compliance with hygiene measures, prolonged stay in ICUs,
decreased resistance surveillance due to diagnostic focus on
COVID-19 and overloading of healthcare systems [7].

The infections caused by MDR carbapenemase-produc-
ing bacteria have limited treatment options [8]. This prob-
lem existed before the onset of SARS-CoV-2. In some
countries (Israel, Greece, Italy, Turkey) the incidence of
carbapenem-resistant (CR) *Klebsiella pneumoniae* exceeded
50% before the pandemic [9]. The European Centre for
Disease Prevention and Control reported a statistically sig-
nificant trend for increasing the rate of CR *K. pneumoniae*
 invasive isolates in Europe during the period 2015–2020
(from 6.8% in 2015 to 10% in 2020), with the highest rates
detected in Greece (66.3%), Romania (48.3%) and Italy
(29.5%) [10]. Before the pandemic, Bulgaria also reported
CR *K. pneumoniae* isolates, associated with blood stream
infections. During the same period, the rate of CR blood
isolates of *Klebsiella pneumoniae* significantly increased
from 3.2% in 2015 to 28.1% in 2020 [10–12].

In 2020 the World Health Organization announced the
threat of antimicrobial resistance as one of the most pressing
challenges of our time, further exacerbated by the COVID-
19 pandemic [13]. This fact determines the surveillance of
antimicrobial resistance and the investigation of its mecha-
nisms as priorities for both individual patient care and
infection control strategies on global, national and regional
levels.

The aim of this study was to evaluate the epidemiological
relationship between carbapenem-resistant *Enterobacteriaceae* isolates, obtained from patients hospitalized in COVID-19
wards and ICUs in Varna University Hospital, Bulgaria
during the period April 2020–July 2021 and to investigate the
main carbapenem resistance mechanisms in these isolates.

**MATERIALS AND METHODS**

A total of 143 non-duplicate CR *Enterobacteriaceae* isolates were obtained from hospitalized patients in the ICUs and
the COVID-19 wards in the hospital during the studied
period: *K. pneumoniae* (n = 135), *Klebsiella oxytoca* (n = 2),
*Enterobacter cloacae* complex (n = 4), *Escherichia coli* (n = 2).
Based on the following criteria - resistance profile,
type of the ward and specimen, year of isolation, a total of 45
CR isolates were studied: *K. pneumoniae* (n = 37), *K. oxy-
toca* (n = 2), *E. cloacae* complex (n = 4) and *E. coli* (n = 2).
The isolates were obtained from urine samples (n = 23),
blood (n = 4), respiratory samples (n = 6), wound
secretions (n = 6), punctates (n = 3), feces (n = 1), semen
(n = 1) and from the hospital environment (n = 1). Two
clinical isolates of *E. cloacae*, previously identified as
representatives of two dominant hospital clones from the
period 2014–2017, were included in the study for compari-
sion. Species identification and antimicrobial susceptibility
testing were done by Phoenix automated system (Beckton
Dickinson, USA) and interpreted according to the European
Committee on Antimicrobial Susceptibility Testing
(EUCAST) recommendations. Confirmation of species
identification was performed by MALDI Biotyper Sirius
(Bruker, Germany). The susceptibility to colistin was
determined by the broth microdilution method (Erba
Lachema, Czech Republic). Multiplex PCR was performed to
detect the presence of genes encoding carbapenemases from
different classes (*bla*KPC, *bla*IM, *bla*VIM, *bla*NDM, *bla*OXA-48) [14]. Epidemiological typing was done by ERIC PCR [15].
The ERIC patterns of the studied isolates were subjected to
UPGMA analysis. Similarity of >70% was used as a
threshold for clonal relatedness of the isolates. The study
was approved by the Ethical Committee of Varna Medical
University (124/06.01.2023).

**RESULTS**

A total of 45 isolates (*K. pneumoniae*, n = 37, *K. oxytoca*,
n = 2, *E. cloacae* complex, n = 4), *E. coli*, n = 2) were studied
for their main CR mechanisms and genetic relatedness.

The rates of antimicrobial resistance in the studied
collection of CR isolates, presented in increasing order, were
as follows: colistin, 7%; amikacin, 52%; trimethoprim/sul-
phametoxazole, 56%; ceftazidime/avibactam, 60%; gentamicin,
69%; tobramycin, 98%; fluoroquinolones (ciprofloxacin,
levofoxacin), 100%.

In the CR *K. pneumoniae* group, 23 isolates (62.2%)
carried *bla*KPC, 13 (35.1%) - *bla*NDM, 10 (27.0%) - *bla*VIM,
and 9 isolates (24.3%) were positive for both *bla*KPC and
*bla*VIM. The *bla*KPC was identified also in the two isolates of
*K. oxytoca* and *bla*VIM in all *E. cloacae* complex isolates.
The two CR *E. coli* isolates possessed *bla*KPC and *bla*OXA-48 genes
(Table 1).

Eighteen different ERIC types (A–R) were detected
among the isolates of *K. pneumoniae*, some presented as
clusters of identical and/or closely related isolates (A, B, C,
D, E, F) (Table 1). Twelve isolates demonstrated unique
ERIC profiles. Type C was the predominant, found in 8 isolates,
collected from patients, hospitalized in 5 hospital
wards. ERIC type D was identified in 4 isolates from 4 hospi-
tal wards. *K. pneumoniae*, isolated from the hospital
environment in the ICU in March 2021 also demonstrated
ERIC type D. Similarly, ERIC types A and B were detected in
different hospital wards. ERIC types A and E were identified
in 2020, while D and F – in 2021. In contrast, isolates with
ERIC profiles B and C were detected during the whole
studied period (Table 1).

All four *E. cloacae* complex isolates were demonstrated
with different ERIC profiles (Table 1). Two of these isolates
exhibited identical profiles with those of previously isolated
*E. cloacae*, representatives of two dominant hospital clones
from the period 2014–2017. One ERIC profile was found in
*K. oxytoca* and *E. coli* (Table 1).
COVID-19 patients in the pre-pandemic year in Serbia, years [16]. Comparing COVID-19 patients and non-
the beginning of the pandemic in comparison to previous
period of time. Data published by Cuntrò demonstrated
increased use of empirical antimicrobial therapy for a long
pandemic, the high number of critically ill patients and the
of the hospital and the medical staff during the two years of
in the rate of CR Enterobacteriaceae

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>ERIC type (n)</th>
<th>bla genes (n)</th>
<th>Year of isolation (n)</th>
<th>Department (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K. pneumoniae</td>
<td>A (n = 2)</td>
<td>blakPC + blavIM (n = 8)</td>
<td>2020n = 2</td>
<td>Neurologyn = 1; INUn = 1</td>
</tr>
<tr>
<td></td>
<td>B (n = 4)</td>
<td>blakPC (n = 4)</td>
<td>2020n = 2; 2021n = 2</td>
<td>Pediatric wardn = 1; Pediatric</td>
</tr>
<tr>
<td></td>
<td>C (n = 8)</td>
<td>blakPC + blavIM (n = 8)</td>
<td>2020n = 4; 2021n = 4</td>
<td>Hemodialysisn = 1; Nephrologyn = 1;</td>
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<tr>
<td></td>
<td>D (n = 6*)</td>
<td>blanNDM (n = 6)</td>
<td>2021n = 6</td>
<td>ICU = 2; Cardiologyn = 1</td>
</tr>
<tr>
<td></td>
<td>E (n = 2)</td>
<td>blanNDM (n = 2)</td>
<td>2020n = 2</td>
<td>INUn = 2; Hematologyn = 1; ICU = 2;</td>
</tr>
<tr>
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<td>F (n = 3)</td>
<td>blakPC (n = 3)</td>
<td>2021n = 3</td>
<td>INUn = 2; Nephrologyn = 1;</td>
</tr>
<tr>
<td>unique profiles</td>
<td>(n = 12)</td>
<td>blakPC (n = 7); blanNDM (n = 3); blavIM (n = 1)</td>
<td>2020n = 10; 2021n = 2</td>
<td>Hematologyn = 1; Therapeutic wardn = 2</td>
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<tr>
<td></td>
<td></td>
<td>blakPC + blavIM (n = 1)</td>
<td></td>
<td>ICU = 1; Hemodialysisn = 1; Pediatric</td>
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<td>K. oxytoca</td>
<td>A (n = 2)</td>
<td>blakPC (n = 2)</td>
<td>2021n = 2</td>
<td>Neurologyn = 1; Gastroenterologyn = 1;</td>
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<td>E. cloacae complex</td>
<td>a** (n = 1)</td>
<td>blavIM (n = 1)</td>
<td>2020n = 1</td>
<td>Reumatologyn = 1</td>
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<tr>
<td></td>
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<td>blavIM (n = 1)</td>
<td>2021n = 1</td>
<td>IPCun = 2</td>
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<tr>
<td></td>
<td>c (n = 1)</td>
<td>blavIM (n = 1)</td>
<td>2021n = 1</td>
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<tr>
<td></td>
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<td>blavIM (n = 1)</td>
<td>2021n = 1</td>
<td>Therapeutic wardn = 1</td>
</tr>
<tr>
<td>E. coli</td>
<td>A (n = 2)</td>
<td>blakPC + blaoxa-48 (n = 2)</td>
<td>2021n = 2</td>
<td>Urologyn = 1;</td>
</tr>
</tbody>
</table>

Abbreviations: ICU, Intensive Care Unit; INU, Intensive Neurology Unit; IPU, Intensive Pediatric Unit; * isolate from the hospital environment; ** ERIC profiles identical with the ERIC profiles of previously isolated E. cloacae, representatives of two dominant hospital clones from the period 2014–2017.

DISCUSSION

The University Hospital “Saint Marina” is a 1380 bed-hos-
pital in Varna city, located in the Northeastern Bulgaria. The
hospital provides healthcare for 1 500 000 population.
During the COVID-19 pandemic (2020–2021), nineteen
COVID departments were structured (including two ICUs)
and more than 10 500 patients were treated. In the recent
years the representatives of Enterobacteriaceae (K. pneumoniae, E. coli, E. cloacae complex) are the most frequently
isolated bacterial species from various clinical samples,
collected from patients in the hospital. During the period
2015–2021, a significant trend of increasing the rate of CR
K. pneumoniae was detected, ranging from 0.7% in 2015 to
9% and 19% in 2020 and 2021, respectively. During the same
period only single isolates of CR E. coli and E. cloacae
complex were identified, especially during the last two years
(2020–2021). Possible reasons for such dramatic increase
in the rate of CR Enterobacteriaceae are the overloading
of the hospital and the medical staff during the two years of
pandemic, the high number of critically ill patients and the
increased use of empirical antimicrobial therapy for a long
period of time. Data published by Cuntrò demonstrated
no significant increase in the isolation of carbapenemase-
producing Enterobacteriaceae in patients with COVID-19 at
the beginning of the pandemic in comparison to previous
years [16]. Comparing COVID-19 patients and non-
COVID-19 patients in the pre-pandemic year in Serbia,
Despotovic reported a statistically significant difference in
the carbapenem resistance: 56.8% and 61.1% for imipenem
and meropenem in COVID-19 patients versus 24.5% and
24.3% for non-COVID-19 patients [17]. In the later periods
of the pandemic an increase in the rate of these pathogens
was observed in many regions of the world [8, 18]. In 2020
an increase in the colonization/infection rate with NDM-
producing Enterobacteriaceae in a cohort of COVID-19
patients in an Italian university hospital compared to other
patients was reported by Porretta. This author also found
a significant increase in the duration of hospital stay, asso-
ciated with these co-infections [18]. In another Italian
hospital, the incidence of acquisition of KPC-producing
K. pneumoniae in patients with COVID-19 from the ICUs
increased from 6.7% in 2019 to 50% in 2020 [19].

In our study a total of 143 CR Enterobacteriaceae isolates
were recovered from patients admitted to COVID-19 wards
during the period 2020–2021, with K. pneumoniae being the
dominant bacterial species (94.4%). Similar results were re-
ported in a multicenter observational Italian study [9]. In the
whole tested group of 45 CR isolates, we documented blakPC
as the main gene encoding carbapenemases (60.0%), con-
firming the findings from Romania (44.4%), Brazil (92.3%)
and Italy (52–100%) [7, 9, 19, 20, 21]. This result also cor-
relates with the data reported by Dabrowska et al. in their
extensive review covering the available literature on CR
K. pneumoniae, associated with COVID-19 patients [22].

In addition, genes, encoding metallo-beta-lactamases
(MBL) were detected in 65.9% of K. pneumoniae and E.
cloacae complex isolates in our study (blanNDM, 31.7%;
blavIM, 34.2%). Similarly, Falcone et al. reported 43.9%
MBL-producing enteric bacteria, obtained from hospitalized
COVID-19 patients [9]. The high proportion of MBL producers in the studied collection of isolates could explain the detected high rates of ceftazidime/avibactam resistance in the whole Enterobacteriaceae group (60%).

In our study, genes, encoding OXA-carbapenemases (blaOXA-48) were identified only in single E. coli isolates (4.4%), confirming published results from Romania (11.1%) and Italy (4.8%) [9, 20].

A similar distribution of carbapenemases, mainly blaKPC in K. pneumoniae, blaOXA-48 in E. coli and blaNDM in E. cloacae was reported by Assis et al. [23]. A prevalence of KPC, OXA-48 and VIM carbapenemases among Enterobacteriaceae isolates was also found by Pintado et al. in their study from 2021 [24]. Studies from Italy, France and the USA also demonstrated predominance of KPC and NDM carbapenemases in COVID-19 patients [8, 19, 21, 25, 26].

The increased isolation of CR Enterobacteriaceae during the COVID-19 period in our hospital is a worrying finding, which underlines the necessity of elucidating the molecular epidemiology of the circulating MDR nosocomial isolates. The results from the present study illustrate the successful intrahospital dissemination and persistence of clonally related KPC, VIM and NDM producing K. pneumoniae isolates, representatives of two major cluster groups, but also emergence of CR K. pneumoniae with unique ERIC profiles, carrying blaKPC, blaNDM and blaVIM in 10 hospital wards during the same period. The isolation of NDM-producing K. pneumoniae from the hospital environment, demonstrates the importance of the contaminated environment as a source to acquire and further disseminate the nosocomial pathogen. Intrahospital clonal dissemination of CR K. pneumoniae isolates, affecting problematic patients, including patients with COVID-19, has been reported in Europe and the USA [25–29]. Similarly, nosocomial spreading of NDM-1 and OXA-48 producing K. pneumoniae during the COVID-19 pandemic was documented in France and Spain [30, 31].

Among the studied E. cloacae complex isolates in this study, all blaVIM positive, no clonality was found. However, the identification of two of these isolates as representatives of two ESBL producing E. cloacae complex clones with widespread intrahospital dissemination during the period 2014–2017, is an indication for the potential of these clones to persist in the hospital over long period of time [32]. Similar to our finding, a clonal dissemination of CR E. cloacae complex during the COVID-19 pandemic was reported by different authors: VIM-4 and NDM-1 producing E. cloacae complex in an intensive care unit in an Italian hospital, NDM-1 producing E. cloacae in ICU in France and the USA [28, 29, 31].

CONCLUSION

The carbapenem resistance in the studied collection of isolates is mediated mainly by blaKPC, but also by blaVIM and blaNDM genes. During the COVID-19 pandemic intrahospital dissemination of CR K. pneumoniae, producing carbapenemases of different molecular classes, as well as continuing circulation of dominant hospital clones of MDR E. cloacae complex, identified in the period 2014–2017, but already carrying carbapenemase genes, were found. This study confirms the wide on-going distribution of KPC, NDM, VIM and OXA-48 carbapenemases in Enterobacteriaceae, facilitated by the antimicrobial over-consumption and selective pressure during COVID-19 pandemic period. The identified persistence of endemic MDR clones necessitates continuous epidemiological investigation as an important part of the hospital infection control program.

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Declaration of competing interests: All authors declare no competing interests.

Ethical conduct of research: All studies in the presented manuscript are part of the routine diagnostic and treatment procedures in the hospital. The study was conducted in accordance with the Declaration of Helsinki. The study was approved by the Ethical Committee of Varna Medical University (124/06.01.2023).

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