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RESEARCH ARTICLE

Changes in molecular epidemiology of carbapenem-resistant *Klebsiella pneumoniae* in the intensive care units of a Greek hospital, 2018–2021

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ABSTRACT

The spread of multi-drug resistant (MDR) Gram-negative bacteria, including *Klebsiella pneumoniae,* constitutes a global threat. The most frequent mechanism of acquired carbapenem resistance is the production of carbapenemases, especially KPC, NDM, VIM, IMP and OXA-48. We analyzed the epidemiological trend of carbapenem resistance genes of carbapenem-resistant *K. pneumoniae* (CRKP) strains isolated from critically ill patients in a Greek tertiary hospital.

The study included 150 CRKP isolates collected from 116 (77.4%) patients hospitalized in the adult ICU and 17 (11.3%) each in the pediatric and the two neonatal ICUs between March 2018 and March 2021. Identification and antimicrobial susceptibility testing were performed using VITEK-2. A multiplex lateral flow immunoassay was used for the detection of carbapenemases, while the detection of bla_{VIM}, bla_{KPC}, bla_{NDM}, bla_{IMP} and $bla_{OXA\text{-}48\text{-}like}$ genes was achieved by multiplex PCR.

The bla_{NDM} was mainly detected in adults (54/116, 46.9%), while in children the most often detected gene was bla_{KPC} (24/34, 70.6%). The predominant carbapenem resistance gene during 2018–2019 was bla_{KPC} alone or in combination with bla_{VIM}, reaching 44.4% in 2019, while during 2020–2021 the detection of bla_{NDM} prevailed significantly, reaching 45.5 and 60.7% for 2020 and 2021, respectively.

A shift in the molecular epidemiology of CRKP was seen during 2018–2021, which is probably associated with the recent excessive empiric use of newer antimicrobials. Surveillance studies and proper and strict implementation of infection control measures are highly needed to decrease the spread of MDR bacteria, including CRKP.

KEYWORDS

Klebsiella pneumoniae, carbapenem-resistant, intensive care unit, Greece

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INTRODUCTION

In recent years, the spread of multi-drug resistant (MDR), extensive drug resistant (XDR) and pan-drug resistant (PDR) Gram-negative bacteria, including *Klebsiella pneumoniae*,

constitutes a global threat; the problem is higher in hospitalized patients, especially those in the intensive care units (ICUs) [1–3]. According to the latest report of European Center for Disease Control and Prevention (ECDC) for 2020, Greece presents a high percentage (66.3%) of carbapenem-resistant *K. pneumoniae* (CRKP) [4]. The most frequent mechanism of acquired carbapenem resistance is through the production of carbapenemases, especially *K. pneumoniae* carbapenemase (KPC), New Delhi metallo-β-lactamase (NDM), Verona Integron-Borne metallo-β-lactamase (VIM), imipenemase (IMP) and oxacillinase-48 (OXA-48) [5, 6]. The aim of the present study was to gain an insight into the epidemiology of carbapenem resistance genes of XDR/PDR CRKP strains isolated in critically ill patients in a tertiary hospital in Greece during the last three years (2018–2021).

METHODS

We studied 150 XDR/PDR CRKP isolates collected between March 2018 and March 2021 from blood, urine, bronchial secretions, trauma, or rectal swabs (as part of colonization screening) from patients hospitalized in the adult ICU (116 isolates, 77.4%), the pediatric ICU (17 isolates, 11.3%) and the two neonatal ICUs (17 isolates, 11.3%) of Hippokration Hospital of Thessaloniki, Greece. The median age of the pediatric patients was 2 years (range 14 days–18 years), and that of the adult patients 66 years (range 19–92 years). The age groups of the patients are seen in Table 1.

Identification and antimicrobial susceptibility testing (AST) of *K. pneumoniae* isolates were performed on VITEK-2 (bioMérieux, Marcy-l'Étoile, France) using the GN ID and the AST 318 cards, respectively; the results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) [7]. The minimum inhibitory concentration (MIC) of colistin was determined by a commercially available broth micro-dilutions assay (ComASP Colistin, BMD-Liofilchem srl, Roseto, Italy). The susceptibility to tigecycline was evaluated according to breakpoints approved by US Food and Drug Administration (FDA) [8]. Susceptibility testing to ceftazidime-avibactam was performed using MIC test strips (BMD-Liofilchem srl).

Table 1. Age groups of critically ill patients included in the study

Patients (age range)	Age group (years)	*N* (%)
Neonates (1–28 days)		*17 (11.3)*
Infants/children (29 days–18 years)		*17 (11.3)*
	2–12	14 (9.3)
	13–18	3 (2.0)
Adults (19–92 years)		*116 (77.4)*
	19–45	21 (14.0)
	46–65	35 (23.4)
	66–80	42 (28.0)
	81–92	18 (12.0)

A multiplex lateral flow immunoassay (LFIA) (NG-Test CARBA 5, NG Biotech, France) for the rapid identification of NDM-, KPC-, IMP- and VIM-type and OXA-48-like carbapenemases was used. In addition, a multiplex PCR was used to detect the carbapenem resistance genes bla_{KPC}, bla_{NDM}, bla_{IMP}, and $bla_{OXA\text{-}48\text{-}like}$ [9]. The protocol was modified by adding primers for the detection of $bla_{VIM\text{-}1}$, described by Tsakris et al. [10].

A descriptive and inferential statistical analysis was performed using the statistical package SPSS v. 22.0. The Pearson's chi-squared test and Fisher's exact test were applied for the comparison of qualitative variables. The level of statistical significance was set at $P \leq 0.05$.

RESULTS

The resistance rates of CRKP isolates are seen in Table 2. The majority of adult patients (42/116 patients, 36.2%) belonged to 66–80-year-old age group. Among carbapenemases, KPC and NDM were the most frequently identified by multiplex PCR (37.3 and 36.0% respectively) (Table 3). The agreement between immunoassay and molecular method was 100%.

In general, bla_{NDM} was mainly detected in adults (54/116, 46.9%), while in neonates and children the most often detected gene was bla_{KPC} (24/34, 70.6%). A clear difference

Table 2. Antimicrobial resistance in the CRKP isolates

Antimicrobial	Resistance (N = 150, %)
Ceftazidime/avibactam	94 (62.7)
Imipenem	150 (100)
Meropenem	150 (100)
Amikacin	128 (85.3)
Gentamicin	119 (79.3)
Ampicillin/Sulbactam	150 (100)
Piperacillin/Tazobactam	150 (100)
Aztreonam	148 (98.7)
Cephalosporins	150 (100)
Ciprofloxacin	147 (98.0)
Levofloxacin	149 (99.3)
Fosfomycin	114 (76.0)
Tigecycline	49 (32.6)
Trimethoprim/Sulfamethoxazole	137 (91.3)
Colistin	105 (70.0)

Table 3. Carbapenemase prevalence in the CRKP isolates

Carbapenemase	*N* (%)
KPC	56 (37.3)
NDM	54 (36.0)
KPC+VIM	28 (18.6)
VIM	4 (2.7)
OXA+NDM	4 (2.7)
KPC+NDM	3 (2.0)
OXA-48-like	1 (0.7)
Total	150 (100)

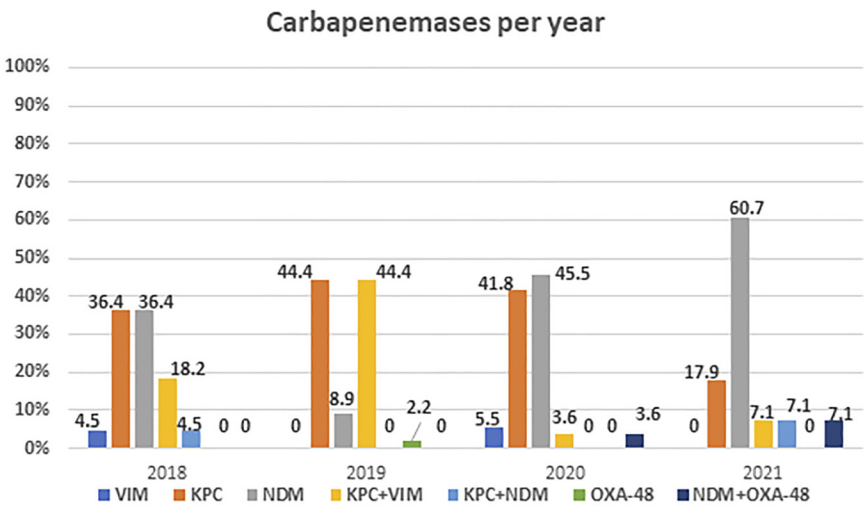

Fig. 1. Prevalence of carbapenemases in CRKP isolates of the study

was observed according to year of detection; the predominant resistance mechanism during 2018–2019 was the production of KPC alone or in combination with VIM, while a significant predominance of NDM production was seen during 2020–2021 ($P < 0.001$) (Fig. 1).

DISCUSSION

KPC-producing CRKP isolates are endemic in Greece for many years [11], while it is worth mentioning that the first KPC outbreak in Greece was recorded in October 2007 in the hospital of the current study [12]. In addition, CRKP strains producing concurrently KPC and VIM have been previously described in the adult ICU of the same hospital in 2013, though at much lower incidence (2/44, 4.5%) [13]. The accuracy of the multiplex LFIA for the detection of KPC and MBL was found high; this finding has been reported previously [14]. Of note, NDM-producing CRKP strains predominated during 2020–2021. Such strains were previously isolated in the adult ICU, but only sporadically [13]. In addition, NDM-producing CRKP were recently reported in a tertiary hospital in the city of Thessaloniki [15], while, CRKP isolates producing simultaneously NDM and other carbapenemases (e.g. OXA-48) have been reported in Greece [16]. The hypothesis of an NDM clone circulating in the Balkans has been recently re-inforced [17]. Despite the fact that NDM-producing CRKP outbreaks have been successfully handled, their virulence and ability to penetrate within the community is high [18]. Changes in the molecular epidemiology of carbapenem-resistant bacteria are common and call for continuous surveillance. Although colistin and tigecycline are considered the two "last resort" antimicrobials presenting significant antimicrobial action against CRKP isolates, their resistance rate is considerably high, raising concern for the future [19, 20]. In addition, pharmacokinetic characteristics of these antibiotics (ie. low blood levels of tigecycline or low cerebrospinal fluid levels of colistin) may prove them inadequate as therapy of specific infections. Changes in the patterns of resistance genes occur also in other Gram-negative bacteria, such as *Acinetobacter baumannii and Pseudomonas aeruginosa*. The prevalence of isolates producing KPC-, VIM-, IPM-, NDM- and OXA-48 carbapenemases is continuously increasing, limiting dramatically the therapeutic options [21–25].

The launch of ceftazidime/avibactam in 2018 has contributed significantly to the management of infections caused by KPC-producing CRKP isolates [26]. However, isolates resistant to ceftazidime/avibactam have emerged, either following antimicrobial exposure or not [27, 28]. The emergence of ceftazidime/avibactam resistant KPC-producing CRKP isolates, combined with the prevalence of NDM-producing strains during 2020–2021, which are by default resistant to ceftazidime/avibactam [27], could explain the increased rates of resistance presented in the current study. Another crucial factor favoring the rapid dissemination of the new resistance mechanism is globalization. Infections with KPC-, VIM-, OXA-48- and NDM-producing *Enterobacterales* in developed countries have been associated with visits and hospitalizations in endemic areas, such as the USA, Greece and Israel for KPCs, Greece for VIMs, Turkey for OXA-48, and the Indian subcontinent for NDMs [29–32].

Epidemiological surveillance and the proper use of antimicrobials could contribute to the control of infections caused by MDR strains. The introduction of novel antimicrobials, such as aztreonam/avibactam, plazomicin, eravacycline, temocillin, cefiderocol, meropenem/vaborbactam and imipenem/relebactam is promising [33, 34]. Strict implementation of infection control measures to combat the spread of MDR bacteria is imperative.

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