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Antimicrobial susceptibility and molecular epidemiology of carbapenem-resistant *Klebsiella pneumoniae* strains isolated in an emergency university hospital

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Abstract

Carbapenemase-producing Klebsiella pneumoniae (CPKP) is one of the important causes of nosocomial infection worldwide. A number of 61 CPKP strains isolated in 2016, from clinical specimens of individual patients in an emergency university hospital from Romania were evaluated by molecular methods. The most isolates of Klebsiella pneumoniae were sensitive to amikacin (77%), and fosfomycinum and nitrofurantoin (55% each).

OXA-48 was the most frequently identified genotype in 73,77% of cases (45 isolates), 13,11% (eight isolates) presented the combinations bla OXA-48 and bla NDM, 1,63 % (one isolate) presented blaOXA-48 and bla KPC, 8,19 % (five isolates) presented bla KPC, and in 3,27 %, (two isolates) was recorded bla NDM, all by use of the Xpert Carba-R Assay. The XbaI PFGE profile-based dendrogram of 20 K. pneumoniae strains selected from 61 strains extended to the 58% similarity level overall. However, the 88% criterion resolved 8 pulsotypes with 4 single-strain pulsotypes (D, E, F, and H) and 4 multiple-strain pulsotypes (A, B, C, and G). Our results confirm an epidemic expansion of a major OXA-48 positive clone in hospitals from Romania. The prudent use of antibiotics and the introduction of laboratory screening of patients for colonisation with carbapenem resistant bacteria will help to control the spread of these bacteria in the hospital.

Keywords: *Klebsiella pneumoniae;* antimicrobial susceptibility tests; carbapenemase gene families *KPC, NDM, VIM, IMP-1*, *OXA-48;* pulsed-field gel electrophoresis (PFGE)

1. Introduction

Carbapenemase-producing *Klebsiella pneumoniae* can cause clinical infections, (ventilator-associated pneumonia, urinary tract infections, sepsis, intra-abdominal infections, surgical wounds infections, (HIRAKATA & al. [1]), or asymptomatic colonization, especially in immunocompromised patients. The risk factors for the development of infection or colonization are lengthy hospitalization, use of broad spectrum cephalosporins or carbapenems, invasive procedures (mechanical ventilation, venous catheters), malignancy, diabetes (HUSSEIN & al. [2], YIGIT & al. [3]). Screening of high-risk patients to detect rectal colonization has been recommended as an infection control modality. Hospitalized patients infected or colonized with carbapenemase-producing bacteria should be placed on contact precautions and the standard measures must be implemented (hand hygiene, minimizing the use of invasive devices, and antimicrobial stewardship). In Romania, an epidemic expansion of a major OXA-48 positive clone which involves hospitals in different regions of the country has been reported between 2010-2014, (SZEKELY & al. [4]; GHEORGHE & al.[5]; LIXANDRU& al. [6]).

In this study we evaluated the antimicrobial susceptibility patterns and also the molecular epidemiology of 61 carbapenemase-producing *Klebsiella pneumoniae* strains isolated in 2016 from clinical specimens of individual patients in an emergency university hospital from Romania.

2. Material and Methods

Bacterial isolates and antimicrobial susceptibility tests

A number of 61 *Klebsiella pneumoniae* strains were isolated from various clinical specimens collected from individual patients, admitted during 2016 in an emergency university hospital. The isolates investigated were obtained from samples originating from lower respiratory tract (n = 20), urine (n = 30), blood (n = 4), peritoneum (n = 1), wound (n = 5), sputum (n = 1) from patients admitted in the Intensive Care Unit (32 isolates), General Surgery (4 isolates), Internal Medicine (3 isolates), Gastroenterology (2 isolates), Neurology (12 isolates), Nephrology (2 isolates), Neurosurgery (1 isolate), Hematology (1 isolate), Orthopedics (1 isolate), Emergency Unit (1 isolate), Cardiovascular Surgery (2 isolates). The isolates were tested as carbapenem- resistant bacteria by Kirby - Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute guideline, 2016, CLSI [7] and supplementary MICs were determined using the MicroScan system (Beckman Coulter, USA), in the laboratory of the Emergency University Hospital, Bucharest, Romania.

The Kirby-Bauer disk diffusion method was performed using the following antibiotics: ampicillin (10µg), amoxicillin–clavulanat (20/10µg), piperacillin (100µg), piperacillin-tazobactam (100/10µg), cefuroxime (30 µg), cefotaxime (30µg) ceftazidime (30µg), cefepime (30µg), ertapenem (10µg), meropenem (10µg), imipenem (10µg), gentamicin (10µg), tobramycin (10µg), amikacin (30µg), ciprofloxacin (5µg), levofloxacin (5µg), norfloxacin (10µg), trimethoprim-sulfamethoxazole (1.25/23.75µg), nitrofurantoin (300µg). Ertapenem, imipenem and meropenem disks were used in phenotypic tests for carbapenemase production detection. Usually, carbapenemase-producing *Klebsiella pneumoniae* isolates are intermediate or resistant to one or more carbapenems using the current interpretive breakpoints. Ertapenem non-susceptibility is the most sensitive indicator for carbapenemase production along with resistance to one or more third subclass of cephalosporins (eg, cefotaxime, ceftazidime, ceftriaxone). The MICs for ertapenem, imipenem obtained with MicroScan system were compared with interpretive criteria for carbapenems described in CLSI 2016. Isolates with imipenem and meropenem MICs of 2-4 µg/ml or above, or ertapenem MIC 2 µg/ml are possibly carbapenemase producers and were characterized with molecular assays.

Molecular investigations

Detection and differentiation among the most prevalent and clinically relevant five carbapenemase gene families *KPC, NDM, VIM, IMP-1* and *OXA-48* was performed to confirm the results of phenotypic assays concerning the carbapenemases production. The molecular profiles of 61 carbapenem non-susceptible isolates in pure culture from various clinical samples were evaluated by real-time polymerase chain reaction, with the Xpert Carba-R Assay (Cepheid, USA). This assay is also used in hospitals as a qualitative *in vitro* diagnostic test for detection of patients at high risk for intestinal colonization with carbapenem - non-susceptible Gram-negative bacteria prior or during the admission, as an epidemiological measure to control the spread of this microorganisms, for the *KPC, NDM, VIM, OXA-48*, and *IMP-1* gene sequences.

20 isolates of *K. pneumoniae* were sent to Cantacuzino National Institute of Research for genome macrorestriction fingerprinting tests, in order to analyze the relatedness of these isolates. The genetic relationship between 20 *K. pneumoniae* isolates was determined by

pulsed-field gel electrophoresis (PFGE) after total chromosomal DNA digestion with *XbaI*. The PFGE protocol used was based on the PulseNet 1-day standardized PFGE protocol for *Escherichia coli* O157:H7, Salmonella, and *Shigella*, *(*RIBOT & al. [8]). PFGE was carried out with a CHEF Mapper system (Biorad, Hercules, California, U.S.A.) and the following running conditions: 6 V/cm for 19 h at 14°C, with the pulse time ramped linearly from 2.16 s to 54.17 s. *Salmonella* serotype Braenderup H9812 was used as a DNA size marker, as recommended by PulseNet (HUNTER & al. [9]). PFGE profiles were analysed with BioNumerics v. 6.6 software (Applied Maths, Kortrijk, Belgium) and a dendrogram was generated using the unweighted pair-group method with arithmetic averages (UPGMA) algorithm. The Dice similarity coefficient was used to analyze the similarities of the banding patterns with a band tolerance setting of 1.5 %. Pulsotypes designations (e.g. A, B, etc.) were assigned at the ≥ 88% profile similarity level, corresponding to an approximately 4-band difference.

3. Results and discussions

The most isolates of *Klebsiella pneumoniae* were sensitive to amikacin (n=47), fosfomycinum (n=34), nitrofurantoin (n=34), norfloxacin (n=31), tetracycline (n=22), tobramycin (n= 22), trimethoprim-sulfamethoxazole (n=16). *OXA-48* was the most frequently identified genotype in 73,77% of cases (45 isolates), 13,11% (eight isolates) presented the combinations *bla OXA-48* and *bla NDM*, 1,63 %, (one isolate) presented *blaOXA-48* and *bla KPC*, 8,19 % (five isolates) presented *bla KPC*, and in 3,27 % (two isolates) was recorded *bla NDM*, all by use of the Xpert Carba-R Assay (Tab.1) .

The *Xba*I PFGE profile-based dendrogram of the *K. pneumoniae* strains extended to the 58% similarity level overall. However, the 88% criterion resolved 8 pulsotypes with 4 single-strain pulsotypes (i.e. D, E, F, and H) and 4 multiple-strain pulsotypes (i.e. A, B, C, and G) (Fig. 1). Two of the latter (i.e. A and G) contained clusters of 2 and 4 strains, respectively, with indistinguishable profiles. While the pulsotype A cluster comprised strains recovered from blood and wound secretion of patients hospitalized in different units, three of the pulsotype H cluster strains, originating from urine specimens and tracheal secretion, were isolated from patients hospitalized in the same unit.

Within each of the multiple-strain pulsotypes, the strains shared the same carbapenemase gene. Specifically, membership in A, C, and G pulsotypes was confined to OXA-48 producers and NDM-positive strains were associated with pulsotype B.

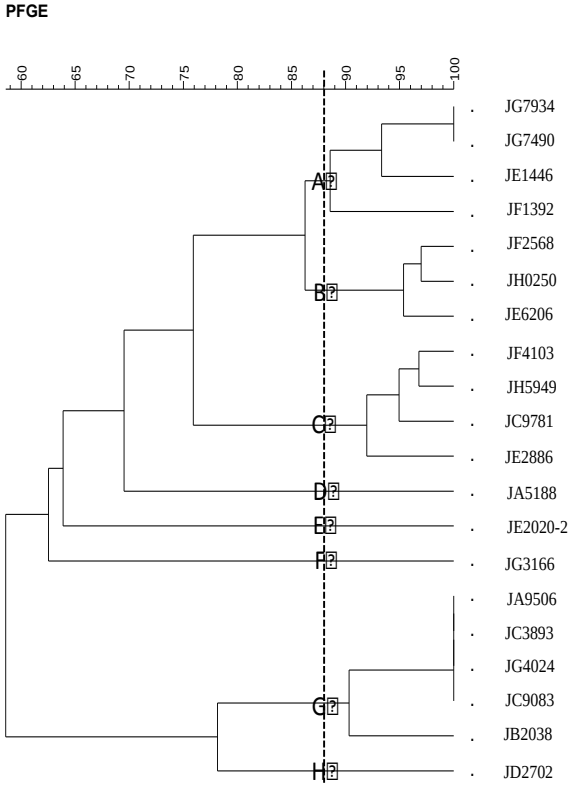
Clinical importance have carbapenemases from classes A, B (metallo-beta-lactamases MBLs), and D. From the class A beta-lactamases, *Klebsiella pneumoniae* carbapenemase (*KPC*) was first detected in North Carolina, USA in 1996 and it was later identified in outbreaks in USA, European countries (HOENIGL & al. [10]), South America (NORDMANN & al. [11]), Asia (WEI & al. [12]), and Australia (CHANG & al.[13]). Metallo-beta-lactamases were initially described in Japan in 1991 (WATANABE & al. [14]), and they require the presence of zinc for hydrolysis of beta-lactams (*IMP-1* imipenemase, *VIM* - verona integron metallo-beta-lactamase, *NDM* - New Delhi metallo-beta-lactamase). *NDM* was first described in December 2009 in a Swedish patient hospitalized in India with an infection due to *K. pneumoniae* (YIGIT& al. [15]; YONG & al. [16]). *OXA-48* type enzymes from class D beta-lactamases have the ability to hydrolyze oxacillin, and the first isolate of *K. pneumoniae* with *OXA-48* was identified in Turkey in 2001 (POIREL & al. [17]). Since then, it has been identified in the United States, Europe, and Africa.

Table 1. Resistance genes for isolates of *K. pneumoniae*

LRT (n=20) 19 ICU, 1 NEU	Urine (n=30) 7 ICU, 3 IMED, 2 GEN, 11 NEU, 1 NEF, 2 GSU, 1 NEUS, 1 HEM, 1ORT, 1 EU	Blood (n=4) 3ICU, 1 GSU	Peritoneum (n=1) 1 ICU	Wound (n=5) 2 ICU, 1GSU, 2 HSU	Sputum (n=1) 1 NEF
OXA 48, n=16 KPC, n=1 NDM, n=-1 OXA48-NDM, n=1 OXA48-KPC, n= 1	OXA48, n=26 KPC, n=2 OXA48-NDM, n=1 NDM=1	OXA48, n=2 KPC, n=1 OXA48-NDM, n=1	KPC, n=1	OXA48, n=1 OXA48-NDM, **n**=4	OXA48-NDM, n=1

LRT(lower respiratory tract) , EU (Emergency unit), GEN (Gastroenterology), GSU (General surgery), GY (Gynecology), HSU (Heart surgery), HEM (Hematology), ICU (Intensive Care Unit), IMED (Int. Medicine), NEF (Nephrology), NEU (Neurology), NEUS (Neurosurgery), ORT (Orthopedics)

Figure 1 – Pulsed-field gel electrophoresis (PFGE) results



ID No.	Sample/Department/ Genes detected
JG7934	Wound / HSU / OXA 48
JG7490	Blood / ICU / NDM +OXA48
JE1446	Urine/ ICU/ OXA 48
JF1392	Urine /ORT/ OXA 48
JF2568	Urine / IMED / NDM
JH0250	LRT/ ICU / NDM
JE6206	LRT/ ICU / OXA 48
JF4103	Urine / ICU/ OXA 48
JH5949	LRT/ ICU/ OXA 48
JC9781	LRT/ ICU/ OXA 48
JE2886	LRT/ ICU/ OXA 48
JA5188	Blood/ HSU /KPC
JE2020	LRT/ ICU/ OXA 48
JG3166	Urine/ GSU/ NDM
JA9506	Urine /NEU / OXA 48
JC3893	LTR/ NEU / OXA 48
JG4024	Urine / NEU / OXA 48
JC9083	LTR/ ICU/ OXA 48
JB2038	LTR/ ICU/ OXA 48
JD2702	Urine/ ICU/ OXA 48

4. Conclusions

The carbapenem resistance genes of *K. pneumoniae* most frequently detected in our study were *OXA 48* - 73,77% of cases (45 isolates), while 13,11%, (eight isolates) presented the combinations *bla OXA-48* and *bla NDM.* Our results confirm an epidemic expansion of a major *OXA-48* positive clone in hospitals from Romania.
The prudent use of antibiotics and the introduction of laboratory screening of patients for colonisation with carbapenem resistant bacteria will help to control the spread of these bacteria in the hospital.

Conflict of interest: No conflict of interest declared

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