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Research Article

Phenotypic detection of Carbapenem-resistant *Klebsiella pneumoniae* strains isolated from blood cultures

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Abstract. Objective: The objectives of the present study were to describe and compare the results obtained by two phenotypic methods (the modified carbapenem inactivation method and the combination disk test) of carbapenem-resistant *Klebsiella pneumoniae* (CR-KP) isolated from blood cultures in hospitalized patients with invasive infections. Material and Method: Between October 2016 and September 2017, 2032 pairs of blood cultures compatible with the BACT/ALERT® 3D automated microbial detection systems were collected (bioMérieux, Inc., Durham, NC). Based on the minimal inhibitory concentration (MIC) values obtained for Ertapenem, Imipenem and Meropenem, we selected 18 carbapenem non-susceptible *Klebsiella pneumoniae* (KP) strains, according to EUCAST standards criteria (2016-2017). Isolates identification and antibiotic susceptibility testing (AST) were performed with the VITEK 2® COMPACT (bioMérieux, Inc., Durham, NC). The modified carbapenem inactivation method (mCIM) was the phenotypic test performed to highlight the carbapenemase-producers. We also performed the combination disks test (CDT), a phenotypic test designed for qualitative *in vitro* detection of carbapenem microbial resistance mechanisms. Results: We considered 18 non-duplicate KP strains that fulfilled the Meropenem (MRP) inclusion criterion (MIC≥ 0.5 µg ml-1). All KP strains showed high-resistance to Ertapenem (ERT) (MIC≥8 µg ml-1). The MIC's distribution values at MRP were: three isolates had MIC 2 µg ml-1, one with MIC 4 µg ml-1 and 14 isolates ≥16 µg ml-1. The MIC's at Imipenem (IMI) were: two isolates- 2 µg ml-1; two isolates- 4 µg ml-1, two isolates 8 µg ml-1 and 12 isolates ≥16 µg ml-1. 11 out of 18 strains showed high-resistance to all three carbapenems tested (MIC≥16 µg ml-1). The mCIM assay showed that 18 strains were positive, suggesting that all the investigated strains were carbapenemase-producers. We obtained the following results by CDT: seven (38.89%) out of 18 were KPC-producers,10 (55.56%) out of 18 were temocillin not-susceptible and showed no synergy between meropenem and meropenem + inhibitors, suggesting the OXA-48 production. One isolate (5.55%), showed a noninterpretable profile. Conclusions: To this day there is insufficient information in Romania regarding the circulation of CR-KP by geographical areas and at a national level. Our results showed that in our area the most CR-KP are OXA-48-like and KPC producers.
This data confirmed the observation that carbapenem-MICs distribution signaled a resistant phenotype which had to be confirmed with another method. We noticed a connection between the low-level resistance of carbapenem and OXA-48 KP producers. Because of that, we recommend to test all KP that are not wild-type profile by a phenotypic method. Our results suggested that performing two different phenotypic assays will improve the performance of lab CR-KP detection.

Key Words: carbapenem-resistant *Klebsiella pneumoniae* (CR-KP), minimum inhibitory concentration (MIC), carbapenem resistance (CR), the modified carbapenem inactivation method (mCIM), the combination disk test (CDT)

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Introduction

In the last few years, the importance of microbiological diagnosis has increased secondary to the detection of multidrug resistant *Enterobacteriaceae* and especially CR-KP strains. Thus, detection with simple and inexpensive methods has become a priority. The increasing incidence of CR-KP infections represents a public health threat. Multidrug resistance is a problem concerning the administration and effectiveness of antibiotic therapy.
As stated by P. Nordmann and collaborators, β-lactamases that are capable of hydrolyzing carbapenems are also capable to inactivate all beta-lactams. According to these authors, carbapenem resistance may be mediated by the carbapenemases production or by the decrease in membrane permeability associated with the increased expression of an enzyme with low activity on carbapenems. These authors consider that knowing and controlling the circulation of microorganisms that are carbapenemase producers is important because CR genes can be easily transmitted through mobile genetic elements between microorganisms (Nordmann et al 2012).
Ambler and K. Bush have classified β-lactamases based on their functional and molecular characteristics. A functional classification proposed by Bush in 1988, divided β-lactamases into four groups. The 2nd functional group has many subgroups defined according to their specific substrate or inhibitor profile. Groups 2f

and 3 are comprised of carbapenemases. According to Queenan and Bush, the molecular classification divided β-lactamases in classes. Class A and D include carbapenemases with serine at their active site (eg. KPC, IMI, SME, GES, OXA enzymes). Class B metallo-β-lactamases enzymes (eg. VIM, IMP, GIM, SIM) have the ability to hydrolyze carbapenems, but not Aztreonam. They have Zn 2+ in their active site. The hydrolysis mechanism can be blocked by EDTA or other chelators. They can't be inhibited by Clavulanic Acid and Tazobactam. *Klebsiella pneumonae* has the ability to gain and spread determinants of antibiotic resistance. The policy of infection control and antibiotic therapy for this microorganism is difficult to apply because of its multidrug resistance (Queenan & Bush 2007).

The objectives of this study were to analyze the antibiotic sensitivity profiles of carbapenem-resistant *Klebsiella pneumoniae* isolated from blood cultures in patients with invasive infections and to describe and compare their carbapenem resistance profile by two phenotypic methods (mCIM and CDT).

Material and method

The blood samples were collected from patients hospitalized in the Regional Institute of Gastroenterology and Hepatology (IRGH), Cluj-Napoca, Romania, between October 2016 and September 2017. A number of non-duplicated 2032 pairs of blood cultures were analyzed with the compatible BACT/ALERT® 3D automated microbial detection system (bioMérieux, Inc., Durham, NC). This system used colorimetric technology for reliable detection of microorganisms. This retrospective study was approved in its entirety by the IRGH ethics committee. The included patients in this study gave their informed consent. Isolates identification and antibiotic susceptibility testing were performed with the Vitek 2 Compact System (bioMérieux, Inc., Durham, NC) with ready-to-use VITEK® 2 ID/AST cards.

The Advanced Expert System ™ highlighted CR-suspected KP strains based on MIC values, interpreted according to EUCAST standards criteria 2016-2017. (European Committee on Antimicrobial 2016-2017).

Isolates with MIC values ≥0.5 µg ml-1 for Meropenem (MRP) were further tested to confirm CR by carbapenemase production with two phenotypic methods: CDT (Rosco Diagnostica A/S, Taastrup, Denmark) and mCIM.

1. The modified carbapenem inactivation method

The technical procedure and interpretation of mCIM results are described in the CLSI standards (Dolinsky, 2017). This method is recommended for the control of nosocomial infections and for epidemiological purposes.

Principle: Carbapenemases inactivate the MRP from the disk by hydrolysis (disk 10 µg, Oxoid Ltd, Basingstoke,UK).

The MRP disk is immersed in a dense suspension of the suspected CR strain. We incubated it for 4 hours at 35-37°C. The disk was extracted from the microbial suspension and placed on the surface of a plate with Mueller-Hinton agar previously inoculated with Escherichia coli ATCC 25922 (carbapenem susceptible control strain). After 18-24 hours of incubation, the inhibition diameter zones were analyzed according to CLSI guidelines, as follows:

Carbapenemase negative: the inhibition area around the MRP disk ≥19 mm, meaning that the investigated strain doesn't produce carbapenemase, the carbapenem from the disk is not hydrolyzed and will inhibit the multiplication of *E. coli* ATCC 25922.

Carbapenemase positive: the inhibition area around the disk of MRP 6-15 mm, meaning that the strain produces carbapenemase, the carbapenem from the disk has been hydrolyzed, the multiplication of *E. coli* ATCC 25922 is not inhibited. An inhibition zone of 16-18 mm is also accepted (with pinpoint colonies in the inhibition area).

Carbapenemase indeterminate: the inhibition zone diameter of 16-18 mm or ≥19 mm (with pinpoint colonies in the area).

Quality control was within the CLSI 2017 standard criteria.

2. The combination disk test

CDT is a phenotypic qualitative in vitro method used to confirm Enterobacteriaceae CR-mechanisms. CDT detects the classes A, B and D carbapenemases (eg. KPC, MBL and OXA-48) by the agar diffusion method.

There were five tablets:

1. Meropenem 10 µg (MRP10), alone
2. Meropenem 10 µg + Boronic Acid (MRPBO); Boronic Acid is a KPC inhibitor.
3. Meropenem 10 µg + Cloxacilin (MRPCX); Cloxacilin is an AmpC inhibitor.
4. Meropenem 10 µg + Dipicolinic Acid (MRPDP); Dipicolinic Acid is a Metallo-ß-lactamase (MßL) inhibitor.
5. Temocillin 30 µg (T); it is not a specific inhibitor but it has been shown that the OXA-48 enzyme confers a high level of resistance to temocillin.

We interpreted our results according to the manufacturer's instructions (Rosco Diagnostica A / S, Taastrup, Denmark, see table 1) (ROSCO, 2017).

3. Statistical analysis

Data were summarized using descriptive measures as absolute or relative frequencies (%) and graphical representations as dot plot. Statistical analysis was performed in R software, version 4.0.0 (reference: R Core Team (2020) (A language and environment for statistical computing, Vienna, Austria).

Table 1. Interpretation of the difference between the inhibition zones diameters

The inhibition zones diameters (mm)					
Meropenem disk	**MRPBO**	**MRPCX**	**MRPDP**	**Temocillin - inhibition zone diameter**	**Interpretation**
MRP 10 µg	≥ 4	≥5	≤3	≥12	**AmpC+porin loss**
MRP 10 µg	**≥4**	≤3	≤3	≥12	**KPC**
MRP 10 µg	<4	≤3	**≥5**	≥12	**MßL**
MRP 10 µg	≤3	≤3	≤3	≤ 12	**OXA-48 like**

Table 2. MIC values for 18 CR-KP isolates (µg ml-1)

No of strain of CR-KP	MIC values: µg ml-1								
	ATM	**MRP**	**IMI**	**ERT**	**FEP**	**CAZ**	**CTX**	**TZP**	**AMC**
KP1	≥64	2	2	≥8	32	≥64	≥64	≥128	≥32
KP2	≥64	≥16	≥16	≥8	≥64	≥64	≥64	≥128	≥32
KP3	≥64	≥16	4	≥8	≥64	≥64	≥64	≥128	≥32
KP4	≥64	≥16	8	≥8	≥64	≥64	≥64	≥128	≥32
KP5	≥64	≥16	≥16	≥8	≥64	≥64	≥64	≥128	≥32
KP6	≥64	≥16	≥16	≥8	≥64	≥64	≥64	≥128	≥32
KP7	≥64	≥16	4	≥8	≥64	≥64	≥64	≥128	≥32
KP8	≥64	≥16	≥16	≥8	≥64	≥64	≥64	≥128	≥32
KP9	≥64	≥16	≥16	≥8	≥64	≥64	≥64	≥128	≥32
KP10	≥64	2	2	≥8	≥64	≥64	≥64	≥128	≥32
KP11	≥64	≥16	≥16	≥8	≥64	≥64	≥64	≥128	≥32
KP12	≥64	≥16	≥16	≥8	≥64	≥64	≥64	≥128	≥32
KP13	≥64	≥16	≥16	≥8	≥64	≥64	≥64	≥128	≥32
KP14	≥64	4	8	≥8	≥64	≥64	≥64	≥128	≥32
KP15	≥64	≥16	≥16	≥8	≥64	≥64	≥64	≥128	≥32
KP16	≥64	2	≥16	≥8	≥64	≥64	≥64	≥128	≥32
KP17	≥64	≥16	≥16	≥8	≥64	≥64	≥64	≥128	≥32
KP18	≥64	≥16	≥16	≥8	≥64	≥64	≥64	≥128	≥32

ATM = aztreonam; MRP = meropenem; IMI = imipenem; ERT = ertapenem; FEP = cefepim; CAZ = ceftazidime; CTX = cefotaxime; TZP = piperacillin-tazobactam; AMC = amoxicillin-clavulanic acid

Table 3. Results of the modified carbapenem inhibition test and the disk combination test; MIC values for three carbapenems (meropenem, imipenem and ertapenem)

No of strain of CR-KP	MIC_{MRP}	MIC_{IMI}	MIC_{ERT}	mCIM	CDT	TEM (mm)
KP1	2	2	≥8	**6**	**NI**	17
KP2	≥16	≥16	≥8	**6**	**KPC**	17
KP3	≥16	4	≥8	**6**	**OXA**	9
KP4	≥16	8	≥8	**6**	**OXA**	9
KP5	≥16	≥16	≥8	**6**	**OXA**	9
KP6	≥16	≥16	≥8	**6**	**KPC**	9
KP7	≥16	4	≥8	**6**	**KPC**	9
KP8	≥16	≥16	≥8	**6**	**OXA**	9
KP9	≥16	≥16	≥8	**6**	**OXA**	9
KP10	2	2	≥8	**6**	**OXA**	9
KP11	≥16	≥16	≥8	**6**	**OXA**	9
KP12	≥16	≥16	≥8	**6**	**KPC**	24
KP13	≥16	≥16	≥8	**6**	**KPC**	19
KP14	4	8	≥8	**6**	**OXA**	10
KP15	≥16	≥16	≥8	**6**	**KPC**	16
KP16	2	≥16	≥8	**16**	**OXA**	9
KP17	≥16	≥16	≥8	**6**	**OXA**	9
KP18	≥16	≥16	≥8	**6**	**KPC**	16

MRP = meropenem; IMI = imipenem; ERT= ertapenem, TEM: the inhibition zone diameter around the temocillin disc (mm); CDT: the disk combination test establishes the type of synergy between meropenem and meropenem + inhibitors: KPC-strain that produces carbapenemases of class A (eg KPC), OXA-lack of synergy between meropenem and meropenem + inhibitors; mCIM column: the inhibition zones (mm) around meropenem disc; NI: noninterpretable test.

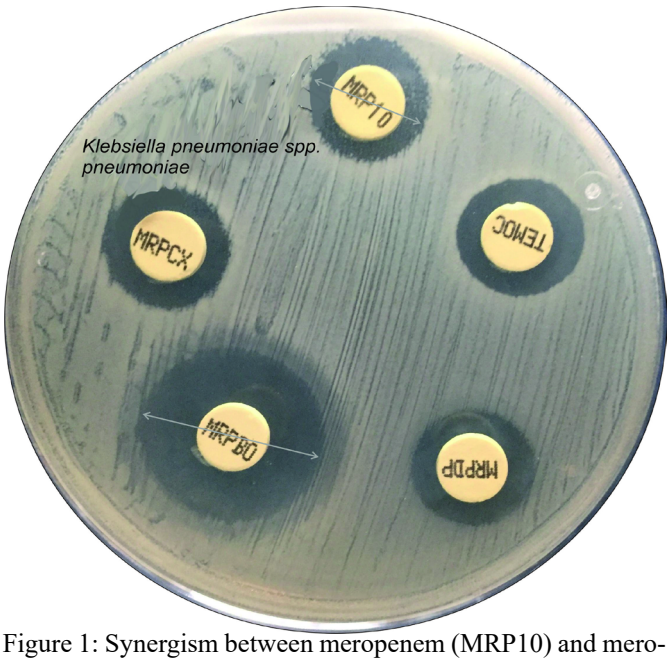

Figure 1: Synergism between meropenem (MRP10) and meropenem + boronic acid (MRPBO). The difference between the inhibition zones diameters is more than 4 mm. The isolate is probably a KPC producer.

Results

We studied 303 (14.91%) positive blood cultures (without duplicates) from 2032 pairs of blood cultures collected consecutively, between September 2016 and September 2017. We identified 153 (50.49%) Gram negative bacilli (BGN) out of which 111 (36.63%) were *Enterobacteriaceae* members. 47 strains were *Klebsiella pneumoniae* (KP) (data partially published at the 11th National Conference of Microbiology and Epidemiology, Sibiu, November 8-10, 2018. Poster)

We analyzed 18 CR-KP not-repeat strains (inclusion criterion: Meropenem MIC ≥ 0.5 µg ml-1), considering the MIC values distribution for different classes of antibiotics (see Table 2) and we tested them with two phenotypic methods to detect and establish the CR mechanism.

The analyzed MIC values for beta lactam antibiotics showed the following:

1. Cephalosporins generation III and IV (Ceftazidime, Cefoperazone, Cefepim); all the strains (18) were resistant (R) with MIC≥64 µg ml-1

2. β-lactams with β lactamase inhibitors (Amoxicillin / Clavulanic Acid and Piperacillin / Tazobactam), all strains were R/ with MICs ≥32 µg ml-1 and ≥128 µg ml-1, respectively)

3. Aztreonam: all strains were R, MIC ≥64 µg ml-1.

4.Carbapenems:

a) Meropenem: 14 (77.8%) strains were R with (MIC ≥16 µg ml-1) and 4 (22.2%) IS with (MIC 2-4 µg ml-1);

b) Ertapenem: all strains were R (MIC ≥8 µg ml-1);

c) Imipemem: 12 (66.7%) strains were R (MIC ≥16 µg ml-1) and 6 (33.3) IS (MIC 2-8 µg ml-1);

The mCIM assay showed the following results: all strains (18) were positive, the inhibition zone around the meropenem disk being 6 mm for 17 isolates and 16 mm for one KP. In conclusion, all the investigated strains (100%) have CR by producing carbapenemases, enzymes that hydrolyzed the meropenem from the disc (see table 3, mCIM column)

The CDT results interpretation followed the manufacturer's instructions. The obtained results are listed in table 3.

Thus, seven (38.89%) out of 18 strains produce the KPC enzyme, showing synergism between meropenem and meropenem - boronic acid. Boronic acid inhibits the KPC enzyme, a serum carbapenemase class A (see figure 1). Out of all the strains, 10 (55.56%) do not show synergy between meropenem and meropenem + inhibitors and the inhibition zone around temocillin disk was 9 mm diameter. This profile suggests the OXA-48 carbapenemases production, a class D member. We considered the temocillin inhibition zone only if there was no synergy between meropenem and meropenem + inhibitors. One isolate (5.55%), K1, showed a noninterpretable profile (see figure 2 for a CDT results image).

Discussion

Because the morbidity and mortality from KP-CR infections is increasing, it is imperative to establish rapid, robust and reproducible methods of microbiological diagnosis regarding the detection of these strains and the effective control of their spread knowing their regional circulation.

The widespread use of carbapenem-type reserve antibiotics in antibiotic therapy has led to carbapenem-resistance, first in *P. aeruginosa and Acinetobacter spp*, and later in *Enterobacteriaceae* (van der Zwaluw et al 2015).

Currently, ß-lactamases are widespread in almost all Gram-negative pathogenic species, being responsible for therapeutic failure, due to ß-lactam resistance (Pfeifer et al 2010).

The extended resistance to other classes of antibiotics (Aminoglycosides, Fluoroquinolones and Trimethoprim/Sulfamethoxazole), an aspect also found on our analyzed strains (data not shown), dramatically limits the therapeutic options (Giske et al 2011).

Resistance to carbapenems is mediated mainly by hydrolytic enzymes. Other mechanisms leading to the same effect are: production of β-Lactamase type AmpC (chromosomal or acquired)/ESBLs combined with porin mutation, antibiotic efflux system and alteration of penicillin binding proteins (PBPs) (Aguirre-Quiñonero & Martínez-Martínez 2017).

Some enzymes hydrolyze carbapenems weakly, with MICs below the accepted limit for resistance and sometimes below the proposed limit for screening breakpoints. Some authors have noted the usefulness of the combination of temocillin (≥12 mm) and Piperacillin / Tazobactam (≥ 16 mm) in the detection of OXA-48 (Aguirre-Quiñonero & Martínez-Martínez 2017) (Martínez-Martínez 2019).

OXA-48 carbapenemases hydrolyze penicillins (high-level) and carbapenems (low-level), they are not susceptible to β-lactamase inhibitors and do not hydrolyze broad-spectrum cephalosporins. If membrane modified porin is associated, resistance to carbapenems may be high (Poirel et al 2012; Codjoe & Donkor 2017).

It is demonstrated that the genetic structure carrying the gene encoding OXA-48 is easily transferable between bacterial species. According to the EUCAST guidelines, carbapenemases confer resistance to all ß-lactams, being different in terms of the degree of expression, structure and action on ß-lactams. These enzymes have been classified into serine carbapenemases and

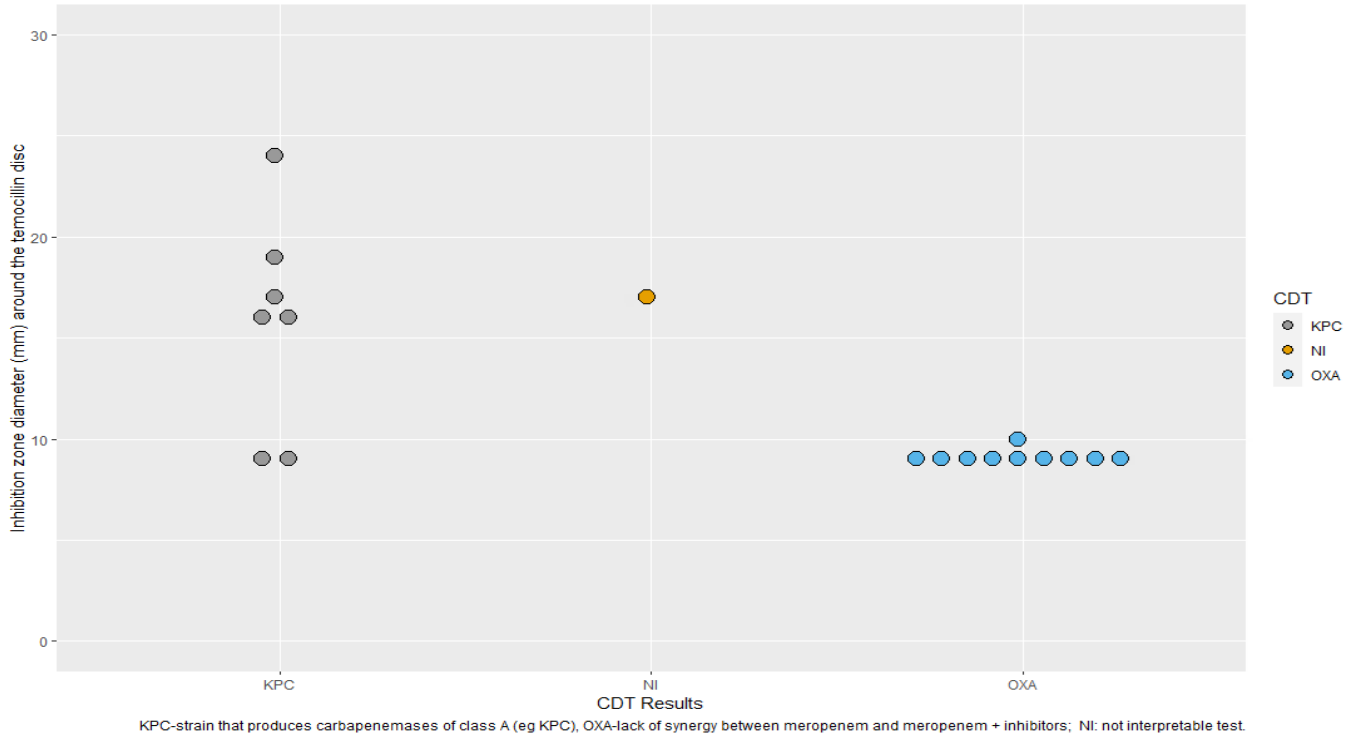

Figure 2. Statistical Analysis - Temocillin disk zone diameters (mm) stratified by CDT results

metallo-beta-lactamases, considering their structure and action mode (Skov & Skov 2012).

The CDT uses Temocillin, a synthetic β-lactam, stable in the presence of ESBL and AmpC. This is not an absolute marker for *in vitro* OXA-48 detection. It has been found that, a Temocillin increased MIC associated with third-generation cephalosporin resistance may be suggestive of OXA-48 presence (Bakthavatchalam et al 2016).

It is important for epidemiological purposes to detect and limit the rapid spread of OXA-48 producing bacteria. The antibiotic sensitivity profile does not always reveal CR, especially low-level resistance, and therefore, their detection becomes a challenge for the laboratory.

KP strains with Imipenem or Meropenem sensitivity but with Ertapenem resistance represent a big problem regarding their true resistance to carbapenems. Our study confirms that Ertapenem sensitivity can be used to detect CR suspected strains.

We recommend to check the carbapenemases production for all KP isolates that are resistant to Ertapenem, even if their phenotype is IS to Meropenem and / or Imipenem, Aztreonam or cephalosporins generations 3 and 4. Some authors consider that the detection based only on the CMI value for Ertapenem has poor specificity (Nordmann et al 2012).

EUCAST guidelines version 2.0, July 2017, consider Ertapenem as having high sensitivity but low specificity because an isolate with ESBLs and AmpC can be Ertapenem-resistant. That is why we recommend to test all *Kebsiella pneumoniae*, except the wild-type profile ones, at least with the mCIM in order to detect carbapenemase-producing strains with low-expressed resistance (European Committee on Antimicrobial 2017).

The Advanced Expert System (AES Vitek 2 Compact, bioMérieux, Inc., Durham, NC) does not differentiate between enzymatic CR and CR by other mechanisms. We did not record false positive results regarding the phenotype reported by Vitek2 Compact. For efficiency, we used AST-cards that test at least two carbapenems (ERT and IMI / MRP). We noticed that, testing only one carbapenem is not enough (false negative results are possible if Meropenem has CMI ≤ 0.5 µg ml-1). Also, the inadequate inoculum size (smaller than recommended) leads to a false sensitivity result.

In our study, all strains showed high resistance to Ertapenem (MIC ≥8 µg ml-1). 11 strains out of 18 KP, showed high resistance to all carbapenems tested. Six out of 11 KP strains were KPC (a serine carbapenemase class A) and five were OXA-48 (a serine carbapenemase class D) producers.

Three KP strains (KP no.1, 10 and 14) showed low level resistance (IS profile) to MRP and IMI, two of them with OXA-48 profile (KP no.10 and 14) and one with a noninterpretable profile (KP no.1). Three isolates (KP no. 3, 4, 7) presented low level resistance only to IMI, but just two strains among them were OXA-48 producers (KP no. 3, 4).

Six isolates were KPC producers and all of them showed high resistance to all the carbapenems tested (KP no. 2, 6, 12, 13, 15, 18).

Ten isolates were OXA-48 like producers but only five of them were resistant to all the tested carbapenems (KP no. 5, 8, 9, 11, 17). This data confirmed the observation that MICs signaled a resistant phenotype concerning carbapenems which has to be confirmed with another method. We noticed a connection between low-level carbapenem resistance and OXA-48 KP producers for some isolates.

Interestingly, KP no. 1 has a special profile. KP no. 1 is a carbapenemase-producing strain, as demonstrated by mMIC test. The disk combination test did not determine the type of hydrolytic enzyme for this strain. We can speculate that it is CR through a complex mechanism, which also associates a carbapenemase.

We noticed that the disk phenotypic test cannot always determine the carbapenemase type, even if it highlights a possible carbapenem-resistance by the enzymatic mechanism. The KP strains no. 1, 10 and 14 had an IS profile to MRP, while IMI, KP10 and KP14 presented a positive OXA-48 profile at the CDT assay.
According to Aguirre-Quiñonero (2017), the sensitivity for CDT was 92.1% and the specificity 82.5% (Aguirre-Quiñonero & Martínez-Martínez 2017). For the identification of classes A, B and D, other authors reported a specificity of 95%, 90% and 100%, respectively, with a sensitivity of 96-100%, concluding that this test is a solid phenotypic confirmation for *Enterobacteriaceae* (van Dijk et al 2014). The lack of synergy between Meropenem and Meropenem + BA / DPA with an inhibition zone of Temocillin ≤ 10 mm identified OXA-48 producers with 100% sensitivity and specificity (Bakthavatchalam et al 2016).
Pierce et al (2017) found a sensitivity of 99-100% with a specificity of 100% regarding the mCIM (Pierce et al 2017). The test is cheap, reproducible, simple to perform and to interpret. It does however require an incubation time. The interpretation of the test cannot be done before 8-10 hours and the test confirmation is achieved after 24 hours of incubation time.
EUCAST and CLSI standards describe several phenotypic methods for detecting carbapenemase production. EUCAST Version 2.01 July 2017 establishes a clinical breakpoint for Meropenem (≤ 2µg ml-1) and Ertapenem (≤ 0.5 µg ml-1) as well as a cut-off screening ≤ 0.125 µg ml-1 for both carbapenems.
Some authors consider that none of the phenotypic tests in use is sufficiently sensitive to detect OXA-48 like carbapenemase-producing isolates (class D). High resistance to Temocillin (MIC > 64 mg L-1) and Piperacillin-Tazobactam found in Enterobacteriaceae associated with an R or IS profile for at least one carbapenem may be suggestive of OXA-48 production (Hrabák et al 2014).
Limits: Our retrospective study should be regarded as a pilot one. It used descriptive statistical methods in order to compare the two phenotypic methods. A future analytical study should be performed, with a genotypic method as the golden standard for carbapenemase detection assay.

Conclusions

The number of studies concerning CR-KP strains in Romania is insignificant. At this time there is insufficient information regarding the circulation of Enterobacteriaceae strains and in particular CR-KP by geographical areas and at a national level. Our results showed that most strains are producing enzymes such as OXA-48-like and KPC.
An applicable national protocol for each microbiology lab is required for the detection of carbapenemase-producing *Enterobacteriaceae.*
We compared two phenotypic tests, mCIM versus the disk combination test (ROSCO) and we have found a correlation between them.
We consider that both tests are reproducible and the interpretation of the results is not subjective if the working conditions are strictly respected.
Both tests are easy to perform, inexpensive and accessible to all laboratories in the country.
The carbapenem inactivation assay highlights the antibiotic hydrolysis by carbapenemases without determining the type of enzyme.
The combination disk test contains specific inhibitors of carbapenemase activity and differentiates between carbapenemase classes if KP produces a single type of carbapenemase. If the same microorganism produces two or more enzymes, the differentiation is no longer possible. Recently, the manufacturer also introduced combinations of specific inhibitors in a single disk allowing the detection of KPC + MBL-producing strains. We have not tested these disks.
The selection of suspicious strains can be made in the laboratory by using one or both phenotypic methods analyzed by us depending on the purpose: carbapenemase type detection, highlighting the presence of enzymes responsible for the carbapenems hydrolysis, or both.
The regional incidence and the epidemiological purpose contribute to the choice regarding the working method for screening and/or routine.
Determining the type of enzyme for epidemiological and nosocomial infection control requires molecular diagnosis (e.g., PCR). Molecular identification is 100% specific but it is expensive and not accessible to all laboratories. It requires qualified personnel and only a limited number of genes can be detected by this method.
Phenotypic methods can highlight the hydrolytic activity on carbapenems or the presence of carbapenemases by using specific inhibitors.
The choice of routinely using phenotypic methods depends on resources, training and number of staff, regional epidemiological status and the usefulness of microbiological diagnosis.

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