Original Paper

# A Comparative Study on Antibiotic Resistance of Klebsiella Strains from Surgical and Intensive Care Wards

OANA MARIANA CRISTEA[1], O.M. ZLATIAN[1], S.N. DINESCU[2], A.T. BĂLĂŞOIU[3], CARMEN AVRAMESCU[1], MARIA BĂLĂŞOIU[1], MIHAELA NICULESCU[4], DANIELA CALINA[5]

[1]*Department of Microbiology, University of Medicine and Pharmacy of Craiova, Romania*

[2]*Department of Epidemiology, University of Medicine and Pharmacy of Craiova, Romania*

[3]*Ophtalmology clinic, County Clinical Emergency Hospital of Craiova, Romania*

[4]*Department of Anatomy, University of Medicine and Pharmacy of Craiova, Romania*

[5]*Department of Clinical Pharmacy, University of Medicine and Pharmacy of Craiova, Romania*

**ABSTRACT**: Introduction. Klebsiella infections are common in Intensive Care Unit (ICU) and surgical wards. In order to establish the prophylaxis protocols, we must know the prevalence of infections and the antibiotic resistance profiles. Material and methods. This cross-sectional study included isolates from patients in County Clinical Emergency County Hospital, Craiova, Romania: 1254 isolates from the ICU and 1040 isolates from surgical wards. We used an automated method (Phoenix analyzer, Becton-Dickinson, USA) with antimicrobial testing according to CLSI 2014. We tested by disc diffusion the ESBL and carbapenemases production, using kits ESBL Confirm ID and KPC/Metallo-beta-lactamase/OXA-48 Confirm (ROSCO Diagnostica, Denmark). The patients in ICU were also screened at admission for carbapenemase producting strains by PCR (GeneXpert® II, Cepheid, Sunnyvale, CA, USA) for the carbapenemases: KPC, IMP-1, VIM-1, NDM, OXA-48. Results. Klebsiella strains were more prevalent in ICU (20.81%) vs. surgical wards (16.34%) and they were resistant in high percentages at: cefuroxime (95.81% vs. 87.21%),ceftazidime (91.70% vs. 84.71%), cefepime (84.2% vs. 69.82%). The highest differences in resistance were observed for Tygecycline (Risk Ratio (RR) = 7.69), Imipenem/Cilastatine (RR=3.36), Cefoperazone with sulbactam (RR=2.58), Ciprofloxacine (RR=2.11), Gentamycin (RR=2.05) and Ertapenem (RR=1.93). The ICU strains showed MDR in 48.57% of cases vs. 23.57% in surgery strains. The prevalence of ESBL production was 82.4% in ICU vs. 32.3% in surgical wards. The prevalence of carbapenemase producing strains was 43.68% in ICU vs. 23.53% in surgical wards. Conclusions. The infections with Klebsiella spp. are more frequent in ICU compared with surgical wards and their antibiotic resistance is greater.

***KEYWORDS**: antibiotic resistance, Klebsiella, Intensive Care Unit, Ventilatory Associated Pneumonia*

## Introduction

Klebsiella spp. is a bacteria that in the recent years has become the most resistant enterobacteria, especially to beta-lactams, aminoglicosides, fluoroquinolones and carbapenemes, due to the ability to collect resistance plasmids. Klebsiella is one of the main germs involved in hospital acquired infections (HAI).

Endotracheal intubation and mechanical ventilation are the most important factors for developing nosocomial pneumonia because they permit the direct access of microorganisms to the lower respiratory tract. The patients in Intensive Care Unit (ICU) have frecquently various conditions that lower the immunity and favorize the colonisation/infection of the lower respiratory tract.

Surgical site infection (SSI) continues to represent a significant portion of healthcare-associated infections, since they account for 14% to 16% of all nosocomial infections and are among the more common complications of surgical care. They have a high impact on morbidity, mortality, and cost of care.

In order to establish the prophylaxis protocols, we must know the antibiotic resistance profiles of the germs involved in surgical site infections.

The antibiotic resistance of bacteria is underlied by several mechanisms, depending on the antibiotic class.

**Resistance to beta-lactam antibiotics**

One of the mechanisms of resistance to beta-lactam antibiotics is the secretion of β-lactamases, classified according to a scheme proposed by Bush *et al* based on molecular properties of the gene and enzyme rather than relying solely on phenotypic hydrolysis properties[1]. Extended spectrum beta-lactamases (ESBLs) isolated from various

Enterobacteriaceae[2] are defined as β-lactamases that have the ability to hydrolyse penicillins, broad- and extended-spectrum cephalosporins, and monobactams, inhibited by clavulanic acid.

ESBLs are currently divided in TEM-type ESBLs, SHV-type ESBLs and other types.

AmpC β-lactamases are a group of enzymes commonly isolated from Gram negative bacteria resistant to extended-spectrum cephalosporin. AmpCs are typically chromosomally encoded in many Gram negative bacteria like Escherichia coli, Citrobacter freundii and Enterobacter spp., but also in plasmids[3]. AmpCs hydrolyse broadband extended-spectrum cephalosporins but they are not inhibited by clavulanic acid or other inhibitors of β-lactamases.

Some of the ESBLs confer high-level resistance and can be easily detected as resistant (or intermediate) by disk diffusion. But the ESBLs may provide low levels resistance (MIC 1-2 μg/ml) to monobactams and third generation cephalosporins that and this can be easily overlooked by routine antimicrobial susceptibility methods[4]. These isolates may not reach current breakpoints for resistance, but can be clinically resistant to beta-lactams[5].

Since some ESBLs are more active on CAZ, while others are more active on CTX, the choice of cephalosporins tested can also affect the ability of laboratories to detect resistant strains[5].

**Resistance to carbapenems**

Resistance to carbapenems is mainly due to carbapenemases, beta-lactamases that can significantly hydrolyze at least imipenem or meropenem. The carbapenemases are classified according to Ambler scheme involved in acquired resistance are of Ambler classes A, B and D (Table 1). They may be plasmid or chromosomally encoded.

***Table 1. Ambler classification of carbapenemases [1]***

| | | 3rd gen cephalosporin | MICs μg/mL | | | Inhibited by | | |
|---|---|---|---|---|---|---|---|---|
| **Ambler** | | | | | | | | Boronic |
| **class** | Enzymes | | AZT | IMP | MRP | CLAV | EDTA | acid |
| **A** | NmcA | S | 4 | 2-16 | 2-8 | ± wk | no | yes |
| | Sme-1 to Sme-3 | S | 4-64 | 2-16 | 0.25-8 | ± wk | no | yes |
| | IMI-1 to IMI-2 | S | S | 2-64 | 4-32 | + | no | yes |
| | KPC-1 to KPC-4 | 2-32 | 2-64 | 4-16 | 4-16 | + or wk | no | yes |
| | GES-2 to GES-5 | 2-32 | 16-R | 0.25-16 | 0.5-16 | + or 0 | no | yes |
| **B** | IMP 1-16 | 2-32 | S-R | 0.5-128 | 0.25-R | no | yes | no |
| Metallo-beta- | VIM 1-12 | 2-64 | S-R | 1-R | 0.5-R | no | yes | no |
| lactamases | SPM-1 | 2-256 | 4 | R | R | no | yes | no |
| | GIM-1 | 16-32 | 8-16 | > 8 | > 8 | no | yes | no |
| | SIM-1 | 2-256 | 128 | 8-16 | 16 | no | yes | no |
| **D** | OXA 23-27 | > 256 | > 256 | 4-64 | 4-128 | ± wk | no | no |
| Oxacillinases | OXA 40-48 | S-R | S-R | 2-64 | 0.25-64 | wk | no | no |
| | OXA 54-55 | S | S | 4 | 0.25 | wk | no | no |
| | OXA-6O | S | R | 0.5 | 2 | no | no | no |
| | OXA-58 | 4-128 | 2-32 | 3-32 | 2-64 | no | no | no |

The carbapenemases from the KPC family confer a greater resistance to cephalosporins from the third generation than to carbapenems[6].

Intermediate or resistant Klebsiella spp. to ertapenem or meropenem should be considered resistant to all carbapenems[7]. E.coli that harbors KPC was identified in nine patients in New York. ESBL CTX M15 was also encountered in three of these isolates[8].

The carbapenemases from class D are enzymes classified as OXA-types (oxacillinase activity). They are important for clinicians because the potential for clinical failure of imipenem treatment (Class D, OXA-55 carbapenemase)[9].

## Objectives

Assessing the prevalence of colonization / lower respiratory tract infection with bacteria of the genus Klebsiella patients from the ICU.

Assessing the prevalence of colonization / infection of wounds with this enterobacteria in hospitalized patients from surgical wards.


DOI: 10.12865/CHSJ.42.02.09

Comparing the antibiotic resistance profiles of Klebsiella strains from ICU and surgical wards. Comparison of detection efficiency of carbapenemase production of Klebsiella strains by a phenotypic method – double disk test and a genotypic method – Polymerase Chain Reaction (PCR).

## Material and methods

This cross-sectional study included isolates from patients in County Clinical Emergency County Hospital, Craiova, Romania: 1254 isolates from the ICU and 1040 isolates from surgical wards. We collected from ICU patients mainly tracheal aspirates/endotracheal tubes and wound secretions and only secretions from abscesses or wounds from surgical patients. The strains were identified by an automated method (Phoenix analyzer, Becton-Dickinson, USA) with antimicrobial testing according to CLSI 2014. In all strains we tested by disc diffusion the ESBL and carbapenemases production, using kits ESBL Confirm ID, AmpC Confirm Kit and KPC/Metallo-beta-lactamase/OXA-48 Confirm (ROSCO Diagnostica, Denmark). The patients in ICU were also screened at admission for carbapenemase producting strains by PCR (GeneXpert® II, Cepheid, Sunnyvale, CA, USA) for the carbapenemases: KPC, IMP-1, VIM-1, NDM, OXA-48. We used PCR to identify the genes: KPC, IMP-1, VIM-1, NDM, OXA-48 and AmpC (Institutul Cantacuzino, Bucuresti.

**Detection of ESBL production**

We screened for the production of Extended Spectrum Beta-Lactamases (ESBL) by the double disk method using a dedicated kit from ROSCO DIAGNOSTICA which contained paper discs with Ceftazidime (30µg), Ceftazidim/acid clavulanic (30/10µg), Cefotaxim (30µg) and Cefotaxim/acid clavulanic (30/10µg). After incubation at 35°C in aerobic atmosphere the test was considered positive if any of the discs containing antibiotics combined with clavulanate have an inhibition area with at least 5 mm greater than the discs containing antibiotics alone.

*Interpretation*. Strains that contain Inhibitor Resistant TEM Beta-lactamases (IRT) give antibiotic resistance patterns similar to TEM 1 or 2 or SHV 1, but are resistant to Amoxicillin with Clavulanate. IRTs are detected mainly in E. coli and Klebsiella pneumoniae.

They are resistant to Amoxicillin + Clavulanate (zone diameter for Amoxicillin+Clavulanate < 17 mm) and are susceptible to cephalosporins: Cefazolin, Cefoxitin, Cefotaxime (Fig.1).

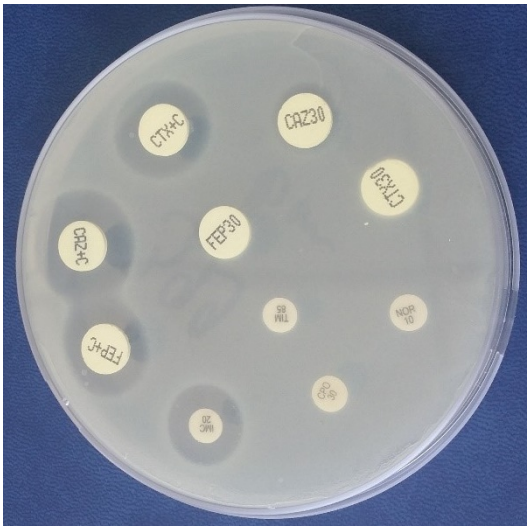


***Fig.1. Combination disks test for detection of ESBL production in Klebsiella pneumoniae***

AmpC confirmation by combined disk test

The double disk method consists in applying on an inoculated plate Cefotaxime, Cefotaxime+Boronic, Ceftazidime, Ceftazidime+Boronic, Cefotaxime+Cloxacillin and Ceftazidime+ Cloxacillin disks.

*Interpretation*: An inhibition zone for Cefotaxime+Cloxacillin ≥ 5 mm than Cefotaxime and/or a zone for Ceftazidime+Cloxacillin ≥ 5 mm than Ceftazidime indicates secretion of an AmpC. An inhibition zone for Cefotaxime+Boronic acid ≥5 mm than Cefotaxime and/or an inhibition zone for Ceftazidime+Boronic acid ≥5 mm than Ceftazidime indicates secretion of an AmpC. (Fig.2).

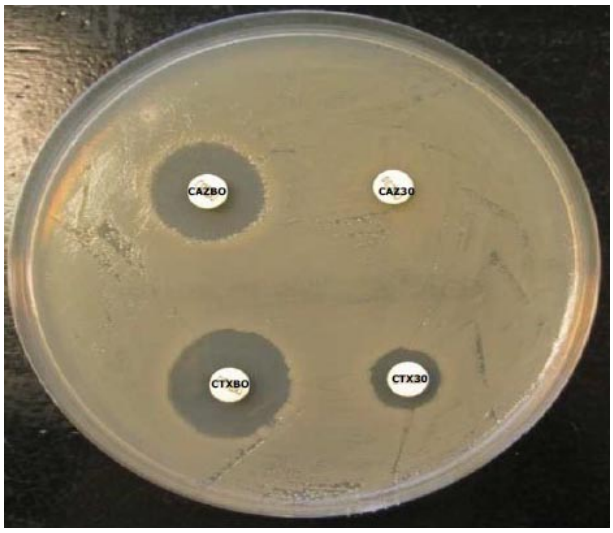


***Fig.2. Confirmation of AmpC production in Klebsiella spp. by combination disks test***

In many cases of isolates with carbapenemases, the carbapenems MICs are around the susceptible breakpoint and the resistance is difficult to detect - particularly with automated systems. Therefore are needed special zone breakpoints for the first line screening of Enterobacteriaceae strains that on Mueller-Hinton Agar with McFarland 0.5 inoculum have reduced susceptibility to Imipenem (zone < 23 mm or MIC > 1 μg/ml) or Meropenem (inhibition zone < 25 mm or MIC > 0.5 μg/ml) or Ertapenem (zone ≤ 22 mm). These strains should be suspected of producing carbapenemases Most of the strains with enzymes KPC and GES are highly resistant to Ceftazidime. The most sensitive indicator for possible production of carbapenemase is Ertapenem, but other resistance mechanisms are involved in approximately 20% of cases.

**Detection of acquired carbapenemases Ambler class A**

Carbapenemases from the class A are penicillinases more active on Imipenem than Meropenem and that also give resistance to other penicillins, cephalosporins and Aztreonam. Class A carbapenemases are inhibited by Boronic acid and consequently these enzymes are detected by the synergy with meropenem or Imipenem [10,11].

Another inhibitor of class A carbapenemases is the Clavulanate and therefore they can be detected by the synergy with imipenem. The family of enzymes KPC confer a greater resistance to third generation cephalosporins than carbapenems[6]. The strain producing Class A carbapenemases show synergy between the disks of Imipenem and Boronic acid (distance from edge to edge is 6 mm). This test is performed on strains with zones of inhibition ≤21 mm for Imipenem 10 μg disks (screening test).

It is recommended to screen multiple colonies for KPC detection since the strains carbapenemase susceptible and resistant may co-exist[12].

**Double disk synergy test**. The test uses disks with Meropenem alone and combinations of Meropenem with Boronic acid, Cloxacillin and Dipicolinic acid (DPA). If the inhibition area of Meropenem + Boronic acid is ≥ 5 mm then Meropenem alone, Meropenem+DPA and Meropenem+Cloxacillin it is suspected the production of a KPC carbapenemase. The inhibitions zones of Meropenem+Boronic acid and Meropenem+Cloxacillin ≥ 5 mm, than Meropenem alone and Meropenem+DPA (INSERTION Figure 3) show hyperproduction of AmpC + porin loss, or efflux [6]. The inhibition zone of Meropenem + DPA ≥ 5 mm than Meropenem, indicates the production of a metallo-β-lactamase (MBL) (Table 2).

A reduced susceptibility to Ertapenem, the synergy between Boronic acid and carbapenems, and lack of synergy between Cloxacillin and carbapenems indicate the presence of a KPC enzyme (or other class A carbapenemase). The strains that produce high level AmpC + impermeability have synergy between Cloxacillin and carbapenems. The strains that produce ESBL + impermeability will have synergy between Amoxicillin + Clavulanate and carbapenems or cephalosporins (Fig.3).

Strains producing oxacillinases will currently show zones of inhibition < 22 mm with Ertapenem and/or <25 mm with Meropenem Neo-Sensitabs. Most are resistant to Aztreonam.

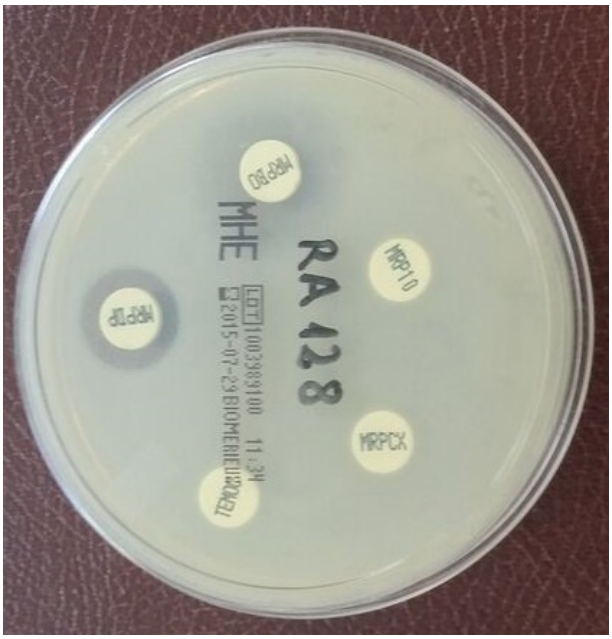


***Fig.3. Double disk test for detection of carbapenemase production***


DOI: 10.12865/CHSJ.42.02.09

***Table 2. Interpretation of the double disk test for detection of carbapenemase production***

| Carbapenemase | MRP+DPA | MRP+BOR | MRP+Cloxa |
|---|---|---|---|
| Metallo-β-lactamases | Synergy | No synergy | No synergy |
| KPC | No synergy | Synergy | No synergy |
| AmpC impermeability | No synergy | Synergy | Synergy |
| Oxacillinases | No synergy | No synergy | No synergy |

The results of antimicrobial testing were stored and analyzed using Whonet 5.6 software (World Health Organisation). Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) 20.0 software (IBM Corporation, New York, USA).

## Results

We identified 261 Klebsiella strains in ICU (prevalence = 20.81%) and 170 strains in surgical wards (prevalence = 16.34%).

We can observe from Table 3 and Fig. 4 that Klebsiella strains were resistant in high percentages at: cefuroxime (95.81% in ICU vs. 87.21% in surgical wards), Ceftazidime (91.70% vs. 84.71%), Cefepime (84.2% vs. 69.82%). Relatively low resistance was observed to Tygecycline (15.38% vs. 2%), Imipenem/Cilastatine (41.10% vs. 12.24%), Cefoperazone with sulbactam (51.60% vs. 20.00%), Ciprofloxacin (58.63% vs. 27.84%), Gentamycin (65.63% vs. 32.03%) and Ertapenem (56.85% vs. 29.38%). The highest differences in resistance were observed for Tygecycline (Risk Ratio (RR) = 7.69), Imipenem/Cilastatine (RR=3.36), Cefoperazone with sulbactam (RR=2.58), Ciprofloxacin (RR=2.11), Gentamycin (RR=2.05) and Ertapenem (RR=1.93). There were no significant differences of resistance to Meropenem, Ceftazidime, Cefuroxime, Cefpirome and Amoxycillin/clavulanate. We can see that for any given antibiotic more than 50% of the ICU strains were resistant.

***Table 3. Comparison between antibiotic resistance of Klebsiella strains to various antibiotic classes in ICU and surgical wards***

| ATB | Class | ICU | Surgical wards | Relative Risk (RR) |
|---|---|---|---|---|
| Amikacin | Aminoglycosides | 23.08% | 15.03% | 1.54 |
| Amoxicillin/Clavulanate | Beta-lactam+Inhibitors | 89.82% | 77.85% | 1.15 |
| Aztreonam | Monobactams | 71.16% | 49.17% | 1.45 |
| Cefazoline | Cephems | 94.12% | 81.99% | 1.15 |
| Cefepime | Cephems | 84.52% | 69.82% | 1.21 |
| Cefoperasone/Sulbactam | β-lactam+Inhibitors | 51.60% | 20.00% | 2.58 |
| Cefpirome | Cephems | 89.29% | 76.92% | 1.16 |
| Ceftazidime | Cephems | 91.70% | 84.71% | 1.08 |
| Ceftriaxone | Cephems | 85.22% | 59.52% | 1.43 |
| Cefuroxime | Cephems | 95.81% | 87.21% | 1.10 |
| Ciprofloxacin | Quinolones | 58.63% | 27.84% | 2.11 |
| Colistin | Lipopeptides | 51.24% | 33.33% | 1.54 |
| Ertapenem | Penems | 56.85% | 29.38% | 1.93 |
| Gentamycin | Aminoglycosides | 65.63% | 32.03% | 2.05 |
| Meropenem | Penems | 70.39% | 66.67% | 1.06 |
| Norfloxacin | Quinolones | 75.00% | 40.00% | 1.88 |
| Ofloxacin | Quinolones | 66.35% | 43.48% | 1.53 |
| Piperacillin/Tazobactam | β-lactam+Inhibitors | 67.46% | 36.76% | 1.84 |
| Sulfametoxazols/Trimetoprim | Folate pathway inhibitors | 53.04% | 30.00% | 1.77 |

| | | | | |
|---|---|---|---|---|
| Ticarcillin/Clavulanate | β-lactam+Inhibitors | 99.42% | 100.00% | 0.99 |
| Tygecycline | Tetracyclines | 15.38% | 2.00% | 7.69 |
| Imipenem/Cilastatine | Penems | 41.10% | 12.24% | 3.36 |

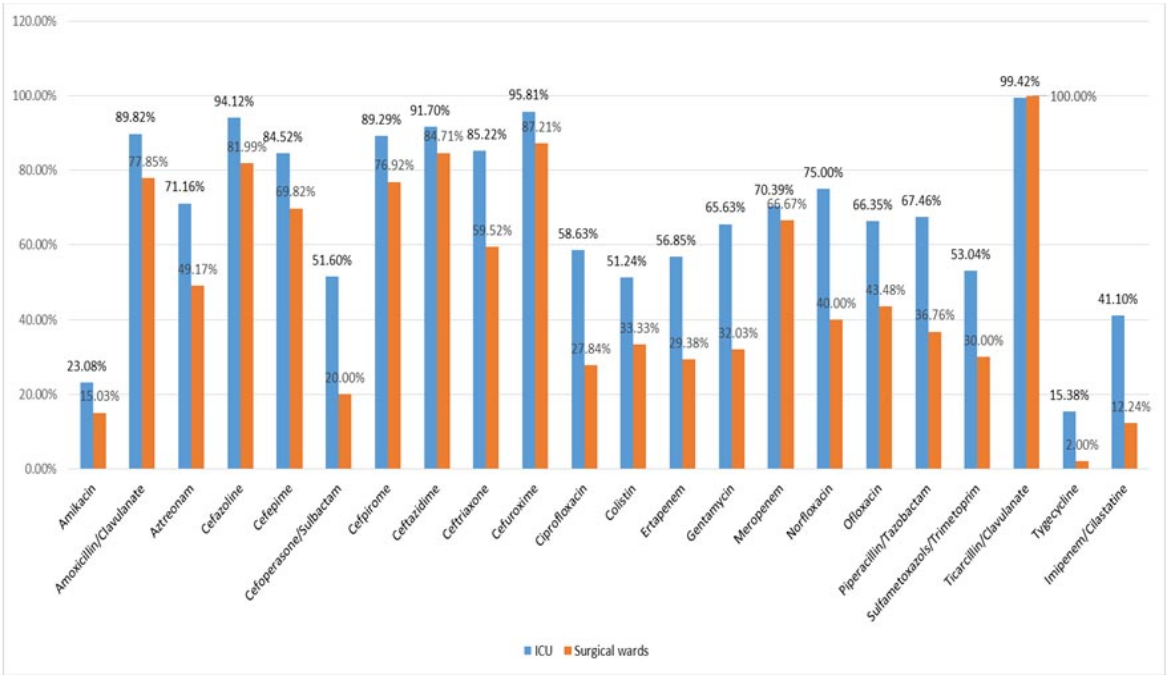


***Fig.4. Comparison between antibiotic resistance of Klebsiella strains to various antibiotic classes in ICU and surgical wards***

We can observe from Table 4 and Fig. 5 that the resistance to all antibiotic classes were higher in the ICU compared with surgical wards. The explanation is the high prevalence of hospital multidrugresistant organisms (MDROs) in the ICU unit, but also the antibiotic treatment of these patients, which often involves higher dosage and longer duration compared with the patients in surgical wards. The resistance to tetracyclines (represented by Tygecycline) was much higher in ICU (RR=7.69), due to the exclusive use of this antibiotic in the ICU. We also remark the higher resistance to carbapenems (RR=2.03) that is definitely due to the carbapenemase producing organisms (CPOs) from the ICU. It is well known that in ICU the MDROs and CPOs frequently spread from patient to patient due to the poor state of the immune system and their persistence in the environment.

***Table 4. Comparison between antibiotic resistance of Klebsiella strains to various antibiotic classes in ICU and surgical wards***

| **Antibiotic class** | **Surgery** | **ICU** | **Risk Ratio** | **Dif %** | **Z** | **p** |
|---|---|---|---|---|---|---|
| Aminoglycosides | 22.78% | 56.06% | 2.46 | 33% | -8.12 | <0.001 |
| Beta-lactam+Inhibitors | 54.44% | 76.52% | 1.41 | 22% | -7.63 | <0.001 |
| Monobactams | 49.17% | 71.16% | 1.45 | 22% | -3.99 | <0.001 |
| Cephems | 75.78% | 89.73% | 1.18 | 14% | -8.47 | <0.001 |
| Quinolones | 32.05% | 65.45% | 2.04 | 58% | -21.23 | <0.001 |
| Lipopeptides | 33.33% | 51.24% | 1.54 | 18% | -1.79 | 0.03 |
| Penems | 30.60% | 62.00% | 2.03 | 31% | -7.04 | <0.001 |
| Quinolones | 32.05% | 65.45% | 2.04 | 33% | -8.82 | <0.001 |
| Folate pathway inhibitors | 30.00% | 53.04% | 1.77 | 23% | -3.3 | <0.001 |
| Tetracyclines | 2.00% | 15.38% | 7.69 | 13% | -2.48 | 0.006 |


DOI: 10.12865/CHSJ.42.02.09

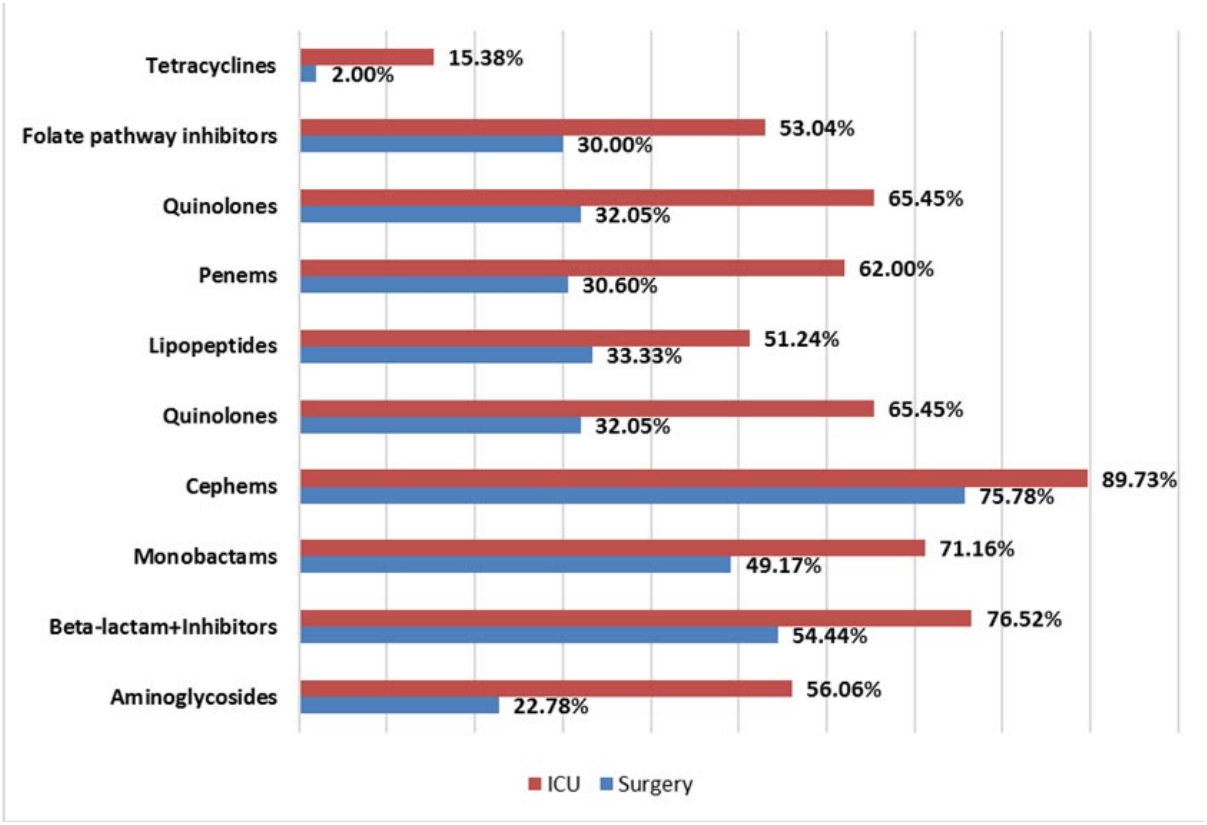


***Fig.5. Comparison between antibiotic resistance of Klebsiella strains to various antibiotic classes in ICU and surgical wards***

In Fig. 6 we can see that 23.87% of the Klebsiella strains were wild-type phenotype, 52.24% were PAZA (penicillinase producing) phenotype, 16.60% - CAZA (chromosomial cephalosporinaze producing) phenotype, 4.15% - ESBL phenotype, 2.00% - PACA (penicillinase and cephalosporinase producing) phenotype and 0.69% IMP (impermeability) phenotype. Actually, the ESBL were far more better detected by the specialized double disk test.

Aminoglicoside resistance phenotypes identified in Klebsiella strains were: KTG, KTNt TA GT A G KTGNt, KTGANt and the wild-type phenotype that was sensitive to all tested aminoglycosides (Fig.7). The quinolone phenotypes were: I, II, III, IV (Figure 8).

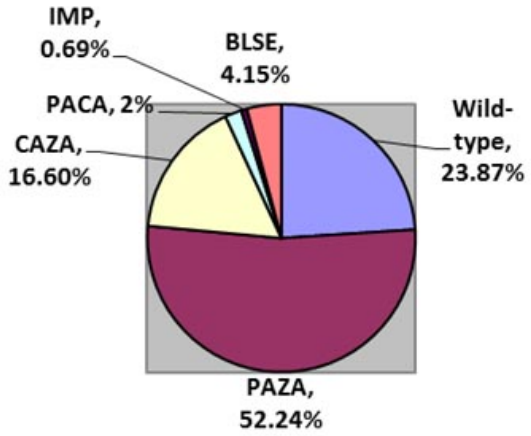


***Fig.6. Resistance phenotypes to beta-lactams identified in Klebsiella strains***

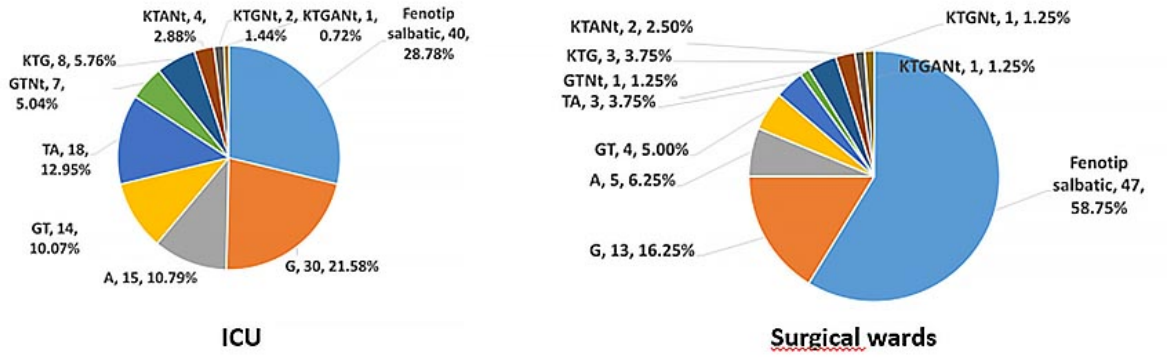


***Fig.7. Resistance phenotypes to aminoglicosides identified in Klebsiella strains***

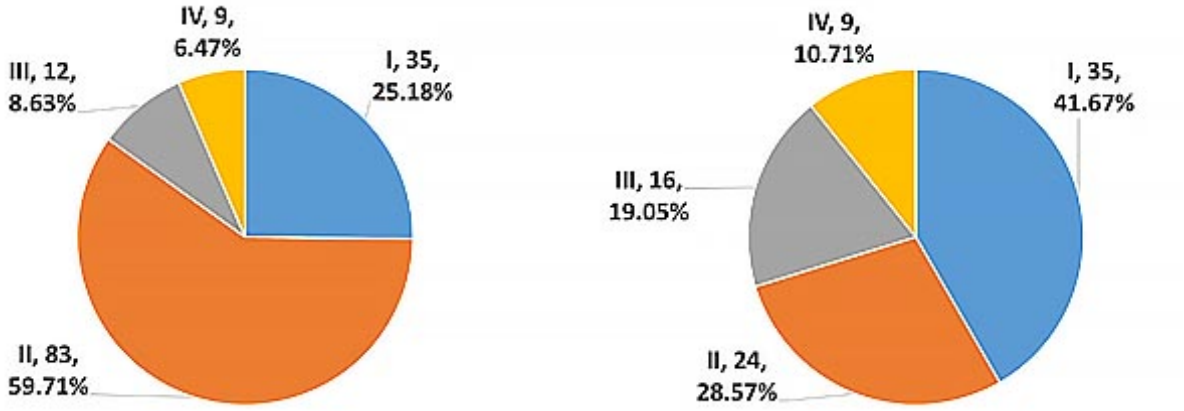


***Fig.8. Resistance phenotypes to quinolones identified in Klebsiella strains***

There were also associations of resistance phenotypes: ESBL + CARBA-R, ESBL + Aminoglycosides, ESBL + AmpC, ESBL + Aminoglycosides + Fluoroquinolones and a few cases of pan-resistance (Fig. 9).

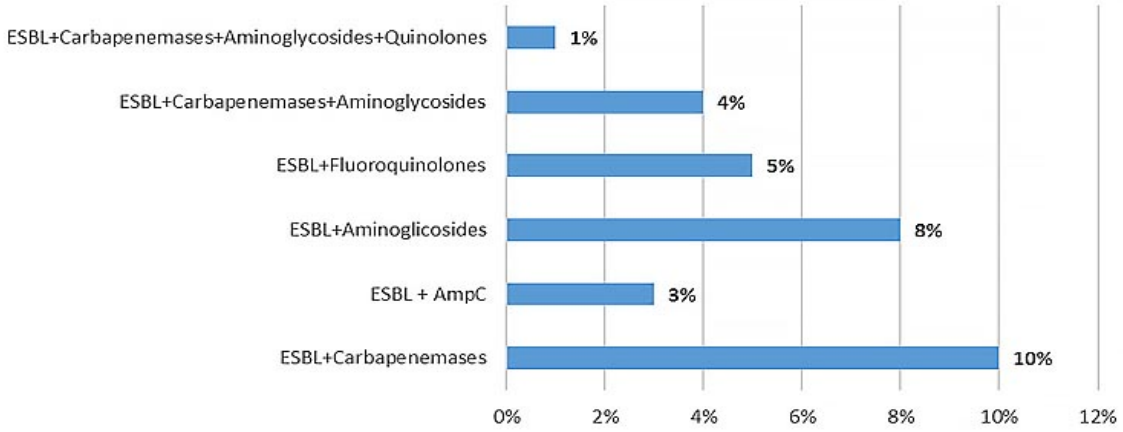


***Fig.9. Resistance mechanisms identified in Klebsiella strains***


DOI: 10.12865/CHSJ.42.02.09

The strains isolated from ICU patients showed MDR in 48.57% of cases (vs. 23.57% in surgery strains), as the antibiotic resistance index had a median of 0.59 in strains from surgical wards and 0.82 in those from ICU. The prevalence of ESBL producing strains was 82.40% in ICU vs. 32.3% in surgical wards (Fig.10).

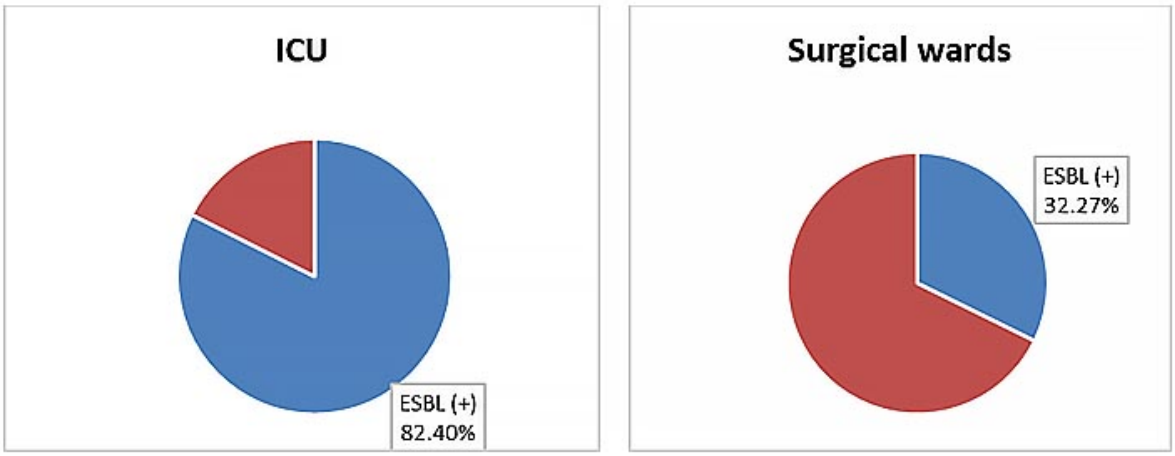


***Fig.10. Prevalence of ESBL-producing Klebsiella strains in the ICU and surgical wards***

AmpC production was almost twice more frequent among the Klebsiella strains isolated from the ICU (11.63%), compared with those isolated from the surgical wards (5.71%) (Fig.11). None of the strains were positive by PCR for AmpC. Various factors can give false positive results.

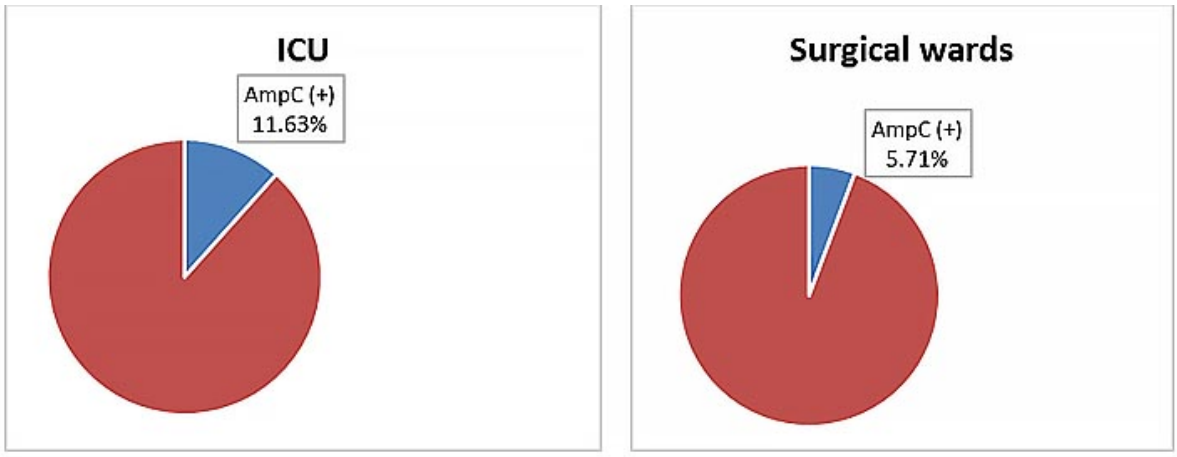


***Fig.11. Prevalence of AmpC-producing Klebsiella strains in the ICU and surgical wards***

The prevalence of carbapenemase producing strains was 43.68% in ICU vs. 23.53% in surgical wards (Fig.12). All strains that tested positive for carbapenemases by the phenotypic method, also were positive by PCR. Conversely, only 86 from the 114 carbapenemase producing strains in ICU (75.43%) and 59 from the 70 carbapenemase producing strains in surgical wards (84.29%) that were confirmed by PCR were also positive by double disk test (Table 5). The sensitivity of double disk method for detection of KPC was 92.34%, for MBL 87.50% and for OXA-48 of 65.56%.

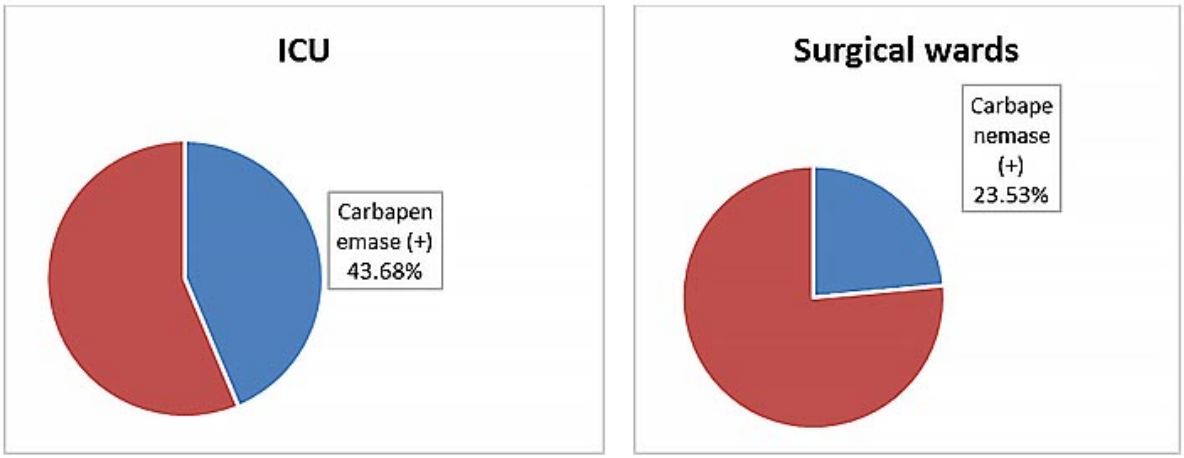


***Fig.12. Prevalence of carbapenemase-producing Klebsiella strains in the ICU and surgical wards***

***Table 5. Comparison between the phenotypic and genotypic detection of carbapenemases***

| | ICU | | | | Surgical wards | | | |
|---|---|---|---|---|---|---|---|---|
| | **Double disk(+)** | **Double disk (-)** | **Sensitivity** | **Specificity** | **Double disk (+)** | **Double disk (-)** | **Sensitivity** | **Specificity** |
| PCR (+) | 86 | 28 | 75.44% (66.49-83.02%) | 91.84% (86.17-95.71%) | 59 | 11 | 84.29% (73.62-91.89%) | 89.00% (81.17-94.38%) |
| PCR (-) | 12 | 135 | | | 11 | 89 | | |

## Conclusions

Klebsiella spp. infections had a greater prevalence in the ICU than in surgical wards. The antimicrobial resistance was greater in the ICU strains, due to the intensive use of antibiotics in this ward, compared with other clinics, sometimes as prophylaxy to prevent infections that can easily develop in these patients with severe pathology and weakened immune system.

The antimicrobial resistance phenotypes encountered in ICU were ESBL, CARBA-R, association between ESBL + CARBA-R, ESBL + Aminoglycosides, ESBL + AmpC, ESBL + Aminoglycosides + Fluoroquinolones and a few cases of pan-resistance.

There is a strong concordance between phenotypic and genotypic methods for detection of carbapenemase production on Klebsiella strains. The double disk test can be successfully used in screening for carbapenemase production, even if the genotypic method remains the golden standard. The double-disc test for detection of AmpC production was not reliable in our experience.

### Acknowledgements

All authors had an equal contribution to this paper.

This study was supported by the internal grant of University of Medicine and Pharmacy of Craiova, Romania, no. 15 from 15/01/2015, entitled “The influence on the antibiotic therapy on emergence of multidrug resistance in germs involved in infections of the patients from intensive care unit of the County Clinical Hospital of Craiova, Romania”.

## REFFERENCES

1. Ghafourian, S., Sadeghifard, N., Soheili, S. & Sekawi, Z. Extended Spectrum Beta-lactamases: Definition, Classification and Epidemiology. Curr. Issues Mol. Biol. 2014; 17, 11–22.
2. Bradford, P. A. Extended-spectrum beta-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin. Microbiol. Rev. 2001; 14, 933–51.
3. Philippon, A., Arlet, G. & Jacoby, G. a. Plasmid-determined AmpC-type β-lactamases. Antimicrob. Agents Chemother. 2002; 46, 1–11.
4. Henshke-Bar-Meir, R. et al. Assessment of the clinical significance of production of extended-spectrum beta-lactamases (ESBL) by Enterobacteriaceae. Infection. 2006; 34, 66–74.
5. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement. Clinical and Laboratory Standards Institute. 2014; 32.
6. Smith Moland, E. Plasmid-mediated, carbapenem-hydrolysing beta-lactamase, KPC-2, in Klebsiella pneumoniae isolates. J. Antimicrob. Chemother. 2003; 51, 711–714.

 DOI: 10.12865/CHSJ.42.02.09

7. Bratu, S. et al. Emergence of KPC-possessing Klebsiella pneumoniae in Brooklyn, New York: epidemiology and recommendations for detection. Antimicrob. Agents Chemother. 2005; 49, 3018–20.
8. Urban, C. et al. Carbapenem-resistant Escherichia coli harboring Klebsiella pneumoniae carbapenemase beta-lactamases associated with long-term care facilities. Clin. Infect. Dis. 2008; 46, e127–e130.
9. Hocquet, D. et al. Nationwide investigation of extended-spectrum beta-lactamases, metallo-beta-lactamases, and extended-spectrum oxacillinases produced by ceftazidime-resistant Pseudomonas aeruginosa strains in France. Antimicrob. Agents Chemother. 2010; 54, 3512–5.
10. Gomez, S. A. et al. Clonal dissemination of Klebsiella pneumoniae ST258 harbouring KPC-2 in Argentina. Clin. Microbiol. Infect. 2011; 17, 1520–4.
11. Tsakris, A. et al. First occurrence of KPC-2-possessing Klebsiella pneumoniae in a Greek hospital and recommendation for detection with boronic acid disc tests. J. Antimicrob. Chemother. 2008; 62, 1257–1260.
12. Robilotti, E. & Deresinski, S. Carbapenemase-producing Klebsiella pneumoniae. F1000 Prime Rep. 2014; 6, 80.

***Corresponding Author: Zlatian Ovidiu Mircea, University of Medicine and Pharmacy of Craiova, Petru Rares St, No.2, Craiova Romania; telephone number: +40 773 849 195; email: ovidiu.zlatian@gmail.com***