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Review

Multidrug-Resistant Gram-Negative Bacteria – a Problem for Hospital Infectious Pathology

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Abstract

The spread of multidrug-resistant /MDR/ Gram-negative bacteria in the hospital setting is a world-wide problem. In this study we present data about the resistance to antimicrobials of some problematic for hospital infectious pathology bacteria – *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Acinetobacter baumannii* and *Pseudomonas aeruginosa* on the model of a multiprofile hospital. There has been an increase in *ESBLs*-producing *E. coli* and *K. pneumoniae* strains, isolated especially in some so-called "risky units" at the Military Medical Academy /MMA/ for the last years. There were also registered the first strains *E. coli,* producing metallo-beta-lactamase NDM1. The data show specific association between the *blaNDM-1* and *rmtB* genes conferring high-level resistance to all aminoglycosides in these strains. Non-fermenting *A. baumannii* and *P. aeruginosa* strains isolated usually are multiresistant with high-level resistance to carbapenems and other beta-lactams and quinolones, and the resistance to carbapenems in *A. baumanni* strains is associated with the production of *Oxa 23*, *Oxa 58* and *Oxa 72* carbapenemases, but not metallo-beta-lactamases.

Keywords: Gram-negative bacteria, resistance to antimicrobials, ESBLs, carbapenemases

Резюме

Разпространението на множествено-резистентни Грам-отрицателни бактерии в болничната среда е световен проблем. В това проучване представяме данни за резистентността към антимикробни лекарствени средства на някои проблемни за болничната инфекциозна патология микроорганизми - *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Acinetobacter baumannii* и *Pseudomonas aeruginosa* на модел многопрофилна болница. Наблюдава се нарастване на щамовете *E. coli* и *K. pneumoniae*, продуциращи широко-спектърни бета-лактамази /ESBL/, изолирани предимно в т.н. „отделения с повишен риск за развитие на инфекция" във Военно-медицинска академия / ВМА/ през последните години. Регистрирани са също първите щамове *E. coli,* продуциращи металобеталакмаза *NDM-1*. Данните показват специфична асоциация межд *blaNDM-1* and *rmtB* гените, кодиращи високо ниво на резистентност към аминогликозиди в тези щамове. Неферментиращите глюкозата Грам-отрицателни бактерии *A. baumannii* и *P. aeruginosa* се изолират обикновено като множествено резистентни с високо ниво на резистентност към карбапенеми и др. бета-лактамни антибиотици, хинолонови производни, като резистентността към карбапенеми при щамовете *A. baumanni* се свързва с продукцията на *Oxa 23*, *Oxa 58* и *Oxa 72* карбапенемази, но не и с метало-бета-лактамази.

Introduction

Many studies were focused on the significance of the multidrug-resistant Gram-negative bacteria as a cause of severe bacterial nosocomial infections worldwide, with two thirds of the 25 000 annual deaths occurring only in Europe /www.medscape.com/ viewarticle/717606-3/. Initial reports of *ESBLs*-producers in the eighties and early nineties essentially focused on the descriptions of nosocomial outbreaks, mainly in intensive care unit settings, caused by *Klebsiella* and *Enterobacter* spp. which produced various *TEM*- or *SHV- ESBL* subtypes. More recently, novel *ESBLs* coding genes (e.g. of the *CTX-M* family) derived from plasmid mobilization of environmental or soil organisms

have emerged worldwide in several enterobacteria – *Enterobacter* species, particularly in *E. coli.* (Schoevaerdts *et al*., 2011). Since 2000, there has been a rapid increase in *Enterobacteriaceae* members (*E. coli, Klebsiella pneumonia*) and some carbapenemase-producing nonfermenting Gram-negative bacteria (*Acinetobacter baumannii, Pseudomonas aeruginosa*). According to the CDC, the proportion of *Enterobacteriaceae* that were resistant to carbapenems increased from 0% in 2001 to 1.4% in 2010, with most of the increase recorded in *Klebsiella spp* (CDCVital signs, 2013). Of particular importance for the resistance to carbapenems it is also the registered co-production of multiple beta-lactamases resulting in clinical resistance to all classes of beta-lactams (Bush, 2013).

The aim of this study is to present data on the resistance and multi-resistance of some problematic for hospital infectious pathology Gram-negative bacteria on the model of a multiprofile hospital.

Background

Among all of the bacterial resistance problems, gram-negative pathogens are particularly worrisome, because they are becoming resistant to nearly all drugs that would be considered for treatment. The most serious, life-threatening infections are caused by a group of drug-resistant bacteria that the Infectious Diseases Society of America (IDSA) has labeled the „ESKAPE" pathogens, because they effectively escape the effects of antibacterial drugs. /http://www.medscape.com/. Additionally, in 2013 the CDC categorized the 18 microorganisms that pose the greatest antimicrobial resistance threats to public health into 3 threat groups: urgent, serious, and concerning (Solomon, 2013; CDC, 2013). The pathogens assigned to the urgent and serious categories require more monitoring and prevention activities. The most serious gram-negative infections are healthcare-associated, and the most common pathogens are *Enterobacteriaceae*, *P. aeruginosa,* and *Acinetobacter.* /CDC, 2013/.

Military Medical Academy /MMA/ in Sofia, Bulgaria, is a community hospital with 800 beds. The hospital is a one of the national centres for treatment of trauma, respiratory disease, liver transplant patients. Antibiotic stewardship at the MMA includes all groups of antibiotics, together with carbapenems, quionolones, third and fourth generation cephalosporins.

Multidrug-Resistant *E. coli* and *Klebsiella* pneumoniae

Multidrug-resistant /MDR/, extended-spectrum beta-lactamases (*ESBLs)* producing *Klebsiella* species and *Escherichia coli* have been isolated in hospitals throughout the world. *ESBLs* positive strains are associated with increased mortality, because of the failure to treat infections caused by *ESBLs* positive organisms, due to the limited therapeutic choices (Kim *et al.,* 2002; Paterson *et al*., 2005). Of all "EARSS-specific" pathogens, *E. coli* demonstrated the most worrying trends. *E. coli* isolates with multiple resistance to third generation cephalosporins, fluoroquinolones, and aminoglycosides were registered in Bulgaria in great proportions (EARSS, 2002). The proportion of ESBL-producing *E. coli* strains in the units of the MMA in 2014 was 22.4% and for *K. pneumoniae* – 47%. Most of the *E. coli* strains, producing ESBL, originated from the so-called "risky units" – ICU (Intensive care unit) 29.9%, Anestesiology and Resuscitation clinic /ARC/ – 17.7% and Hepato-Pancreatic surgery /HPS/ – 16.2%. /MMA, 2015./ In a study of bloodstream infections, the proportion of E.coli producing ESBLs increased from 40% in 2002 to 61% in 2009, and the proportion of carbapenem-resistant *K. pneumoniae* increased from 2.4% to 52% (Datta *et al.,* 2012). The resistance patterns of the *E. coli* and *K. pneumoniae* strains isolated in 2014 are presented in Figures 1 and 2.

ESBLs are clearly a matter of global concern. In Europe*, CTX-M ESBLs*, which began to disseminate clinically later than the classical TEM and SHV variants, are now spreading rapidly and are increasingly dominant. While they were found among hospitalized patients and in species more common in the ICU (*K. pneumoniae, E. cloacae*), *ESBLs* are now commonly found in *E. coli* strains even in patients with community-acquired infections (Corgnaglia *et al*., 2008).

The results of a comprehensive study conducted in Bulgaria over a 8-year period /1996-2003/, showed that *CTX-M-3, CTX-M-15* and *SHV-12*, are the most widespread *ESBLs* in *Enterobacteriaceae* strains, especialy in the *E. coli* and *K. pneumoniae* strains investigated (Markovska *et al.,* 2008). Detection of *E. coli* strains, carriers of *CTX-M-15* in the MMA later in 2012 (Poirel *et al.*, 2014) and in 2014 (Pfeifer *et al*., 2015/, suggested persistence of such problematic hospital isolates over a long period of time in the hospital, requiring constant monitoring and control on ESBL-producing *Enterobacteriaceae* strains, because of the importance of the problem, related to the limited options for treatment of such infections (Mshana *et al*., 2011).

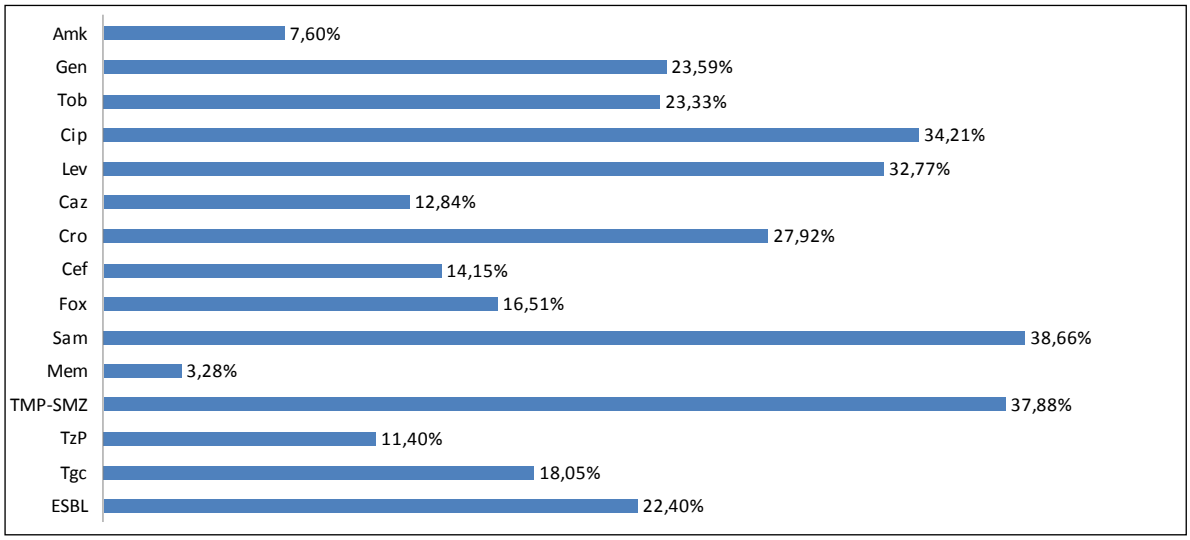

Fig. 1. Resistance of *E. coli* strains to antimicrobials for 2014 amk – amikacin, gen – gentamicin, tob – tobramycin, cip – ciprofloxacin, lev – levofloxacin, caz – ceftazidim, cro – ceftriaxon, cef – cefepim, fox – cefoxitin, sam – ampicillin/ sulbactam, mem – meropenem, tmp-smz – trimetoprim/sulfametoxazol, tzp – piperacillin/tazobactam, tgc - tigecyclin, n-763

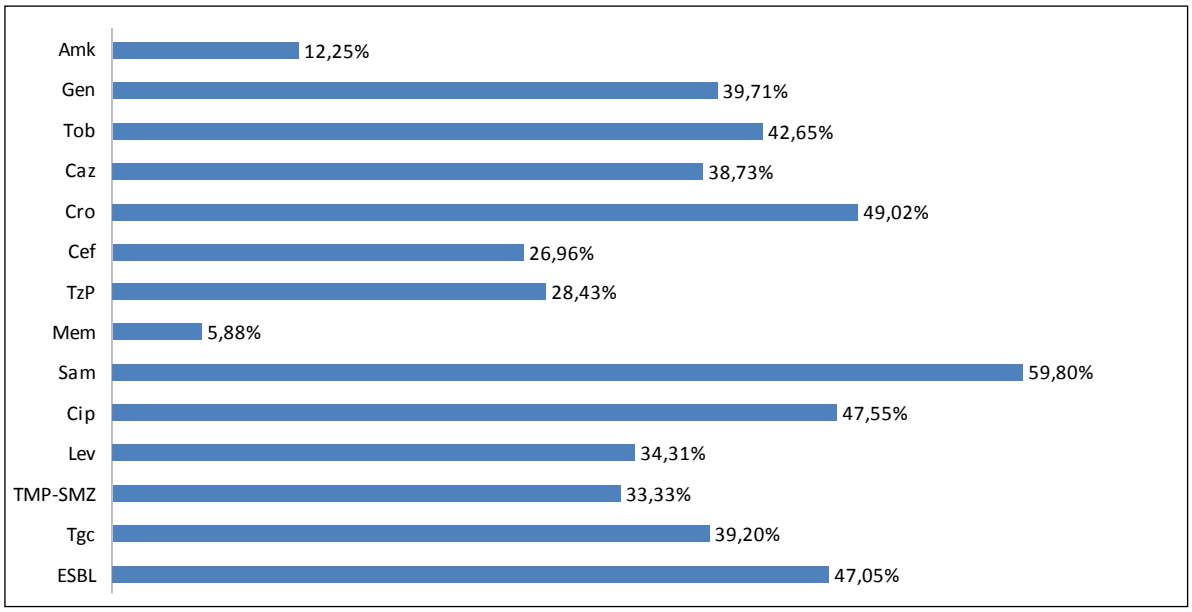

Fig. 2. Resistance of *K. pneumoniae* strains to antimicrobials for 2014 amk – amikacin, gen – gentamicin, tob – tobramycin, cip – ciprofloxacin, lev – levofloxacin, caz – ceftazidim, cro – ceftriaxon, cef – cefepim, fox – cefoxitin, sam – ampicillin/ sulbactam, mem – meropenem, tmp-smz – trimetoprim/sulfametoxazol, tzp – piperacillin/tazobactam, tgc - tigecyclin, n-204

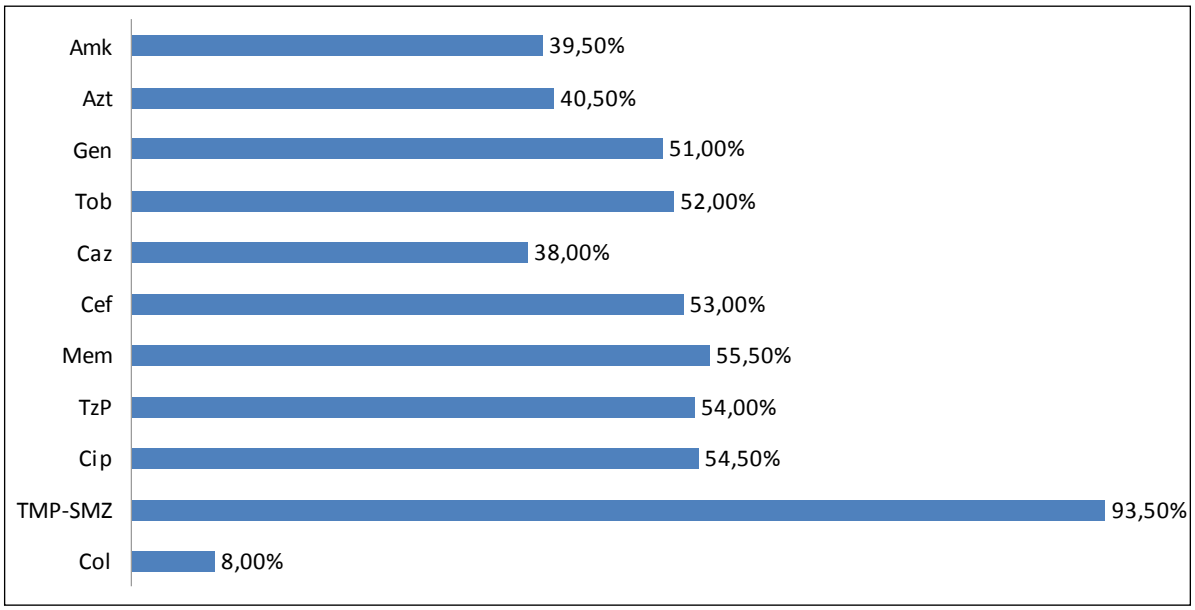

Fig. 3. Resistance of *A. baumannii* strains to antimicrobials for 2014 amk – amikacin, azt – azreonam, gen – gentamicin, tob – tobramycin, caz – ceftazidim, cef – cefepim, cip – ciprofloxacin, sam – ampicillin/sulbactam, mem – meropenem, tzp – piperacillin/tazobactam, tmp-smz – trimetoprim/sulfametoxazol, tgc – tigecyclin, col – colistin, n-200

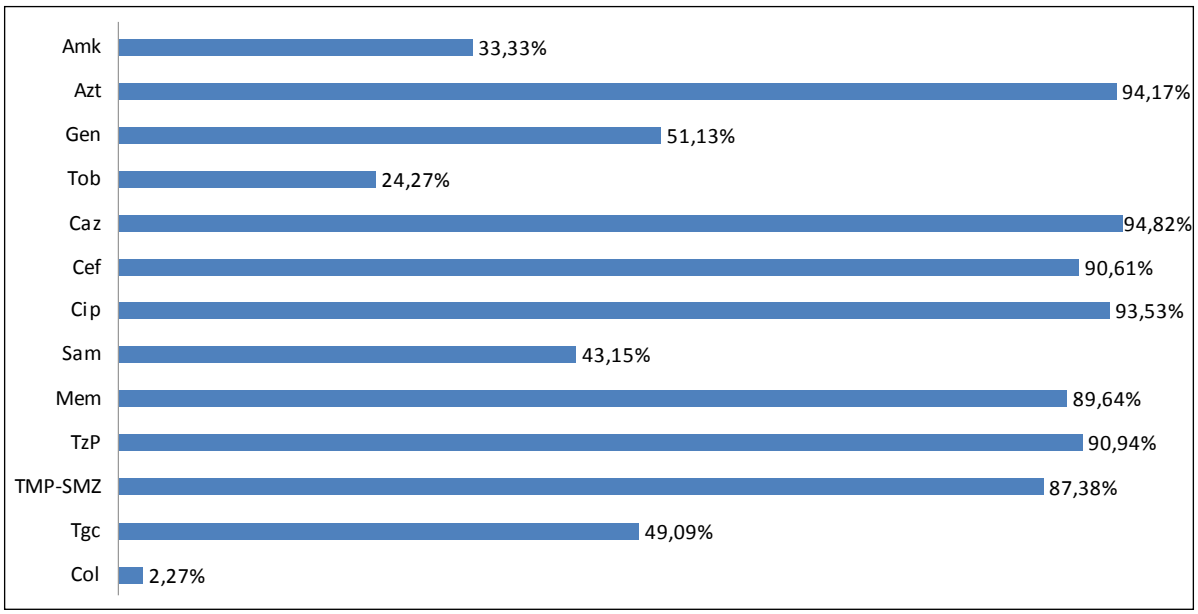

Fig. 4. Resistance *of P. aeruginosa* strains to antimicrobials for 2014 amk – amikacin, azt – azreonam, gen – gentamicin, tob – tobramycin, caz – ceftazidim, cef – cefepim, mem – meropenem, tzp – piperacillin/tazobactam, cip – ciprofloxacin, tmp-smz – trimetoprim/sulfametoxazol, col – colistin, n-309

Carbapenem resistance among common *Enterobacteriaceae* has increased sharply over the past decade. The proportion of *Enterobacteriaceae* resistant to carbapenems increased from 0.0% to 1.4% in 2010, with most of the increase recorded in *Klebsiella spp*. (CDC, 2013).

In comparison with 2008, when the resistance to meropenem registered for *E. coli* was 0.0% and for *K. pneumoniae* - 1.4%, the data for 2014 showed an increase of 3.3% for *E. coli* and 5.9% for *K. pneumoniae*, respectively. In Europe, there are distinct epidemiological situations, corresponding mainly to the diffusion of *OXA-48* producers in France, Belgium, the Netherlands and Turkey, and *KPC* – producing isolates in Italy and Greece (Poirel *et al.,* 2014). In contrast, in 2012 the first NDM-1 carbapenemase-producing *E. coli* strains were described in Bulgaria, together with the extended-spectrum-lactamase *CTX-M-15* and the *16S rRNA* methylase *RmtB*, conferring high-level resistance to all aminoglycosides (Savov *et al.,* 2012; Poirel *et al.*, 2014). All the isolates were clonally related and belonged to the same sequence type, ST101. In addition to being the first to identify *NDM*-producing isolates in Bulgaria, this was the very first study reporting an outbreak of *NDM-1*-producing *E. coli* in the world (Poirel *et al.,* 2014). The recent information on *NDM-1* producers /mostly *K. pneumoniae* isolates/ has often been related to imported cases, with a link to the Indian subcontinent. Therefore, it is very important to note that Bulgarian *E. coli* strains, positive for NDM-1 production, together with those, isolated in Croatia (Mazzariol *et al*., 2012), Kosovo (Struelens *et al*., 2010), Serbia (Jovci *et al*., 2011), Bosnia and Herzegovina (Struelens *et al.,* 2010) and Montenegro (Struelens *et al*., 2010), suggest that the Balkan region is probably another area of endemicity in addition to the Indian subcontinent.

Aminoglycoside resistance has become an expected companion to the NDM-1 beta-lactamase in almost every isolate, but this specific association between the *blaNDM*-1 and *rmtB* genes has been very rarely reported, and mainly in *E. coli*, with a single isolate from Australia (Poirel *et al.,* 2010) and a single isolate from Belgium (Pakistan origin) (Bogaerts *et al.,* 2011). *VIM* producing organisms also exhibit high resistance to aminoglycosides, due to aminoglycoside-modifying enzymes such as acetyltransferases encoded by genes such as *aacA4* found in *P. mirabilis* strains, isolated at MMA in 2014 (Pfeifer *et al.,* 2015). Unfortunately, in many cases most carbapenemases were found with a number of other resistance factors that encode resistance to almost all classes of antibiotics, used to treat infections caused by Gram-negative bacteria and most affected were the aminoglycosides and fluoroquinolones, antibacterials that have been used widely as therapeutic options for variety of Gram-negative infections (Bush, 2013).

Multidrug (pandrug)-resistant *Acinetobacter baumannii* infections

A. baumannii has emerged as one of the most troublesome pathogens for health care institutions on a global scale (Davis *et al*., 2005; Peleg *et al*., 2008; Savov *et al*., 2015). Its clinical significance, especially over the last 15 years, has been propelled by its remarkable ability to acquire resistance determinants, making it one of the organisms threatening the current antibiotic era (Davis *et al*., 2005). The rapid global emergence of *A. baumannii* strains resistant to all β-lactams, including carbapenems, quinolones and other antimicrobials, illustrates the potential of this organism to respond swiftly to changes in selective environmental pressure (Peleg *et al.,* 2008). After performing whole-genome sequencing of a clinical epidemic *A. baumannii* strain found in France /AYE/, an 86-kb resistance island, one of the largest to be described thus far, was identified /*AbaR1/.* Overall, 52 resistance genes were identified, and surprisingly, 45 (86.5%) were localized in the *AbaR1* resistance island (Fournier *et al*., 2006).

Resistance to carbapenems at the MMA in Sofia (89% to meropenem) (Fig. 3) is associated with the production of *Oxa 23* and *Oxa 58* carbapenemases, but not to metallo-beta-lactamases (Stoeva *et al.,* 2009; Savov *et al.,* 2010). The resistance to quinolones was assessed at the DNA level for mutation detection in quinolone-resistance-determining regions /QRDRs/ and the subsequent aminoacid substitution in the *GyrA* and/or the *ParC* enzymes. A strong correlation was found between quinolone resistance and mutations *in gyrA codon 83* and/or in the *parC* gene /*codons 80 or 84*/ (Deccache *et al.*, 2011).

Recently, we have isolated a *A. radioresistens* strain, which can be considered as a cause of opportunistic infection in immunocompromised patients. Additionally, we found that the strain harboured the carbapenemase gene *blaOXA-23* without insertion sequences upstream of this gene but with the sensitivity to imipenem and meropenem (Savov *et al*., 2015). It is not uncommon since *A. radioresistens* is known as a silent source of carbapenem resistance

owing to chromosomally encoded *blaOXA-23*-like genes lacking insertion sequences as described previously (Poirel *et al*., 2008; Boo *et al*., 2009).

Multidrug-resistant *Pseudomonas aeruginosa* infections

P. aeruginosa is a non-fermenting Gram-negative microorganism, which is a major dreaded cause of infection among hospitalized patients, usually with localized or systemic impairment of immune defence. It is a common cause of hospital-acquired infections like pneumonia, urinary-tract infections, wound infections, respiratory tract infections, bloodstream infections, especially in the units with high risk for infection development (Jalal *et al*., 2000; ECDC, 2010). According to our data for 2014, *P. aeruginosa* occupies the 6th place with 6.4% among the first 10 most frequently isolated microorganisms in the MMA and with 15% isolation in ICU, as well (MMA, 2015). *P. aeruginosa* is resistant to the majority of antimicrobial compounds due to its selective ability to exclude various molecules from penetrating its outer membrane and involvement of an active efflux mechanism. It is a problem, because in many cases the *P. aeruginosa* strains isolated were multiresistant - with resistance to: piperacillin/ tazobactam of 54%, cefepime - 53%, and ceftazidime - 38%. The level of the resistance to meropenem is 55% (Fig. 4).

The spread of similar multiresistant strains is very important for big hospital complexes also according to Edalucci et al*.,* 2008. This multiresistance is usually connected with production of metallo-beta-lactamase (MBL) *VIM-2* and also these widespread clones, responsible for human infections, belong to O11 and O12 serotypes (Edalucci *et al.*, 2008). Another problem which is very important for the treatment of infections caused by these microorganisms is the resistance development to quinolones in the last years. Evolution at the molecular level involves the gradual accumulation of mutations /and other changes/ in DNA sequences. The major mechanism of resistance of this bacterium to fluoroquinolones is the modification in *gyrA* gene supplemented by possible changes in *parC* and *mexR* regions. In this sense, we reported the detection of point mutations in *codon 83* of *gyr A* gene, *codons 87* and *136* of the *parC* and *codons 126* and 44 of the *mex*R regulatory gene. Mutations in *gyr A* gene were found in all *P.aeruginosa* strains, resistant to ciprofloxacin (Savov *et al*., 2014).

Perspectives, cooperation against resistant bacteria

Antibiotic resistance, especially in Gram-negative bacteria, is a global problem with many examples of the rapid spread of new resistance between continents. Laxminarayan and colleagues warn that "we are at the dawn of the postantibiotic era", with "almost all disease-causing bacteria resistant to the antibiotics commonly used to treat them" (Laxminarayan *et al.,* 2013). Recent routine surveillance of antibiotic resistance in commensal *E. coli* from food animals in China has documented a major increase of colistin resistance due to a highly mobile, transferrable, plasmid-mediated colistin-resistance gene designated *mcr-1* with the possibility to pass to *K. pneumoniae* and even to *P. aeruginosa* strains. (Liu *et al*., 2016). Later similar *E. coli* strains were isolated from patients, which means that we may soon face a situation without useful antibiotics to treat infections caused by MDR Gram-negative bacteria (Coetzee *et al*., 2016; Prim *et al*., 2016). This multidrug-resistance affects the practice in many different medical disciplines and resolution needs coordination of efforts of the different medical specialists and pharmacists. It is very important and in this connection at the EU-US Summit on November 3, 2009 in Washington, President B. Obama, Jose Manuel Barroso, Fredric Reinfeldt, and Javier Solana agreed to establish a transatlantic task force on urgent antimicrobial resistance issues /EU-US Summit agrees to form transatlantic task force on antimicrobial resistance /www.reactgroup.org/. The task force has to focus on appropriate therapeutic use of antimicrobial drugs in the medical and veterinary communities, prevention of both healthcare and community-associated drug resistant infections, and strategies for improving the pipeline of new antimicrobial drugs, which could be better addressed by intensified cooperation between the US and Europe. Following this, the IDSA Antibiotic Availability Task Force announced the necessity to achieve the development of ten new antibiotics within the next ten years (the 10 × '20 initiative), meaning that the aim is to develop 10 novel drugs for Gram-negative bacteria by the year 2020 /www.reactgroup.org, http://www.medscape.com/. Further, the key elements to be used in this aspect include improvement of infection prevention and control practices in human and animal health, improvement of professional education, training and public engagement, better access to and use of surveillance data, better identification and prioritization of research into antimicrobial resistance and

strengthened international collaboration (Howard *et al*., 2013).

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