

Pseudomonas aeruginosa infections in the "Sfanta Parascheva" Infectious Diseases Hospital of Iasi City

Andrei Vata^{1,2}, Romulus Pruna³, F. Rosu^{1,2}, Egidia Miftode^{1,2}, E.V. Nastase^{1,2},
Luminita Gina Vata¹, Olivia Dorneanu^{1,2}

¹"Gr. T. Popa" University of Medicine and Pharmacy of Iasi City, Romania

²"Sfanta Parascheva" Infectious Diseases Hospital of Iasi City, Romania

³Emergency County Hospital of Piatra-Neamt, Romania

ABSTRACT

Background. *Pseudomonas aeruginosa* is a dangerous, hard to treat pathogen, due to an increasing frequency of resistance to multiple antibiotics. This susceptibility pattern is influenced by multiple factors and it frequently has a regional or local character, different for each hospital or intensive care unit.

Methods. We analysed and compared the characteristics of 62 patients and their isolated *P. aeruginosa* strains, treated in the "Sfanta Parascheva" Infectious Diseases Hospital of Iasi City between January and December 2011 (Group 1 – 25c) and between January and December 2016 (Group 2 – 37c). The susceptibility was tested by disk diffusion test; CLSI standards were used.

Results. The median age was higher in group 2 (63 years) vs group 1 (52 years, $p=0.04$), more men were affected in both groups (59.2 vs 57.5%). In group 2 the strains were isolated from urine (50%), sputum (17.5%), wound secretions (15%), blood cultures (10%) or central venous catheters (7.5%); more than half may be of nosocomial origin; the infection mainly occurred in patients with significant comorbidities, long-term hospitalization (median - 15 days), ICU care or mechanical ventilation. We did not find a significant increase in the resistance rates in group 2 for the tested antibiotics; they remain high for almost every drug: 55 vs 60% for imipenem, 70 vs 62% for meropenem, 31 vs 41.7% for ceftazidime, 68 vs 75% for ciprofloxacin, 50 vs 42.8% for gentamicin, 63.6 vs 45.8% for amikacin, 46 vs 30% for piperacillin-tazobactam. All isolates were susceptible to colistin. The share of MDR isolates was slightly higher in group 2 (52 vs 61%, $p=0.4$). The treatment of these infections was difficult, with an average duration of 15 days; 20% of patients died.

Conclusions. In our hospital, *P. aeruginosa* infections appeared predominantly in elderly patients, often in association with medical care, were associated with multidrug resistance to anti-biotics and sometimes had a guarded prognosis. The antibiotic susceptibility rates did not vary significantly in the two time intervals that we analysed.

Keywords: *Pseudomonas aeruginosa*, infection, antibiotic resistance, intensive care

INTRODUCTION

Pseudomonas aeruginosa is a non-spore-forming single-flagellum gram-negative bacillus, with incredible nutritional versatility. It is strictly aerobic, but it may also exist in anaerobic conditions. It is capable of catabolizing a wide range of organic substrates, which makes it ubiquitous.

It is an opportunistic pathogen for humans, as it often colonizes immunocompromised hosts (namely patients suffering from cystic fibrosis, cancer,

HIV infection) and it has intrinsic resistance to a wide range of antibiotics (1).

P. aeruginosa produces a water-soluble pigment, which, when exposed to ultraviolet radiation, generates a blue-greenish fluorescence (called pyocyanin), hence its name "pyocyanic" bacillus. These cultures have a specific grape or jasmine flower smell.

P. aeruginosa exhibits a circular extremely twisted chromosome in cytoplasm and a very large number of plasmids playing a very important role

Corresponding author:

Eduard Vasile Nastase, MD
E-mail: edubacau@gmail.com

Article History:

Received: 7 September 2018
Accepted: 17 September 2018

in antibacterial resistance, which turns it into a dangerous pathogen. It has a flagellum and pili, which enhance the ability of the bacterium to adhere to mucous membranes and other epithelial cells (2).

It often occurs in water tanks, sewage pipes and sinks, both in and outside hospitals. It may also occur in pools and toilet drains, as warmth enhances its multiplication (3). It is able to form biofilms on various surfaces (metal, plastic, medical implants, tissues) and it communicates with other microorganisms through *quorum-sensing* (1,4).

It may cause serious infections in animals and even in plants. In humans, it is often found in the wounds of patients having suffered burns and it often complicates the respiratory tract infections of patients with cystic fibrosis. Moreover, by altering immunoglobulin structure (Fc section), *P. aeruginosa* may then easily penetrate the inner environment and thus cause systemic infections.

P. aeruginosa is a pathogen commonly associated with healthcare and it has shown increasingly higher resistance to many classes of antibiotics; it often has regional character, specific to each hospital or intensive care unit.

P. aeruginosa's main antibiotic resistance mechanisms are: beta-lactamase production (which are chromosome- or plasmid-mediated, of the AmpC, TEM, SHV, PER, PSE or OXA type; the most recent data have identified 2 carbapenemases, i.e. IMP and VIM), efflux pumps (MexAB-OprM) – which determines resistance to fluoroquinolones, anti-pseudomonas penicillin and cephalosporins, aminoglycoside-inactivating enzymes (acetyltransferases and adenylyltransferases), which causes them to bind poorly to ribosomes (5).

Considering that an increasing number of researches and statics have proven the increase of the resistance rates of *P. aeruginosa* to an ever-higher number of antibiotic classes, our goal is to achieve a comparative analysis of patients infected by this microorganism at two different times (2011 and 2016), in order to describe and characterize the types of infections and the sensitivity to antibiotics of this microorganism, and also in order to determine the dynamics of these parameters.

MATERIALS AND METHOD

We conducted a retrospective cross-sectional study, which included patients diagnosed with *P.*

aeruginosa infection in 2011 (group 1 – 25 patients) and 2016, respectively (group 2 – 38 patients), in the “Sfânta Parascheva” Infectious Diseases Hospital of Iasi City.

The clinical and demographic data were taken from the General Clinical Patients Records in the hospital's archives, whereas the microbiological data were found in the Register of the microbiology laboratory in our hospital. Data related to antibiotics consumption were gathered from the electronic system of the hospital Pharmacy.

Our study included patients in whom a *P. aeruginosa* strain, considered infecting, was isolated in biological products considered naturally sterile or in potentially contaminated products in cases of multiple locations or in cases of related local or systemic inflammatory manifestations.

Literature data have shown that *P. aeruginosa* infections occur especially in immunosuppressed patients, in many cases by associated diseases. In the patients included in our research, these associated diseases were quantified by means of the Charlson Comorbidity Index score.

The disk diffusion test was used to determine the antibiotic sensitivity of the strains, in accordance with the CLSI standards in effect at the time of their isolation. CMI was determined by the E-test method. A strain was considered multidrug-resistant (MDR) if it exhibited no sensitivity to more than one representative of at least 3 different antibiotic classes (piperacillin-tazobactam, ceftazidime, fluoroquinolones, aminoglycosides, carbapenems).

RESULTS

From the demographic point of view, the infections concerned mostly aged patients, the median age was 52 years in 2011 and increased to 63 years in 2016 ($p=0.04$); there were of course exceptions to this trend.

The patients were mainly male. Group 1 included 3 times more men than women, yet the difference was smaller in the second group (the M/F ratio was 3.16 in 2011 and 1.37 in 2016).

As far as location is concerned, urinary tract infections were the most common (12/25 in the 2011 group and 14/37 in the 2016 group), followed by surgical wounds or bedsores (8/25 in the 2011 group and 10/37 in the 2016 group), and respiratory

infections, especially in the second group (2/25 in the 2011 group and 7/37 in the 2016 group) or meningial group in the first group (1/25 in the 2011 group).

TABLE 1. Patients' demographic, clinical and epidemiological characteristics

	Group 1 (2011)	Group 2 (2016)	p
Number of cases	25	37	
Median age (years)	52	63	p=0.041
• Highest	82	84	
• Lowest	4	17	
M/F ratio	3.16	1.37	p=0.14
Location of infection			
• urinary tract	12	14	p=0.45
• wound	8	10	
• blood cultures	1	4	
• catheter	1	3	
• sputum	2	7	
• LCR	1	0	
Nosocomial infection (no. of cases)	14	23	p=0.12

In most cases, these were nosocomial infections, which occurred during the patients' hospitalization in our clinic or in other hospitals, the patients being subsequently transferred to our unit for treatment (table 1).

P. aeruginosa infections concerned patients with significant and often multiple comorbidities (76% in the 2016 group and 79% in the 2016 group, respectively). The mean Charlson score was 8.1 in the first group, and 8.7 in the 2016 group. Some of

these were liver conditions, diabetes, neurological, oncological or kidney conditions.

We also analysed the *P. aeruginosa* infection rate from the viewpoint of the patients' previous hospitalisation in an intensive care unit. Thus, we noticed that 28% and 37% of the patients in the first and second group, respectively, had passed through an intensive care unit, and their infections occurred during or after their discharge from such unit. This percentage was higher in 2016, yet no statistical significance could be proven. We also noticed that 24% of the patients in the first group and 16% of the patients in the second group had been mechanically ventilated, yet no specific correlation could be established between infection location and this procedure.

As far as the resistance to antibiotics of the *P. aeruginosa* strains isolated from the patients included in the study was concerned, we noticed that Ceftazidime and Cefepime were and still are the main anti-pyocyanic cephalosporins belonging to the beta-lactam class of antibiotics, which finding actually supports literature data. Nonetheless, the resistance rates are also high in these antibiotics, as they amounted to 50% to Cefepime in the 2016 group, and they increased from 31.8% in 2011 to 41.7% in 2016 as far as Ceftazidime is concerned.

Piperacillin-tazobactam, a drug which is little used in our clinic, also belongs to the anti-*Pseudomonas* beta-lactam antibiotics class. We want to point out though that it is the most potent anti-

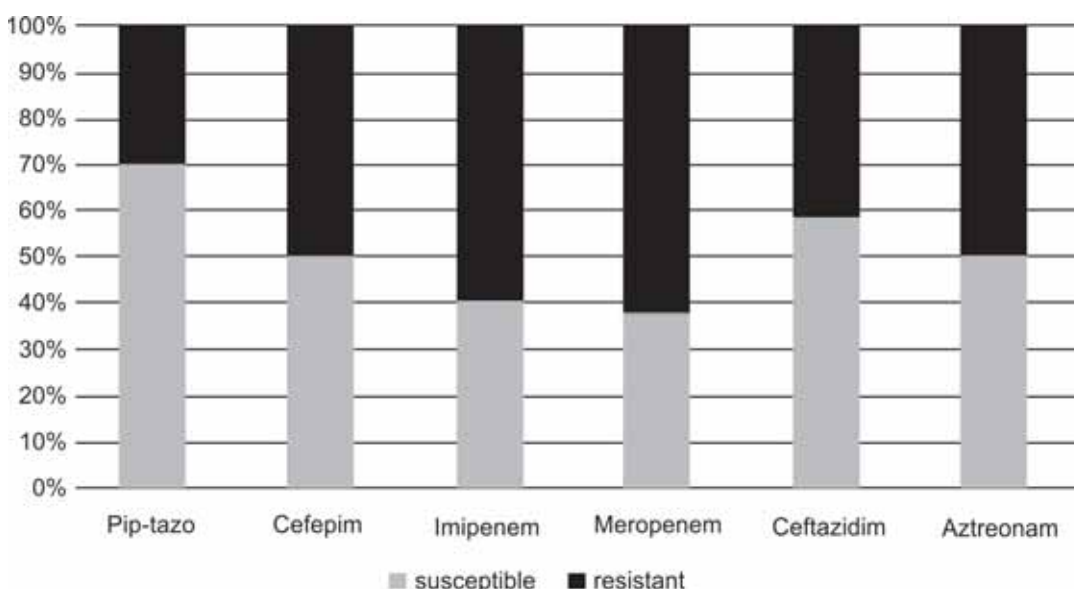


FIGURE 1. Sensitivity of *P. aeruginosa* strains in the second group (2016)

Pseudomonas drug in its class and it has the lowest resistance (30% in the 2016 group, which was about 16% lower than the 46.1% found in the 2011 group – a difference with no statistical significance).

The resistance of *P. aeruginosa* strains to carbapenems is a particularly important aspect. Imipenem showed an increase from 55% in the 2011 group to 60% in the 2016 group, whereas Meropenem exhibited a statistically insignificant decrease from 70% to 62%.

TABLE 2. Beta-lactam resistance dynamics (% resistant strains)

	Pip-tazo	Cefepime	Imipenem	Meropenem	Ceftazidime	Aztreonam
Group 2	30	50	60	62	41.7	50
Group 1	46.1	ND	55	70	31.8	35.3
p	0.23	-	0.73	0.81	0.74	0.35

The resistance of isolated strains to systemic aminoglycosides exceeded 40% (42.8% to gentamicin and 44% to amikacin). The resistance rates were even higher to fluoroquinolones: 75% to ciprofloxacin, 84.6% to ofloxacin and 64% to levofloxacin. All the strains that we tested were sensitive to colistin (Figure 2).

As compared to the first group, the strains isolated in the patients included in the second group exhibited lower resistance rates to aminoglycosides and ciprofloxacin (no statistical significance). All the strains were sensitive to Colistin (table 3).

TABLE 3. Resistance dynamics to other classes of antibiotics (% resistant strains)

	Gentamicin	Tobramycin	Amikacin	Ciprofloxacin	Colistin
Lot 2	42.8	65	45.8	75	0
Lot 1	50	66.7	63.6	68	0
p	0.63	-	0.17	0.85	-

The multi-drug resistance rates in the patients included in the first group amounted to 52%, and they were even higher in those belonging to the second group, namely 61% (p=0.5).

No noteworthy differences were revealed between the two groups as concerns the duration of the antibiotic therapy, the median duration being 15 days in the second group and 16 days in the first.

The lethality rate of the *P. aeruginosa* infection remained relatively constant: 20% in the 2011 group and 18.4% in the 2016 group.

DISCUSSIONS

P. aeruginosa infections are a challenge nowadays, especially in view of the antibiotic resistance profiles of its strains. In addition, infections with this microorganism involve a high degree of severity also due to the type of patients affected by them. Aged individuals with significant co-morbidities and with particularly severe conditions requiring hospitalization in intensive care units or requiring oral-tracheal intubation and mechanical ventilation are the most exposed.

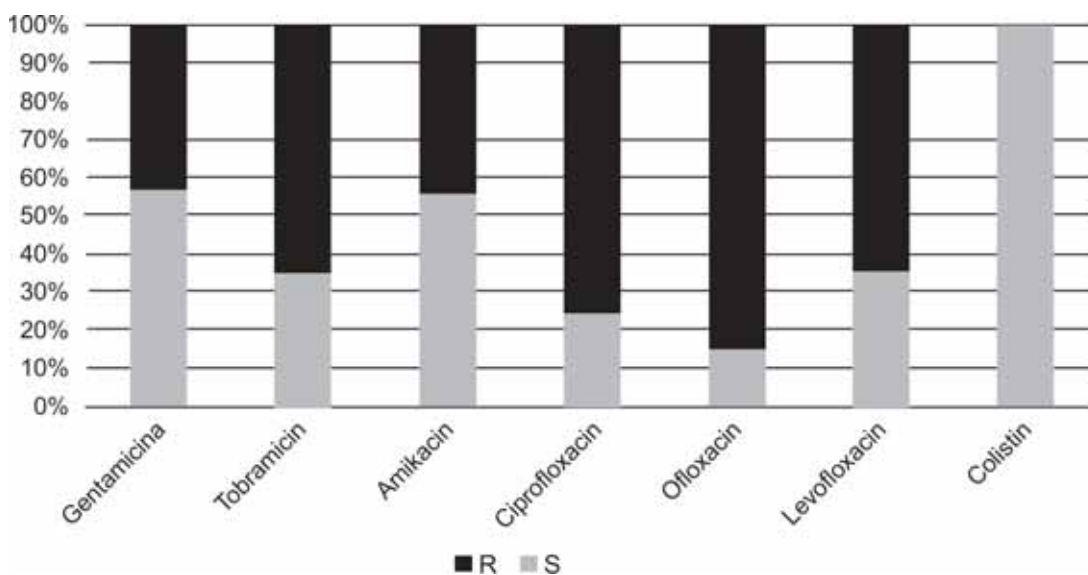


FIGURE 2. Sensitivity to other antibiotics classes: group 2 – 2016

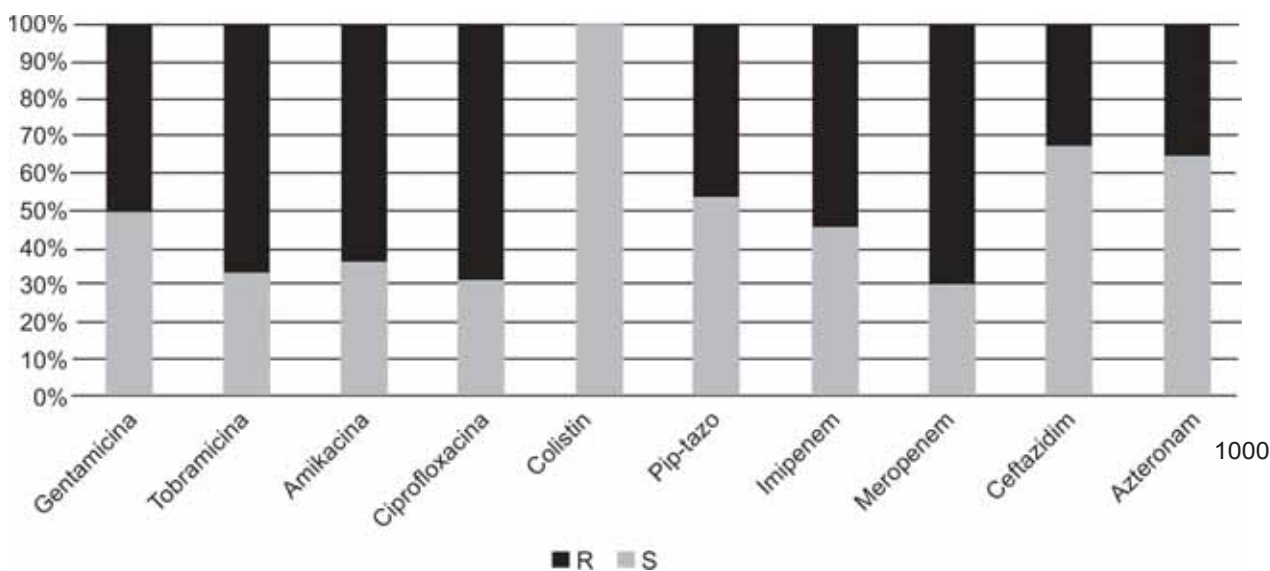


FIGURE 3. Sensitivity of *P. aeruginosa* strains in the first group (2011)

A problem identified by this study is the extremely high rate of carbapenem-resistant *P. aeruginosa* strains. Regardless of the carbapenem chosen, the fact that about 60% of the strains were already resistant is a worrying fact, given the position of carbapenems in the class of backup antibiotics. The main mechanism of resistance to carbapenems is the production of carbapenemases, as the genes encoding these enzymes may be carried via plasmids (6,7). The selection pressure of these strains may be generated by excessive consumption of antibiotics. Whereas for the use of carbapenems in the treatment of various conditions, the selection pressure for carbapenemase-producing strains appears to be a logical hypothesis to correlate the increase in the incidence of carbapenem-resistant strains with the excessive use of other classes of antibiotics, there is no precise link. However, a study conducted in a USA farm on pigs extensively treated with cephalosporins revealed a statistically significant correlation between the rate of occurrence of gram-negative bacilli strains producing carbapenemases and the consumption of cephalosporins (3).

As far as the Infectious Diseases Hospital of Iasi City is concerned, between 2011 and 2016, the Ceftazidime consumption showed an approximately 5-fold increase, whereas the Imipenem and Meropenem consumption was approximately 7 times higher.

According to the ECDC statistics, in comparison to the situation in the European Union, Roma-

nia reported resistance rates of *P. aeruginosa* strains isolated from invasive infections to Ceftazidime of 44.2% (1st place, followed by Bulgaria, Greece, Slovakia), to Piperacillin-Tazobactam of 48.8% (1st place, followed by Bulgaria, Slovakia, Italy), to Carbapeneme of 51.6% (1st place, followed by Slovakia, Croatia, Greece), to Fluoroquinolone of 51.7% (1st place, followed by Slovakia, Croatia and Bulgaria), and to aminoglycosides of 50.6% (1st place, followed by Bulgaria, Croatia, Slovakia) (8).

As regards MDR strain ratios, Romania ranks 1st once again - 66% (followed by Bulgaria, Croatia, Slovakia, Greece), while the EU median value is 12.9% (8).

The comparative analysis of the data at national level and of those gathered in the “Sfânta Parascheva” Infectious Diseases Hospital of Iasi City reveals similar resistance rates (8,9), the only exception being Piperacillin-Tazobactam in which only 30% of the strains were resistant - precisely the low-consumption antibiotic in this hospital.

CONCLUSIONS

In the “Sfânta Parascheva” Infectious Diseases Hospital of Iasi City, *P. aeruginosa* caused infections, frequently healthcare associated, especially in aged individuals with significant associated conditions and in those hospitalized in intensive care units requiring mechanical ventilation. Antibiotic resistance rates are currently high, with 61% of the 2016 strains being MDR. What is worrying is that

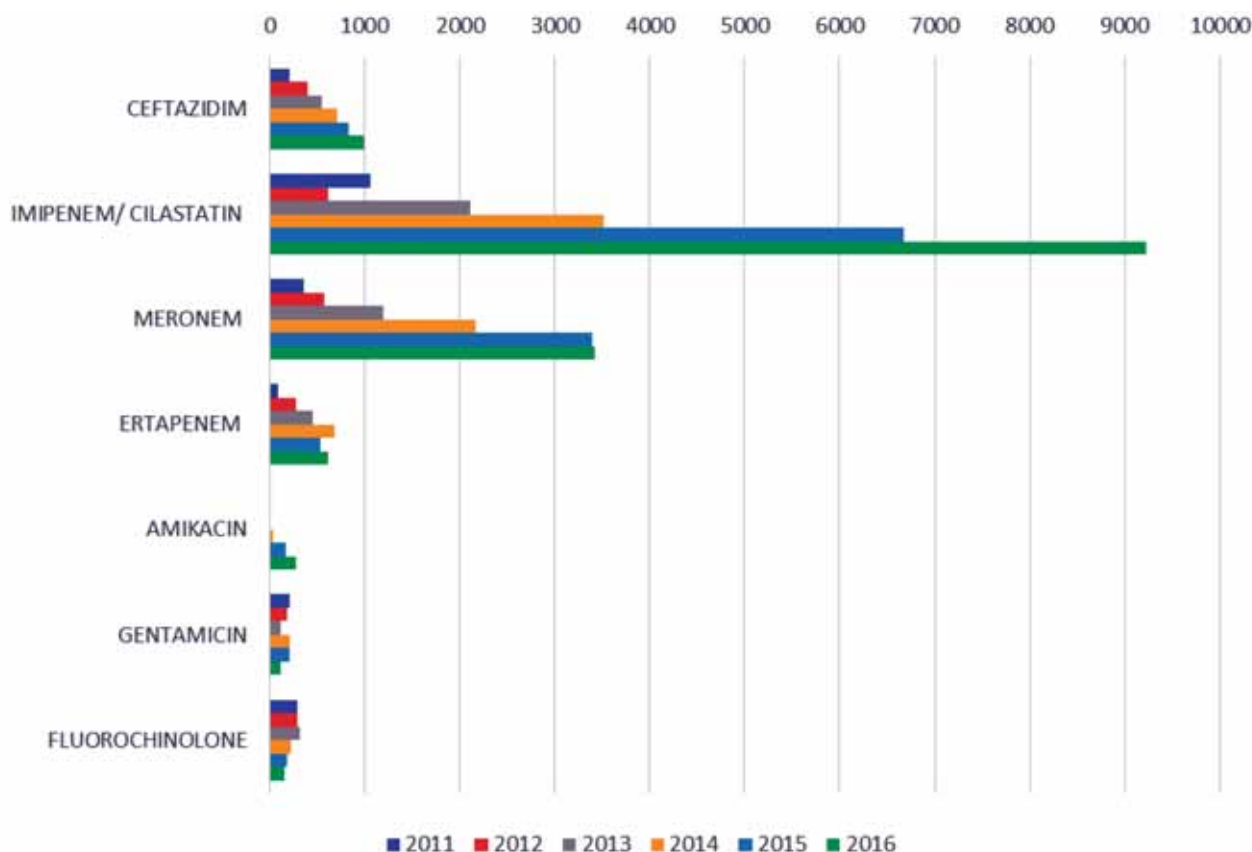


FIGURE 4. Median selected antibiotics consumption between 2011 and 2016 in the Infectious Diseases Hospital of Iasi City (DDD/100BED/DAYS)

we rank, both at regional and at national levels, at the top of this unfortunate European classification. The degree of antibiotic resistance did not vary significantly between 2011 and 2016. We can still use

Colistin as a backup antibiotic for this type of infection, as no resistant strains have been identified so far to this antibiotic.

REFERENCES

- Gellatly SL, Hancock RE. *Pseudomonas aeruginosa*: New insights into pathogenesis and host defenses. *Pathog Dis.* 2013;67(3):159-73. doi: 10.1111/2049-632X.12033
- Lyczak JB, Cannon CL, Pier GB. Establishment of *Pseudomonas aeruginosa* infection: lessons from a versatile opportunist. *Microbes Infect.* 2000;2(9):1051-60.
- Mollenkopf DF, Stull JW, Mathys DA, Bowman AS, Feicht SM, Susan V. Grooters, Daniels JB, Wittuma TE. Carbapenemase-Producing *Enterobacteriaceae* Recovered from the Environment of a Swine Farrow-to-Finish Operation in the United States. *Antimicrobial Agents and Chemotherapy.* 2016;61, 2 e01298-16
- Moradali MF, Ghods S, Rehm BHA. *Pseudomonas aeruginosa* Lifestyle: A Paradigm for Adaptation, Survival, and Persistence. *Front. Cell. Infect. Microbiol.* 2016; 7:39. doi: 10.3389/fcimb.2016.00039
- Klockgether J, Tümmler B. Recent advances in understanding *Pseudomonas aeruginosa* as a pathogen. *F1000Research.* 2016, 6:1261 doi: 10.12688/f1000research.10506.1
- Mereuță AI, Bădescu AC, Dorneanu OS, Iancu LS, Tuchiluş CG. Spread of VIM-2 metallo-beta-lactamase in *Pseudomonas aeruginosa* and *Acinetobacter baumannii* clinical isolates from Iași, Romania. *RJLabM* 2013; 21(4): 423-430.
- Dortet L, Flonta M, Boudehen YM, Creton E, Bernabeu S, Anaïs V, Thierry N. Dissemination of Carbapenemase-Producing *Enterobacteriaceae* and *Pseudomonas aeruginosa* in Romania. *Antimicrobial agents and Chemotherapy.* 2015, 59, 11: 112-114
- <http://atlas.ecdc.europa.eu/public/index.aspx>
- Gavriliu LC, Popescu GA, Popescu C. Antimicrobial resistance of *Pseudomonas aeruginosa* in a Romanian hospital at the dawn of multidrug resistance *Braz J Infect Dis.* 2016;20: 5