

Etiology and resistance patterns of *Pseudomonas aeruginosa* strains isolated from a Romanian hospital

Wisam Abdulameer Najm^{1,2}, Alexandra Bolocan^{3,*}, Diana Ionescu¹, Bogdan Ionescu¹, Irina Gheorghe¹, Otilia Banu⁴, Dan Mihailescu¹, Adina Decuseara¹

¹Faculty of Biology, University of Bucharest; Research Institute of the University of Bucharest, Romania

²University of Babylon, PO Box: 4, - Babylon - Hilla, Iraq

³Emergency Institute of Cardiovascular Diseases "Prof. Dr. C.C. Iliescu", Bucharest, Romania.

⁴Emergency University Hospital, Bucharest, Romania; "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania

*corresponding author e-mail address: bolocan.alex@gmail.com

ABSTRACT

Pseudomonas aeruginosa is a Gram-negative, aerobic, non-fermentative opportunistic bacterium that causes a wide variety of infections in immunocompromised individuals. The objective of this study was to determine the etiology and antibiotic resistance patterns of the *P. aeruginosa* strains isolated from patients with nosocomial infections diagnosed in the Emergency Institute for Cardiovascular Diseases "Prof. Dr. C.C. Iliescu", Bucharest. The study was performed on 1094 samples acquired from patients with ages between 0-91 years starting from January 2012 to December of 2013. The prevalence of *P. aeruginosa* infections was 18% (197) from the total number of samples. From the 197 *P. aeruginosa* analyzed strains almost half were obtained from tracheal secretions (40%). The antibiotic susceptibility analysis showed a high degree of resistance to the majority of antibiotics tested (35%).

Keywords: cardiovascular surgery, mortality rate, hospitalization days.

1. INTRODUCTION

Pseudomonas aeruginosa is a Gram-negative, aerobic, nonfermentative opportunistic bacterium that is found in soil, water and moist environments in general [1-3]. It has minimal nutritional requirements and can withstand low temperatures [4-6]. *P. aeruginosa* forms biofilms being able to adhere to different substrates [7-10], gaining resistance to environmental factors and a large number of commonly used antibiotics [11-13].

P. aeruginosa causes a wide variety of infections in immunocompromised individuals: meningitis malignant otitis externa [14-16], sepsis, endocarditis or osteomyelitis, community-

acquired pneumonia, urinary tract infections and peritonitis [17-21].

P. aeruginosa has a particular tropism for cystic fibrosis (CF) epithelial cells and is resistant to the normal respiratory tract host defenses [22-28].

The objective of this study was to determine the identity and antibiotic resistance patterns of the *P. aeruginosa* strains isolated from patients with nosocomial infections diagnosed in the Emergency Institute for Cardiovascular Diseases "Prof. Dr. C.C. Iliescu", Bucharest.

2. EXPERIMENTAL SECTION

The study starting from January 2012 to December 2013 and was realized on 1094 samples acquired from patients with ages between 0 and 91 years hospitalized in different wards of the Emergency Institute for Cardiovascular Diseases "Prof. Dr. C.C. Iliescu". The tested bacterial strains were obtained from various sources: tracheal samples, wounds, urine, blood, sputum, nasal carriage and soft tissue injuries.

The strains isolation was made on MacConkey Agar, CLED Agar and Columbia Blood Agar medium. The identification strains was based on oxidase and API NE

bioMerieux tests. For identity confirmation, it was used Vitek 2 Compact bioMerieux based on the determination of the metabolic activity of bacteria.

The resistance patterns of the studied strains were determined using the conventional Kirby-Bauer disc diffusion method in accordance with the CLSI guidelines. The antibiotics panel contained: piperacilin (PRL), cefepime (FEP), aztreonam (ATM), ceftazidime (CAZ), ciprofloxacin (CIP), levofloxacin (LEV), meropenem (MEM), amikacin (AK), tobramycin (TOB), gentamicin (CN) and colistin (CO).

3. RESULTS SECTION

The prevalence of *P. aeruginosa* infections was 18% (197), with a higher incidence in the tracheal secretions isolated strains.

The results showed that, from the total of 197 strains of *P. aeruginosa*, isolated in two years, 79 (40%) were isolated from

tracheal secretions, 47 (24%) from wounds, 25 (13%) from urine, 22 (11%) from blood, 17 (9%) from other various secretions, 5 (2%) from sputum and 3 (1%) from nasal secretions (Fig.1).

It was analyzed the incidence of *P. aeruginosa* infections in relation to the clinical departments, from where the samples was obtained. The results of this prevalence study showed that the cardiovascular surgery presented the highest rate of cases (100), followed by the vascular surgery (57 cases), cardiology ward 1 (24), Intensive Care Units -ICU (7), cardiology ward 2 (4), cardiology ward 4 (3) and cardiology ward 3 (2). The highest number of deaths (63) was also recorded in the cardiovascular surgery department (Fig. 2).

Regarding the age of the patients with *P. aeruginosa* infections, there was no patient younger than 20 years and most individuals were over the age of 65 years (109 cases). The age infections distribution also showed that 71 of cases were encountered in patients with ages between 50 and 64 years, 15 cases belonged to the 35 and 49 years category and 2 in the 20-34 age interval (Fig. 3). The average age was found to be 66 years.

The gender criteria pointed out that 151 (77%) males were affected by these infections in comparison to the 46 (23%) females (Fig. 4).

An important part of the study was related to the possible correlations between the gender, age and the hospitalization interval for the patient with *P. aeruginosa* infection.

The mean duration of hospitalization days in the case of patients with *P. aeruginosa* tracheal infections was 62 days, and their average age was of about 65 years. Of these patients 78% were male and 22% female. 15 (20%) patients needed more than 100 days of hospitalization in ICU, 28 (35%) had between 50 and 100 days of hospitalization and 36 (45%) had less than 50 days.

Regarding the patients with wound infections, correlation between age and length of hospital stay revealed the following average values: 65 years and 16 days, respectively. From the total number of patients presenting infections at this site 37 (79%) were males and 10 (21%) females. Most of patients included in this category were hospitalised less than 20 days, 8 people were

hospitalized between 20 and 35 days, but there were two people hospitalized for 87, respectively up to 110 days. The large majority of these patients were aged between 40 and 65 years (48%) (Fig.6).

The patients with soft tissue injuries determined by *P. aeruginosa* required an average of 63 hospitalization days, most needing more than 40 days, with 4 of them remaining 100 days in the hospital ward. The mean age of these patients was around 61 years (Fig. 7).

The mean length of hospital stay in the case of patients with *P. aeruginosa* blood infections was 57 days, and the average age was 67 years. Of these, 13 patients (59%) were male and 9 (41%) females. From all this patients, 10 (45%) required more than 80 days of hospitalization, 7 (32%) between 20 and 60 and 4 (18%) less than 10 days (Fig.8).

In the case of patients with urinary tract infections caused by *P. aeruginosa*, the average hospitalization period was 54 days.

A detailed analysis of the length of hospital stay showed that: 6 (24%) of the patients required between 90 and 130 days, 5 (20%) between 60 and 70 days, 9 (36%) between 10 to 30 days and 3 (12%) less than 10 days. The age criteria showed an average of 65 years. Regarding the gender 20 (80%) were male and 5 were female (Fig. 9).

Of the patients with *P. aeruginosa* infections isolated from sputum, 3 were elderly individuals (75 years) and 2 aged of 40 years. The length of their hospital stay did not exceed 21 days and the average period was 12 days (figure 10).

The analysis of *P. aeruginosa* resistance patterns revealed a resistance of 91.2 % in some cases with up to 36.5% of isolates resistant to 9 out of the 10 tested antibiotics.

Among the fluoroquinolones, ciprofloxacin was the most effective with a susceptibility rate of 34 %. Colistin remained the most effective antibiotic for clinical *P. aeruginosa* strains.

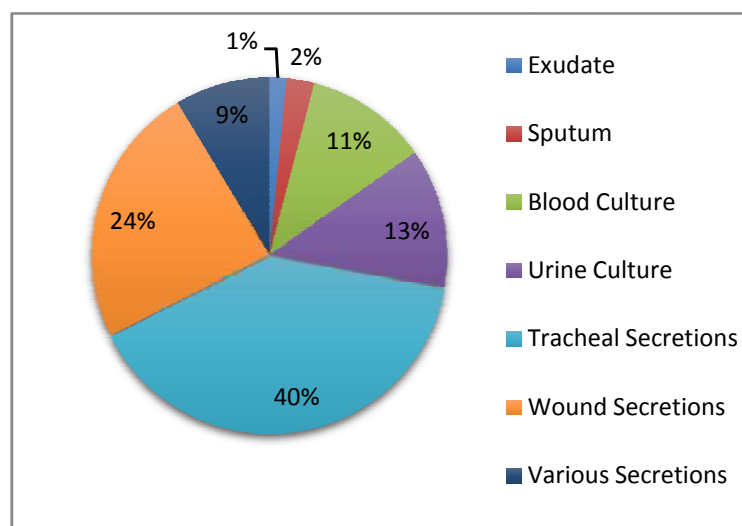


Figure 1. Graphic representation of the isolation sources of *P. aeruginosa* strains.

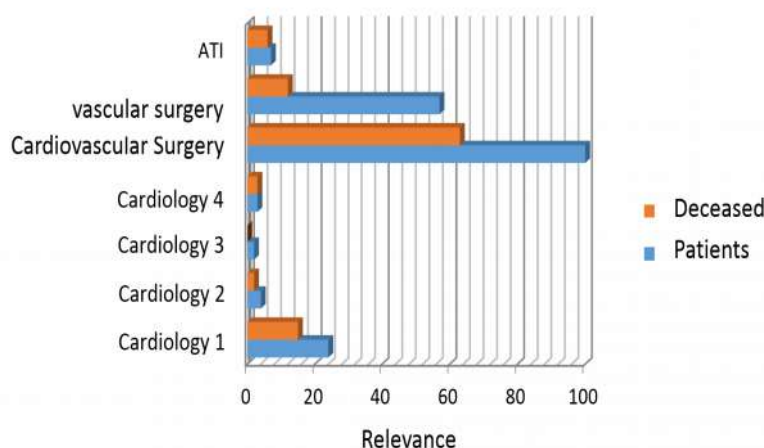


Figure 2. Prevalence of *P. aeruginosa* infections, in relation to different clinical departments and with the number of associated deaths. ATI=ICU.

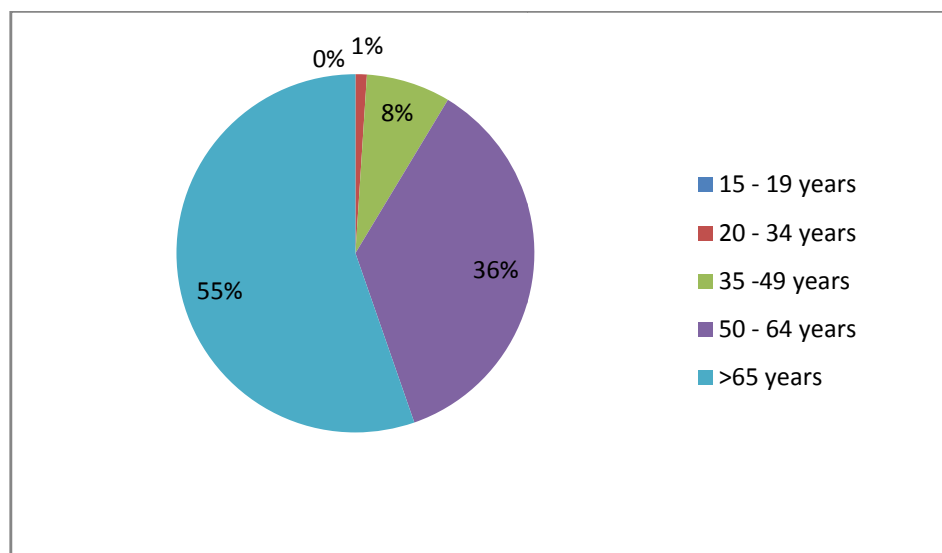


Figure 3. Age distribution of patients with *P. aeruginosa* infections.

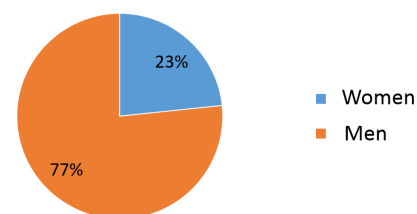


Figure 4. Gender distribution of patients with *P. aeruginosa* infections.

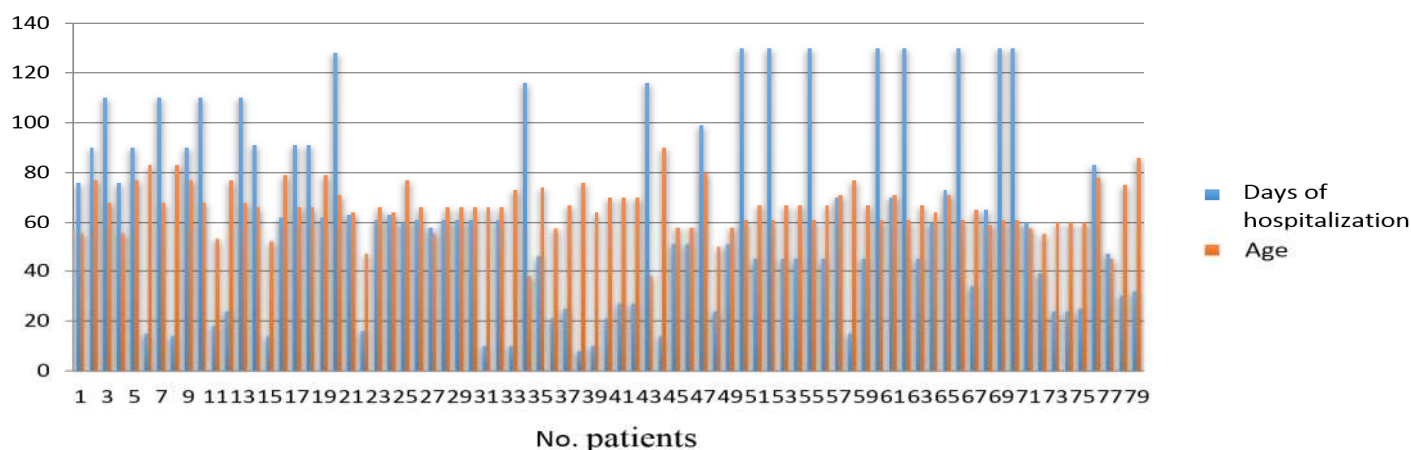


Figure 5. Correlation between the hospitalization days and the age of the patients with *P. aeruginosa* respiratory tract infections.

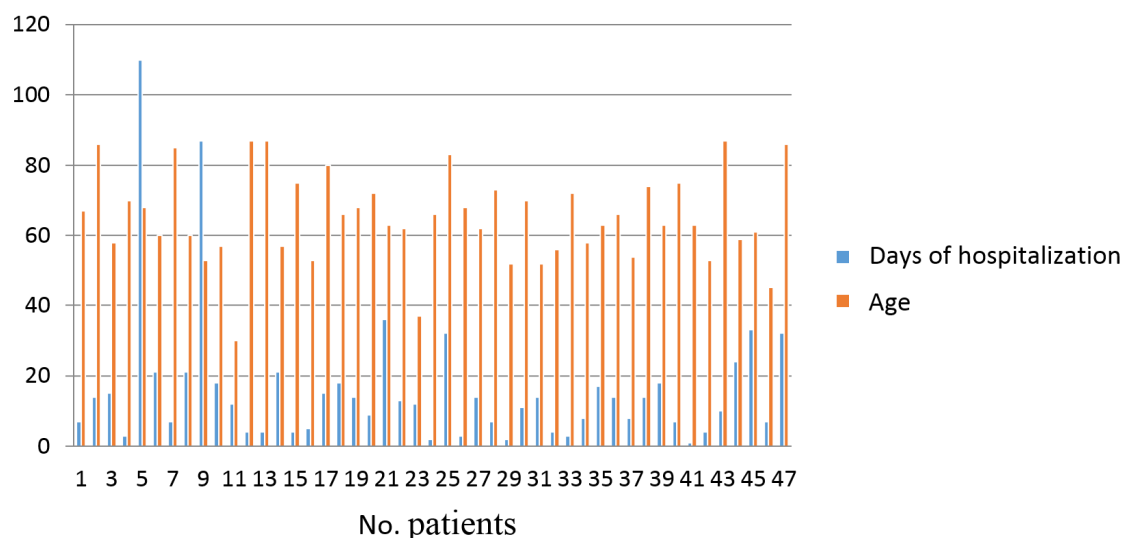


Figure 6. Correlation between the hospitalization days and the age of the patients with *P. aeruginosa* wound infections.

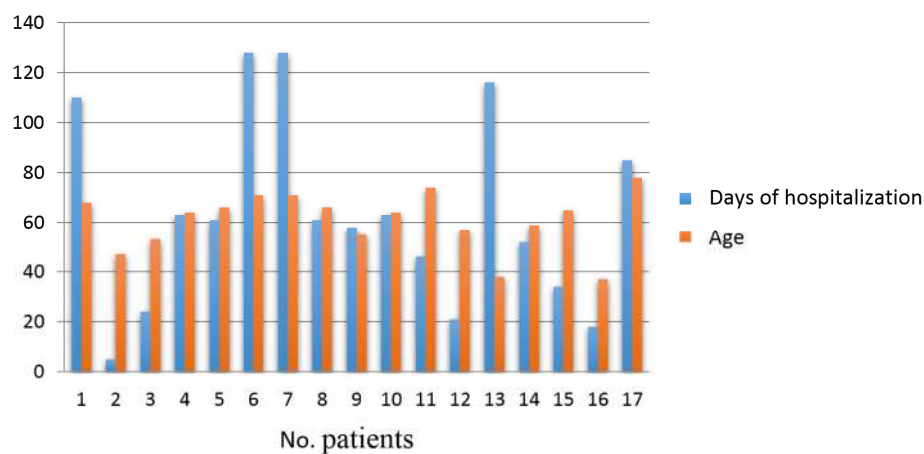


Figure 7. Correlation between the hospitalization days and the age of the patients with *P. aeruginosa* infections isolated from soft tissue secretions.

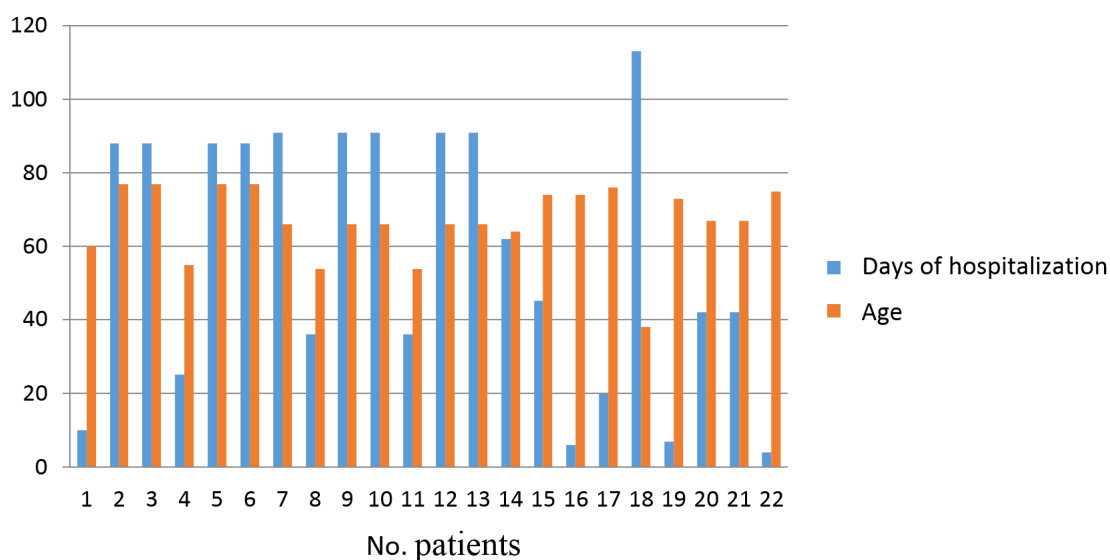


Figure 8. Number of hospitalization days correlated with the age of patients with *P. aeruginosa* positive blood cultures.

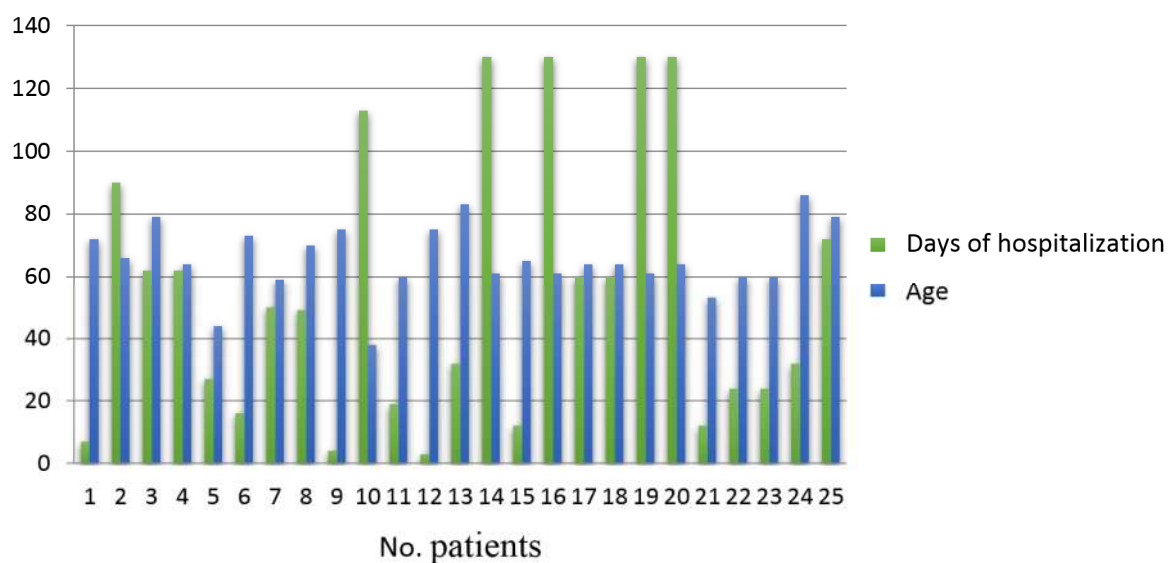


Figure 9. Number of hospitalization days correlated with patient's age in the case of *P. aeruginosa* urinary tract infections.

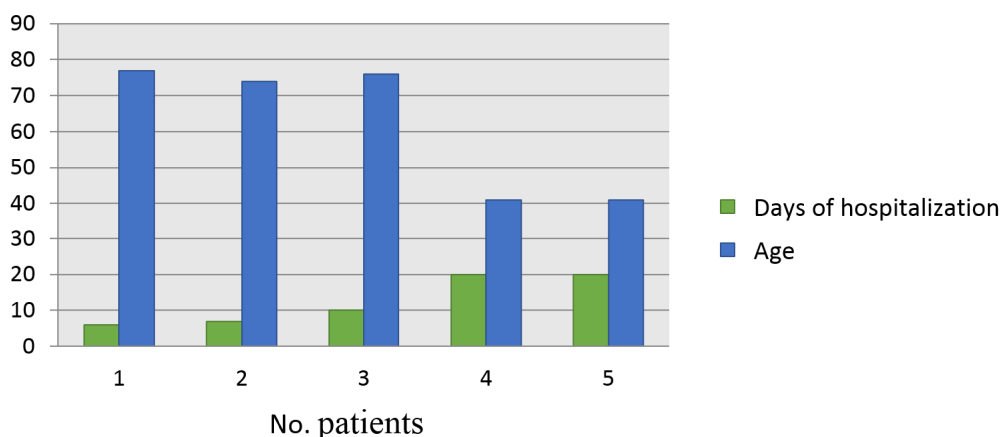


Figure 10. Correlation between the hospitalization days with the patient's age in the case of *P. aeruginosa* infections, isolated from sputum.

4. CONCLUSIONS

From the total of 197 *P. aeruginosa* strains analyzed during a 2 years period, almost half were obtained from respiratory tract secretions (40 %), followed by wound secretions (24%), urine cultures (13%), blood cultures (11%), soft tissue secretions (9%), 2% from sputum samples and subsequently, with the lowest prevalence nasal secretions (1%).

The study highlighted a significant prevalence of *P. aeruginosa* infections in cardiovascular surgery departments, these being the one with the highest mortality rate.

Another important factor taken into account in this study was the possible correlations between the patient related features such gender, age and length of hospitalization with the *P. aeruginosa* infection site. The average hospitalization period was

not influenced by the *P. aeruginosa* infection site with some exceptions (12 days for respiratory tract infections) and was between 50 and 60 days. The male elderly individuals seemed to present a higher risk of infections with *P. aeruginosa* irrespective of the anatomical site.

The *P. aeruginosa* resistance patterns analysis showed a high degree of resistance to the majority of tested antibiotics (85%). Colistin was found to be the most effective antibiotic. *P. aeruginosa* remains one of the most prolific opportunistic pathogens as it has the ability to cause serious infections in immunocompromised patients coupled with a high resistance to commonly used antibiotics and the host's immune defence effectors.

5. REFERENCES

- [1] Allen H. K., J. Donato H. H., Wang K. A., Cloud-Hansen J., Davies E., Handelsman J., Call of the wild: antibiotic resistance genes in natural environments., *Nat Rev Microbiol.*, vol. 8, pag. 251-9, **2010**.
- [2] Aloush V., Navon-Venezia S., Seigman-Igra Y., Cabili S., Carmeli Y., Multidrug-resistant *Pseudomonas aeruginosa*: risk factors and clinical impact. *Antimicrob. Agents Chemother.* vol. 50, pag. 43-48, **2006**.
- [3] Dotsch A., Becker T., Pommerenke C., Magnowska C., Jansch L., Haussler S., Genomewide identification of genetic determinants of antimicrobial drug resistance in *Pseudomonas aeruginosa*. *Antimicrob. Agents Chemother.*, vol. 53, pag. 2522-2531, **2009**.
- [4] Paixão V.A., Barros T.F., Mota C.M., Moreira T.F., Santana M.A., Reis J.N. Prevalence and antimicrobial susceptibility of respiratory pathogens in patients with cystic fibrosis. *Braz J Infect Dis.* vol. 14, pag. 406-9, **2010**.
- [5] Hauser A., *Pseudomonas aeruginosa*, *American Journal of Respiratory and Critical Care Medicine*, vol. 178, pag. 438-439, **2008**.
- [6] Watnick P., Kolter R., Biofilm, city of microbes. *J Bacteriol.*, vol. 182, pag. 2675-2679, **2000**.
- [7] Hassett D., Korfhagen T., Irvin R., Schurr M., Sauer K., Lau G., Sutton M., Yu H., Hoiby N., *Pseudomonas aeruginosa* biofilm infections in cystic fibrosis: insights into pathogenic processes and treatment strategies, *Expert Opinion on Therapeutic Targets*, vol.14, **2010**.
- [8] Govan J.R., Deretic V., Microbial pathogenesis in cystic fibrosis: mucoid *Pseudomonas aeruginosa* and *Burkholderia cepacia*. *Microbiol Rev.* vol. 60, pag. 539-74, **1996**.
- [9] Eftekhar F., Rostamizadeh F., Khodadad A., Henry D., Speert D.P., Isolation and genetic fingerprinting of *Pseudomonas aeruginosa* from Iranian patients with cystic fibrosis using RAPD-PCR. *Iranian Journal of Biotechnology.*, vol. 1, **2003**.
- [10] Lister P., Wolter D., and Hanson N., Antibacterial-Resistant *Pseudomonas aeruginosa*: Clinical Impact and Complex Regulation of Chromosomally Encoded Resistance Mechanisms, *Clin Microbiol Rev.*, vol. 22, pag. 582-610, **2009**.
- [11] Strateva T., Yordanov D., *Pseudomonas aeruginosa* – a phenomenon of bacterial resistance, *J. Med. Microbiol.*, vol. 58, pag. 1133-1148, **2009**.
- [12] Alekshun, M. N., and Levy S. B., Molecular mechanisms of antibacterial multidrug resistance, *Cell*, vol. 128, pag. 1037-50, **2007**.
- [13] Hossain G., Saha S., Rahman M., Singha J., Mamun A., Isolation, Identification and Antibigram Study of *Pseudomonas aeruginosa* from Cattle in Bangladesh, *J. Vet. Adv.*, vol. 3, pag. 180-185, **2013**.
- [14] Ferguson D., Cahill O.J., Quilty B., Phenotypic, molecular and antibiotic resistance profiling of nosocomial *Pseudomonas aeruginosa* strains isolated from two Ir. Hospitals. *J. Med.*, vol. 1, pag. 201-210, **2007**.
- [15] El Solh A., Akinnusi M., Wiener-Kronish J., Lynch S., Pineda L., Szarpa K., Persistent Infection with *Pseudomonas aeruginosa* in Ventilator-associated Pneumonia, *American Journal of Respiratory and Critical Care Medicine*, vol. 178, No. 5, pag. 513-519, **2008**.
- [16] Tripathi P., Banerjee G., Saxena S., Gupta S.M., Ramteke P.W., Antibiotic resistance pattern of *Pseudomonas aeruginosa* isolated from patients of lower respiratory tract infection. *African J. Microbiol. Res.*, vol. 5, pag. 2955-2959, **2011**.
- [17] El Solh A.A., Choi G., Schultz M.J., Pineda L.A., Mankowski C., Clinical and hemostatic responses to treatment in ventilator-associated

pneumonia: role of bacterial pathogens. *Crit Care Med.* vol. 35, pag. 490–496, **2007**.

[18] El Solh A.A., Akinnusi M.E., Wiener-Kronish J.P., Lynch S.V., Pineda L.A., Szarpa K., Persistent infection with *Pseudomonas aeruginosa* in ventilator-associated pneumonia. *Am J Respir Crit Care Med.*, vol.178, pag. 513–519, **2008**.

[19] Fowler R., Adhikari N., Scales D., Lee W., Rubenfeld G., Update in Critical Care 2008, *American Journal of Respiratory and Critical Care Medicine*, vol. 179, pag. 743–758, **2009**.

[20] Weiser T.G., Regenbogen S.E., Thompson K.D., Haynes A.B., Lipsitz S.R., Berry W.R., Gawande A.A., An estimation of the global volume of surgery: A modelling strategy based on available data. *Lancet* vol.372, pag. 139–144, **2008**.

[21] Cai X, Wang R, Filloux A, Waksman G, Meng G., Structural and Functional Characterization of *Pseudomonas aeruginosa* CupB Chaperones. *PLoS ONE*, vol. 6, **2011**.

[22] Allen H. K., Moe L. A., Rodbumrer J., Gaarder A., Handelsman J., Functional metagenomics reveals diverse beta-lactamases in a remote Alaskan soil, *ISME J.*, vol. 3, pag.243-51, **2009**.

[23] Allou N., Cambau E., Massias L., Chau F., Fantin B., Impact of low-level resistance to fluoroquinolones due to *qnrA1* and *qnrS1* genes or a *gyrA* mutation on ciprofloxacin bactericidal activity in a murine model of *Escherichia coli* urinary tract infection. *Antimicrob. Agents Chemother.*, vol. 67, pag. 2438–44, **2009**.

[24] Veesenmeyer J.L., Hauser A.R., Lisboa T., Rello J., *Pseudomonas aeruginosa* virulence and therapy: evolving translational strategies. *Crit Care Med.*, vol. 37: pag. 1777–1786, **2009**.

[25] Mazar J., Cotter P.A., New insight into the molecular mechanisms of two-partner secretion. *Trends Microbiol.*, vol. 15, pag. 508–515, **2007**.

[26] Cezairliyan B., Vinayavekhin N., Grenfell-Lee D., Yuen G.J., Saghatelian A., Ausubel F.M. Identification of *Pseudomonas aeruginosa* Phenazines that Kill *Caenorhabditis elegans*. *PLoS Pathog.*, vol. 9, **2013**.

[27] Williams B.J., Dehnbostel J., Blackwell T.S., *Pseudomonas aeruginosa*: host defence in lung diseases. *Respirology* vol. 15, pag. 1037–1056, **2010**.

[28] Lau G.W., Hassett D.J., Ran H., Kong F., The role of pyocyanin in *Pseudomonas aeruginosa* infection, *Trends Mol Med.*, vol. 10, pag. 599–606, **2004**.

6. ACKNOWLEDGEMENTS

”This work received financial support through the project entitled "CERO – Career profile: Romanian Researcher", grant number POSDRU/159/1.5/S/135760, cofinanced by the European Social Fund for -Sectorial Operational Programme Human Resources Development 2007-2013”.

© 2015 by the authors. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (<http://creativecommons.org/licenses/by/4.0/>).