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PHENOTYPES OF FLUOROQUINOLONE RESISTANCE IN *PSEUDOMONAS AERUGINOSA* ISOLATES FROM A ROMANIAN HOSPITAL

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Abstract

Due to the increased frequency of multidrug-resistant bacterial strains isolated from infectious processes, a constant analysis of their sensitivities to the antibiotics currently used in therapy is required. The aim was to follow the evolution of the resistance phenomena for Pseudomonas aeruginosa strains isolated from infections. Pseudomonas aeruginosa is a current concern for clinicians and epidemiologists due to the intrinsic resistance to several classes of antibiotics, acquiring resistance and limiting therapeutic actions. It is a microorganism of major importance in the nosocomial infections developed both in the human and veterinary spaces. The tests were part of a more extensive study which was aimed at the correlating, identifying common resistance profile of P. aeruginosa strains of human and animal originated. Considering the pathogenic action of this bacterial species both for humans and animals, the data obtained can support the establishment of a mutual strategy to prevent and combat the action of strains having multiple resistance to antibiotics. Efflux transporters have a considerable role in the multidrug resistance (MDR) of P. aeruginosa, an important nosocomial pathogen. The lack of some antibiotics, active towards P. aeruginosa, makes the control of infection the most important measure against the MDR- P. aeruginosa strains. The study batch included strains of P. aeruginosa, out of which only the antibiotic-resistant strains, 61 strains respectively, were selected for the phenotypic characterization in fluoroquinolones.

Key words*: hospital, Pseudomonas aeruginosa, resistance to antibiotics, fluoroquinolones.*

INTRODUCTION

Quinolones (also called 4-quinolones) are the first antimicrobial substances produced synthetically and form a family of compounds that resemble one another due to the existence of the quinolinic nucleus. The first compound from this group that used in therapy was the nalidixic acid.

Quinolones, together with the β – lactam antibiotics and the macrolides, are one of the three main families of antimicrobial agents used in human therapy (Gülhan et al., 2015). Their therapeutic importance has been growing since 1968, the date of marketing the first quinolone represented by the nalidixic acid.

Considering their spectrum of antibacterial activity, limited to Gram-negative bacteria and mainly to Enterobacteriaceae, the nalidixic acid and its derivatives have been used for the treatment of urinary tract infections. The changes to the structure have given rise to quinolones, called the new quinolones or fluoroquinolones (Norfloxacin, Pefloxacin, Ofloxacin, Ciprofloxacin, etc.) whose spectrum of antibacterial activity extends to other Gram-negative species (e.g. *P. aeruginosa*).

Resistance is mediated chromosomally and is due to the modification of the DNA gyrase that becomes insensitive or to the decrease in the penetrability of quinolones due to the modifycation of proteins in the composition of the exterior bacterial membrane (Edson et al., 1999).

In vitro, the wild phenotype is sensitive to all the fluoroquinolones: Norfloxacin, Ofloxacin, Ciprofloxacin and Levofloxacin. Practically, Ciprofloxacin is frequently used in clinical medicine (Ciocan et al., 2015a,b).

The acquired resistance is due to several mechanisms: Impermeability: porins and LPS; the change in the target affinity: the A and B subunits of the DNA gyrase and the C and D subunits of the topoisomerase IV; the active efflux: OprM, OprJ, OprN conferring low-level resistance (Çoban et al., 2009).
The different resistance phenotypes are presented in the table nr. 1 (Jehl et al., 2004).

Tab. 1. Fluoroquinolones resistance of *Pseudomonas* aeruginosa strains

Phenotype	Norfloxacin	Peflox acin	Ofloxacin Levofloxacin	Ciproflo xacin
I	S	S	S	S
II	R/I	I	I	S
III	R	R	R	S
IV	R	R	R	R
Efflux	R	S	S	R

The present study was designed to detect the production of fluoroquinolones resistance phenotypes in *P. aeruginosa* and to evaluate the susceptibility pattern.

MATERIALS AND METHODS

In this study, we analyzed the *P. aeruginosa* strains that were isolated and identified during 2013-2014 in the laboratory hospital from NE Romania. These strains isolated from urine, sputum, tracheal secretions, wounds, blood cultures, catheter, pleural empyema, pneumonectomy.
The following eligibility criteria were listed: the morphological and tinctorial character of Gram-negative bacilli and the certainty of the clinical significance of the isolate based on the pathological product. (Ciocan et al., 2015a).
Bacteria were identified on the basis of the microscopic, culture and biochemical properties (RapID NF test) (Ciocan et al., 2015b).
The study batch included strains of *P. aeruginosa*, out of which only the antibiotic-resistant strains, 61 strains respectively were selected for the phenotypic characterization. The sensitivity to antibiotics of the bacterial strains included in the study was tested by using the diffusimetric method. The interpretation of results was carried out based on the CLSI – 2014 standards (Gilbert D. N. et al., 2003).

RESULTS AND DISCUSSIONS

The studied batch included only isolates of *P. aeruginosa*, out of which the ones having a profile of resistance to antibiotics were selected for the phenotypic characterization. Thus, 793 of ***P. aeruginosa* strains** out of which (Fig. 1):

- ***Sputum***: 3052 bacterial cultures – 227 strains of *P. aeruginosa;*
- ***Urocultures***: 3715 bacterial cultures – 331 strains of *P. aeruginosa;*
- ***Hemocultures***: 784 bacterial cultures – 70 strains of *P. aeruginosa;*
- ***Pus***: 768 bacterial cultures – 165 strains of *P. aeruginosa.*

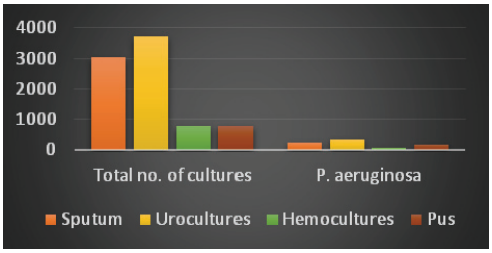

Fig. 1. Isolates of Pseudomonas aeruginosa out of the total number of cultures

Out of the total of 793 strains, only 7.9 % presented a profile of resistance to anitbiotics, respectively 61 strains, being characterized from the phenotypical point of view by means of the diffusimetric antibiogram. (Jehl et al., 2004). Following the tests of sensitivity to antibiotics and the interpretation of results, the following strains presented:

- *15 out of 61 strains are* ***wild type phenotypes*** *(sensitive to all the fluoroquinolones tested)*
- *28 out of the 61 resistant strains are* ***phenotype IV****: they are resistant to all the fluoroquinolones tested.*
- *only one strain presents* ***phenotype III of resistance*** *(sensitive only to CIP, resistant to the rest of the fluoroquinolones tested)*

The resistance profile of *Pseudomonas aeruginosa* strains is presented in Fig. 2:

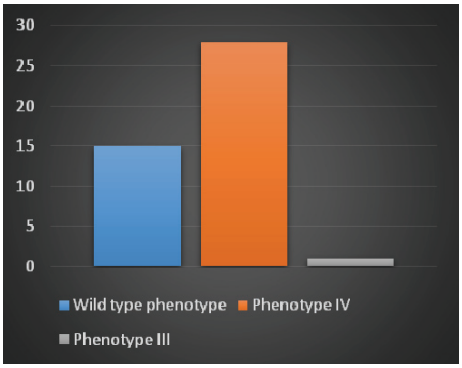

Fig. 2. Pseudomonas aeruginosa strains with resistance profile

CONCLUSIONS

- The diffusimetric antibiogram is a simple method to be executed that enables characterizing strains and classifying them in different phenotypes of resistance, but it is complicated regarding the interpretation of results and their specificity is not as qualitative as when they are characterized from the molecular point of view; the interpretive reading and application of the experts' rules gives the possibility to transform some results registered as sensitive into intermediate or resistant results, in accordance with possible therapeutic failures, therefore, following the tests and phenotypic characterization, we recommend that molecular analyses should be carried out in order to highlight the genes that provide the resistance to these antibiotics tested.
- In this study we have not identified phenotype II and the efflux.
- The resistance to quinolones limits the selection of the drug for the treatment of several infections, resistant to quinolones are most often also resistant to other classes of antibiotics.
- Quinolones are frequently prescribed before the results are known. The prompt reporting of the resistance to them reduces the risk of complications caused by infectious diseases.
- Reporting the susceptibility to different quinolones, we possess the necessary information on the next therapy that will minimize the selection of mutations leading to resistance.
- Efflux transporters have a considerable role in the multidrug resistance (MDR) of Pseudomonas aeruginosa, an important nosocomial pathogen

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