Resistance Phenotypes of *Pseudomonas Aeruginosa* Strains of Human Origin in B – Lactamases

Oana – Alexandra CIOCAN^{1*}, Mihai CARP – CĂRARE¹, Cătălin CARP – CĂRARE¹, Andreea – Paula COZMA¹, Eleonora GUGUIANU¹, Cristina RÎMBU¹, Elena Petraru², Carmen - Valentina PANZARU³

¹Faculty of Veterinary Medicine, Iasi

²"St. Mary" Emergency Hospital for Children "St. Mary", Iasi

³Grigore T. Popa University of Medicine and Pharmacy Iasi

*Corresponding author: veterinarians_phd@yahoo.com

Bulletin UASVM Veterinary Medicine 72(2) / 2015,

Print ISSN 1843-5270; Electronic ISSN 1843-5378

DOI:10.15835/buasvmcn-vm: 11530

Abstract

The use of β – lactam antibiotics is of utmost importance in medical practice. The natural resistance of *Ps. aeruginosa* strains to β -lactamases is important for clinical reasons, however, acquired resistance is very common (Pilly *et al.*, 2006). Acquired resistance comprises either enzymatic mechanisms or non-enzymatic ones (Strateva T *et al.*, 2009)

The aims it is to identifying β -lactam profiles of resistance to *Ps. aeruginosa* strains and the last objective of this study is to evaluate the reliability and accuracy of the VITEK-2 Compact (Biome'rieux) automated identification and susceptibility testing system.

The batch studied included 793 multi-resistant strains of *Ps. aeruginosa*, which were isolated from the Institute of Cardiovascular Diseases in Iași during 2013 – 2014, the isolates being taken from sputa, urine, pus and blood. Those isolates have passed susceptibility test that resistant to one or more antibiotic from third generation cephalosporin and/or resistant to aztreonam and/or intermediate or resistant to one or more antibiotic from carbapenem class.

The results were interpreted with the help of the Vitek2 system, 61 beta-lactam resistance profiles being defined. Sputum is the primary source of isolates with almost 60%, the rest are from other sources i.e. urine, pus, blood, etc.

As a result of the investigations we carried out, out of the total of 793 strains isolated and identified as multi-resistant *Ps. aeruginosa*, 61 strains were confirmed as having beta-lactam resistance profile. The result of this study confirmed that anyone could be infected by β - lactams resistance profile regardless their age, gender, and clinical manifestation.

Keywords: *Pseudomonas aeruginosa*, β – lactamase, strains of human origin, VITEK® 2

INTRODUCTION

β -Lactams are a group of antibiotics acting on the cell wall of a bacterial cell. These include the penicillins, cephalosporins, carbapenems and monobactams. These bind to and inhibit the carboxypeptidases and transpeptidases. All beta - lactam based on a common chemical structure: beta-lactam nucleus. From this nucleus there

are four families: penicillins, cephalosporins or cephem, carbapenem, monobactams.

Beta - lactam have a synergistic effect when administered with aminoglycosides and have an additive effect when administered together with fluoroquinolones.

Resistance to beta - lactams can occur by three mechanisms: the target protein modification,

production of β -lactam - especially observed in gram-negative bacteria - β -lactamase is an enzyme which inactivates β -lactam nucleus, decreased permeability of the outer membrane for Gram negative, so β -lactam no longer have access to the internal wall. (Pilly *et al.*, 2006)

Resistance to β -lactams has probably arisen throughout bacterial history but has become a useful and therefore selected trait since the β -lactam antibiotics came into clinical use.

Acquired resistances are related enzymatic mechanisms (penicillinases, cephalosporinases, ESBLs, metallo-enzymes) or non-enzymatic (impermeability, efflux and targets).

MATERIALS AND METHODS

There were studied strains of *Pseudomonas aeruginosa* isolated and identified during 2013-2014 in Iasi Cardiology hospital laboratory.

The study group included 61 for phenotypic characterization resistant *Pseudomonas aeruginosa* strains of the Institute of Cardiovascular Diseases "Prof. Dr. G.I.M. Georgescu" Iasi.

These strains came from urine, sputum, tracheal secretions, wounds, blood cultures.

Antibiotic sensitivity of strains included in the study were tested using automated systems 2 VITEK®.

Ensemble resistance phenotypes of *Pseudomonas aeruginosa* strains to β -lactams is summarized in Table 1:

RESULTS AND DISCUSSIONS

Between 2013 - 2014 the Institute of Cardiovascular Diseases "Prof. Dr. G.I.M. Georgescu" Iasi were isolated and identified:

- 793 strains of *Pseudomonas aeruginosa* of which:

Tab.1 The main resistance phenotypes of *Pseudomonas aeruginosa* strains to β -lactams

Antibiotic	Wild phenotype	Penicillins	CHN	ESBL	Carbapenemases	Efflux			D2
				OXA/PER		OprM	OprJ	OprN	
Ticarcillin	S	R	R	R/R	R	R	S	S	S
Tica-ac. clav	S	S	R	R/S	R	R	S	S	S
Piperacillin	S	R	R	R/R	S	S	S	S	S
P-tazobactam	S	S	R	R/S	S	S	S	S	S
Cefoperazone	S	R	R	R/R	R	S	S	S	S
Ceftazidime	S	S	R	R/R	R	S	S	S	S
Cefepime	S	S	R	R/R	R	S	R	S	S
Cefpirome	S	S	R	R/R	R	S	R	S	S
Aztreonam	S	S	R	R/R	S	R	S	S	S
Imipenem	S	S	R	S/S	S	S	S	R	R

Fig. 1 *Pseudomonas aeruginosa* isolates from the total culture

Ø Sputum: 3052 bacterial cultures - 227 strains of *Pseudomonas aeruginosa*

Ø Urinalysis: 3715 bacterial cultures - 331 strains of *Pseudomonas aeruginosa*

Ø blood cultures: 784 bacterial culture - 70 strains of *Pseudomonas aeruginosa*

Ø Pus: 768 bacterial cultures - 165 strains of *Pseudomonas aeruginosa*

In this study we have identified 61 strains of *Pseudomonas aeruginosa* resistant strains being characterized these phenotypic test system using VITEK® 2 provides greater certainty of results, including:

Ø 19 strains show resistance phenotype of beta - lactam

Ø 2 strains acquired resistance phenotype type enzyme ESBL OXA

Ø 12 strains acquired resistance phenotype enzymatic: carbapenemases

Ø 8 strains acquired resistance phenotype enzymatic and non-enzymatic of waterproof type

CONCLUSIONS

- Phenotypic testing system using VITEK® 2 provides greater certainty of results and eliminating repetitive manual operations.
- The phenotypic confirmatory tests are highly sensitive and specific compared to genotypic confirmatory tests. However, there are a number of instances whereby the phenotypic confirmatory tests may be falsely positive or negative.
- The most common resistance phenotype in this study β -lactam phenotype.
- The natural resistance of *Ps. aeruginosa* is important and acquired resistance are increasingly common. The bacteria may acquire additional resistance gene from the plasmid, transposon or phage (Jehl *et al.*, 2004).
- One additional important that contribute to the resistance of the strains of *Ps. aeruginosa* especially *aeruginosa* isolates from patients with cystic fibrosis (Hancock *et al.*, 1999) is its mode of growth in the lungs. The aggregates of bacteria in the lungs are surrounded by a layer of polysaccharide alginate (Nichols *et al.*, 1988)

. For this layer can bind cationic antibiotics such as aminoglycosides, restricting diffusion. These microcolonies or biofilms are extremely resistant to antibiotics, due to the mechanisms remain unclear.

- Resistance is mostly due to a combination of endogenous acquired β -lactamases, along with natural up-regulated impermeability and efflux. (Livermore *et al.*, 2006)
- Many organisms now produce multiple β -lactamases, which may reduce the effectiveness of β -lactam/ β -lactamase inhibitor combinations.
- Antimicrobial resistance of the pathogens responsible for a majority of nosocomial infections continue to increase throughout the healthcare system. (Ciocan *et al.*, 2014)

REFERENCES

1. E. Pilly – Maladies Infectieuses et Tropicales (20e edition, 2006) – Chapter 3 – Beta-lactamines
2. Hancock RE. Resistance mechanisms in *Pseudomonas aeruginosa* and other nonfermentative gram-negative bacteria. Clin Infect Dis 1998; 1(suppl): S93-99
3. Henwood CJ, Livermore DM, James D, Warner M. *Pseudomonas* Study Group. Antimicrobial susceptibility of *Pseudomonas aeruginosa*: results of UK survey and evaluation of the British Society for Antimicrobial chemotherapy disc susceptibility test. J Antimicrob Chemother 2001; 47: 789-799
4. Jehl F, Chomarat M, Weber M., Gerard A. De l'antibiogramme à la prescription. 2ème Edition, BioMérieux, 2004
5. Livermore DM, Paterson DL. Pocket guide to extended spectrum β -lactamases in resistance. New Delhi: Springer (India) Private Limited; 2006
6. Nichols WW, Dorrington SM, Slack MP, Walmsley HL. Inhibition of tobramycin diffusion by binding to alginate. Antimicrob Agents Chemother 1988; 32: 518-523
7. Oana – Alexandra C., Classifying *Pseudomonas aeruginosa* Strains of Human Origin in MDR, XDR and PDR by Determining the Resistance to Antibiotics from Seven Groups, Bulletin UASVM Veterinary Medicine 71(2) / 2014
8. Pitt TL, Sparrow M. Survey of antimicrobial resistance of *Pseudomonas aeruginosa* isolates from cystic fibrosis patients in the United Kingdom. Abstracts of the 24th European Cystic Fibrosis Conference. Vienna: ECFS, 2001
9. Strateva Tanya and Yordanov Daniel. 2009. *Pseudomonas aeruginosa* – a phenomenon of bacterial resistance. Journal of Medical Microbiology 58: 1133-1148.