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ANTIBIOTIC RESISTANCE PHENOTYPES OF *PSEUDOMONAS AERUGINOSA* STRAINS ISOLATED IN AN EMERGENCY HOSPITAL

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ABSTRACT: The study of antibiotic resistance phenotypes of 105 *Pseudomonas aeruginosa* strains isolated from inpatients and outpatients. The resistance profile on antibiotic classes reflects the multidrug-resistance of the bacteria: 56,19% of the strains are resistant to fluororquinolones and aminoglycosides; 54,28% are ceftazidime-resistant strains; 47,61 are crabapenem resistant; 46,66 % showed resistance to piperacilin-tazobactam and colistin - resistance.
KEYWORDS: *Pseudomonas aeruginosa,* resistance phenotypes

INTRODUCTION

Pseudomonas aeruginosa, a known nosocomial pathogen, ubiquitous and nonfermentative Gram-negative bacillus, is becoming more resistant to antibiotics and thus is one of the microorganisms with dificult therapeutic response. The pyocyanic bacillus is an undemanding aerobic organism wide-spread in moist environments and especially in hospitals – toiletes, sinks, disinfecting solutions, ophtalmic solutions, incubators, respiratory monitoring equipment, food, flowers, brooms, thermometers.

This pseudomonad can be isolated in the following situations (Berceanu – Văduva, 2018):

- From healthy carriers among medical personell - in high percentage;
- From the indigenous microbiota of healthy people, carriers of bacilli – 6%;
- From hospitalised people – 38%;
- From immunocompromised unhospitalised people – 78%.

In certain situations, although the pyocyanic bacillus was isolated from biological samples, it doesn't have a major clinical significance, being about the colonization of the anatomical site (differentiation with the infection).

Factors favouring the infections with *Pseudomonas aeruginosa* are (Berceanu – Văduva, 2018):

- Immunosupression (patients with diabetes, neoplastics, transplanted);
- Trauma (surgical, burns, open cuts);
- Invasive maneuvers with foreign body implants (urinary catheters, contact lenses, corneal implant, articular and valvular prostheses);
- Liquids administration (dialysis, saline irrigations);
- Cystic fibrosis (mucoviscidiosis) predisposed to colonisation and/or infection with pseudomonads and *Burkholderia cepacia.*

The number of infections caused by *Pseudomonas aeruginosa* is on the rise as well as the rate of deaths caused by bacterial strains resistant to several classes of antibiotics: MDR strains – multidrug-resistant (resistant to at least 2 classes of antibiotics) PDR strains – pandrug-resistant (resistance to more than 3 classes of antibiotics without colistin) and XDR strains – extensively – drug resistant (resistance to all classes of antibiotics) (Iovănescu and colabs., 2017).

The terms MDR, PDR, XDR have been defined in more than one way in PubMed database causing confusions among clinicians (Falagas and colabs., 2006).

The infections caused by the pyocyanic have various locations: urinary tract, cutaneous, soft parts, ocular, cardiac, pulmonary, thrombophlebitis, osteomyelitis, newborn gastroenteritis, often in association with medical care, being associated with antibiotic multi-resistance and implicitly with a reserved prognosis.

The resistance to multiple classes of antibiotics has often a regional or local character being particular to each intesive care unit or each hospital.

Continuous monitoring of susceptibility of *Pseudomonas aeruginosa* strains to antibiotics is crucial to show an efficient guide regarding empirical therapy.

Anti-pseudomonas treatment is difficult to perform due to it's resistance to various antibiotics.

Antibiotics to be tested according to CLSI and EUCAST guides are:

- Penicillins anti-*Pseudomonas*: carbenicillin, piperacillin, ticarcillin
- 3rd generation CFS - ceftazidime
- 4th generation CFS - cefepime
- Fluoroquinolone: ciprofloxacin, ofloxacin
- Carbapenem: imipenem (IMP), meropenem (MEM)
- Aminoglycoside: gentamicin (CN), amikacin (AK), tobramycin (TOB)
- Penicillins with beta-lactamase inhibitors: piperacilin with tazobactam (TZP)
- Ceftolozane/tazobactam is a new ATB introduced in Romania in 2017 with broad spectrum and active on

Gram-negative bacilli and MDR but not with metallo-beta-lactamase –MBL (not carbapeneme-resistant). It is indicated for the treatment of complicated urinary infections, complicated intra-abdominal infections, hospital-acquired pneumonias, ventilator-associated pneumonia. Tazobactam additions provides the bacillum activity against the producers of broad spectrum beta-lactamase (van Duin and colabs., 2016).

Pseudomonas aeruginosa is known for the intrinsic resistance to several classes of antibiotics (Eucast 2018):

- Aminopenicillin – AM
- Beta – lactam +beta-lactamase inhibitors – Amoxicillin+ clavulanic acid, Ampicillin + sulbactam
- 1st and 2nd generation cephalosporins: cefazolin, cefalotin, cefalexin, cefadroxil, cefotaxime, ceftriaxone.
- Carbapenem: Ertapenem
- Chloramphenicol
- Aminoglycoside – kanamycin, neomycin
- Trimethoprim
- Tetracycline, Tigecycline
- Macrolide
- Glycopeptide
- Fusidic acid

The pyocyan gains very easily resistance to antibiotics, the most dengerous ones being the intrahospital strains („the feared germs of the hospital") that are more frequent MDR, PDR or XDR.

The resistance phenotypes of wild type pyocyanic bacillus strains appear by accumulation of resistance mechanisms: production of beta-lactamase-penicillinase, extended-spectrum beta-lactamases, carbapenemase; enzymatic inactivation of aminoglycosides; supraexpression of a protein from the external membrane, OprH that protects the lipopolysaccharide, not allowing the binding of the antibiotic; by impermeability and efflux mechanisms (Pandrea and colabs., 2011).

The virulence factors of the pyocyan are structural component enzymes, toxins.

The life-saving treatment for patients with infections with multi-resistant strains of *Pseudomonas aeruginosa* consist of (Falagas and colabs., 2006) :

- Using guides adapted where possible;
- Latest generation antibiotics in associations and doses according to studies for MDR, PDR strains;
- Using of new antibiotic molecules - Ceftolozane/ Tazobactam for the patients in therapeutic failure having

microbiological data of susceptibility to this antibiotic.

The spreading of resistant germs and understanding the resistance mechanisms, the risk of transmitting multidrug-resistance to other highly pathogenic bacteria leads to major treatment difficulties. Therefore, it is absolutely necessary the strict monitoring of the local epidemiological data and a correct policy of using antibiotics both in hospital and comunity.

The last decade of 2007 – 2017 is representative by pluri-resistance to classic antibiotics especially for germs of the ESKAPE group (*Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Enterobacter sp.*, *Staphylococcus aureus*, *Enterococcus faecium*).

OBJECTIVE

To study the antibiotic resistance of *Pseudomonas aeruginosa* strains, isolated in patients with pulmonary, urinary tract and wound infections and sepsis, hospitalized in The Clinical Emergency Hospital of Arad during January 2018- January 2019.

MATERIALS AND METHODS

There were 274 strains of *Pseudomonas aeruginosa* analyzed, isolated from the following departments (wards):

- Intensive care (ICU):
- Tracheobronchial secretions
- Wound discharge
- Hemocultures
- Urocultures

- Dermatovenerology:

- Wound discharge

- Orthopedics:
- Wound discharge

- Surgery:
- Wound discharge

- Gastroenterology:
- Urocultures

- Palliative care:
- Urocultures
- Wound discharge

- Pulmonology
- Urocultures

- Obstetrics and gynaecology
- Lochia
- Ambulatory
- Wound discharge
- Urocultures

The resistance to antibiotics was tested using the Vitek 2C automated MIC (minimum inhibitory concentration) method and less often using the disc diffusion based method. The Mueller – Hinton medium and Oxoid discs were used for imipenem (IMP), meropenem (MEM), ceftazidime (CAZ), ciprofloxacin (CIP), amikacin (AK), gentamicin (CN), piperacilin/ tazobactam (TZP), colistin (CT). The plates were incubated aerobically at 35+/- 2^0C for 18-24 hrs. For quality control, the reference strain *Pseudomonas aeruginosa* ATCC 27853 was used.

The interpretation of the results was done according tu EUCAST 2018 standards.

Since 1999 there is a system for antimicrobial resistance (AMR) surveillance in Europe (EARS – Europa Antimicrobial Resistance Surveillance) which monitors the antibiotic resistance for 7 pathogenic bacteria: *Streptococcus pneumoniae, Staphylococcus aureus, Enterococcus faecalis, Enterococcus faecium, E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and for 20 associations germs/antibiotics. EARS collects data related to microbial resistance communicated by the european countries and does not tests bacteria.

RESULTS

1.From a total of 274 *Pseudomonas aeruginosa* strains tested, 105/ 38,82% had resistance to one ore more antibiotics – CAZ, CIP, AK, CN, carbapeneme, TZP, CT.

The wild type phenotype of *Pseudomonas aeruginosa* sensible *in vitro* to these antibiotics was found in 169 strains.

2.The distribution of the overall resistance of the pyocyanic bacillus to antibiotics tested on the wards is the following:

- ICU: 43 strains/ 40,95%
- Dermatovenerology: 40 strains/ 38,09%
- General surgery and palliative care: 7 strains each
- Ambulatory: 3 strains
- Gastroenterology: 2 strains
- One strain each: Orthopedics, Rheumatology, Obstetrics and gynaecology.

3.The distribution of antibiotic resistance of the pyocyan in pathology products showed that the most bacterial isolates were from wounds:

- 58 strains in wound discharge
- 35 strains in the tracheobronchial secretions
- 10 strains in urine
- one strain each in blood and lochia

The resistance profile of the 105 bacterial strains on antibiotic classes reflects the multiresistance of the pyocyan.

- 59 / 56,19% strains resistant to CIP and aminoglycosides
- 57/ 54,28% strains ceftazidime-resistant
- 50/ 47,61% to carbapeneme
- 49/ 46,66% to TZP
- 9/ 8,57% colistin-resistant.

4.The analysis of the resistance phenotypes showed that 41 strains (39,04%) were resistant to three or more antibiotic classes (PDR). The share of MDR strains (resistance to minimum 2 classes of antibiotics) is 14,28%/15 isolates.

5.At 2 strains resistance was found to all of the tested antibiotic classes (XDR/ 1,90%). Thus, 2 strains of colistin-resistant were isolated usind the MIC method.

6.The level of carbapeneme resistance in our study was 47,61%, which was 1,33 times lower than the average resistance reported by Romania in 2017. This reported value of 63,4 exceeded the european average by 3,31 times. Romania exceeded the european level of 19,12% recorded in 2017 and also the level reported by each european country in the years 2016 – 2017 (Slovenia 47 %; Latvia 57,1% - in 2017) (Popescu and colabs., 2016; https://atlas.ecdc.europa.eu).

7.The aminoglycoside resistance was 56,19% and Romania reported in 2017 a value of 57,6%. In this regard too, the average european level was exceeded 4,17 times and also the level communicated by each state in 2016 – 2017. Regarding aminoglycoside resistance, Romania is followed by Latvia with 42,9% and Slovakia with 36% (Popescu and colabs., 2016; https://atlas.ecdc.europa.eu).

8.The fluoroquinolone resistance was 56,19%. The level of resistance communicated by Romania/62,1% puts us on the second position after

Latvia 64,3% and ahead of Slovakia/46,9%. In Europe, the level of fluoroquinolone resistance was the highest compared to the other anti-pseudomonas antibiotics. The value communicated by Romania exceeded again the european average by 2,92 times(Popescu and colabs., 2016; https://atlas.ecdc.europa.eu).

9.CAZ resistance: from the 105 strains tested, 57 were ceftazidime – resistant, 54,28% without a major difference from the resistance reported by Romania/ 55,9%. In turn, Romania maintains it's unwanted supremacy in Europe between european states, showing a 3,51 times growth compared to the european average (Latvia 42,9%; Slovakia 35,6%) (Popescu and colabs., 2016; https://atlas.ecdc.europa.eu).

10. Piperacilin-tazobactam resistance: from the 105 bacterial isolates, 49 were TZP-resistant/46,66%. The level communicated by Romania in 2017 – 53,4% was not exceeded (is 1,14 times lower) but Romania was recording an ascending trend during 2016 – 2017 with a growth that exceeded the european average by 2,91 times (Popescu and colabs., 2016; https://atlas.ecdc.europa.eu).

11. The data obtained in our study showed a decrease in activity of these antibiotics used to treat infections caused by *Pseudomonas aeruginosa* reaching in the Clinical Emergency Hospital of Arad a comparable or slightly lower resistance levels than those reported by Romania and elevated relative to other states of the European Union.

Pseudomonas aeruginosa being known as a intrahospital germ and implicitly corroborated with the repeated exposure to antibiotics, increases the probability of acquiring new resistance mechanisms and consequently the selection of resistant strains including to reserve antibiotics – carbapeneme.

We have isolated 2 CT (life saving antibiotic)– resistant strains requireing the necessity of it's optimisation based on pharmacokinetics criteria and also the testing of the antibiotic association efficiency.

DISCUSSIONS

The results of our study confirms the following evolution levels of pyocyanic bacillus in Romania:

- Multiresistance to antibiotics (at least 3 distinct classes) was revealed for 39,04% of the strains.

- The efficiency of the studied antibiotics – CAZ, carbapeneme, TZP, fluoroquinolone, aminoglicoside on the pyocyan was 46% - 56% and therefore their use is risky for the initial treatment of the invasive infections caused by this bacterium.

- The isolation of the 2 CT – resistant *Pseudomonas aeruginosa* strains represents a serious problem and in the same time a major emergency for Romania.

- The increased consumption of CT in mono or poly-therapy caused by the isolation of MDR strains is corelated with the selection of *Providencia stuartii* bacteria with intrinsic resistance to this carbapeneme (Szekely and colabs., 2016).

- The rational use of antibiotics, the implementation of a unitary microbiological diagnostic policy in al laboratories in Romania and increasing the effectiveness of intrahospital infections control could limit the high level of antibiotic resistance in Romania.

CONCLUSIONS

1.Pluri-resistant strains of *Pseudomonas aeruginosa* are predominant in I.C.U., the common aetiologic agent of pneumonia, and in the dermatovenerology department (38 – 40%).

2.There are no singnificant differences regarding the distribution of the resistance to aminoglicosides, fluoroquinolones, ceftazidime, carbapeneme and TZP, this being around 50%.

3.In this context, for critical patients infected with *Pseudomonas aeruginosa*, colistin remains the only therapeutic option (with reduced penetrability and multiple side effects).

4.The 2 XDR isolated from tracheobronchial secretions showed high resistance to colistin.

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