ABSTRACT

Key words

antibioresistance, antibiotyping, identification, Pseudomonas aeruginosa, RAPD

Thesis entitled "Characterization of antibiotic resistance strains of Pseudomonas aeruginosa of human and animal in Romania" is written in 8 chapters and is divided into two distinct parts.

The first part entitled "The current state of knowledge" theory was based on consultation with representative sources and comprises 27 pages, representing 18,75% of the thesis. The information was summarized in two chapters and 6 chapters covering: the ecology and epidemiology of the species *Pseudomonas aeruginosa*, the involvement of bacteria in human and animal pathology; antibiotic resistance isolates of *Pseudomonas aeruginosa* and their mechanisms of antimicrobial action; Phenotypic and molecular testing techniques and identification of *Pseudomonas aeruginosa* strains to identify phenotypes resistant to specific antibiotics used in therapy.

Part II includes "own contributions" presented in 117 pages, representing 81,25% of the work and comprises 6 chapters and 9 chapters, describing the purpose and goals, the institutional framework in which the research took place, methods used for isolating and identifying the strains of *Pseudomonas aeruginosa*, the study antibioresistance strains of *Pseudomonas aeruginosa* of human and animal analysis antibiotic susceptibility of isolates and identification of strains of *Pseudomonas aeruginosa* producing ESBL and MBL; checking a connection between isolates of human origin and animal by phenotypic methods (antibiotyping) and molecular (RAPD-PCR).

Thesis have 24 work tables and 99 of figures, and the bibliography contains 94 references.

Chapter 3 contains "Purpose and research objectives." Research has sought to determine the susceptibility of *Pseudomonas aeruginosa* to antibiotics used in therapy and integration into resistance phenotypes and comparative testing methods phenotypic identification of strains resistant to antibiotics, in order to establish how best to detect and correct early antibioresistance in clinical laboratory conditions, with the ultimate aim antibiotics target Pseudomonas aeruginosa infections.

Objectives: 1. Determination of antibiotic sensitivity profile of *P. aeruginosa* strains isolated from animals and humans; 2. The recommendation, based on research carried out a protocol for the identification and surveillance of *Pseudomonas aeruginosa* strains multidorg-resistant; 3. to link genetic strains of human origin of the animal; 4. timely identification and declaration of *Pseudomonas aeruginosa* infections in both human and veterinary clinics to prevent nosocomial outbreaks and developments; 5. exploitation and dissemination of the results by presenting them at national and international events.

Chapter 4 presents the instrituţional in which the research took place.

In Chapter 5, entitled "Isolation and identification of Pseudomonas aeruginosa strains" are presented the steps taken to identify bacteriological examination and confirmation of Pseudomonas aeruginosa strains isolated. Following investigations carried out were isolated and molecular confirmed 125 strains of Pseudomonas aeruginosa from human patients and 57 strains of Pseudomonas aeruginosa from animals. The strains of human origin were from bronchial aspirate (15%), aspirated gastric (12%), pleural liquids (10%), purulent collections postoperative (21%), runny ear and eyes (20%) sputum samples (10%), urine samples (12%). The animal were collected from ear infections (30%), perianal abscesses (44%), sepsis (14%), conjunctivitis (12%).

Chapter 6 entitled "antibioresistance study of *Pseudomonas aeruginosa* strains of human and animal." Effectiveness of treatment with antibiotics is conditioned primarily by the pathogen susceptibility to the antibiotic in question, property is assessed "in vitro" by antibiotic. The essential purpose of these investigations was to help the therapeutic decision being useful: epidemiological surveillance of bacterial resistance that will guide subsequent schemes antibiotics, comparing phenotypes resistant strains presumed responsible for nosocomial infection correctness identifying bacterial, by putting out of natural resistance. All strains were tested against 13 antibiotics are usually recommended produced by *Pseudomonas aeruginosa* infectious pathology.

Since bronchial aspirates collected from human patients (H-1) were isolated 19 strains of *Pseudomonas aeruginosa* and of which 5.27% was 13 at the same time resistant to all antibiotics. A positive aspect is that 15.78% of tested strains showed sensitivity to all antibiotics tested.

Of gastric aspirates collected from human patients (H-2), they have isolated 15 strains of *Pseudomonas aeruginosa*, being at the same time 6.66% of the strains resistant to 11, 8, 7, 3, 2, one of the antibiotics tested, 60 % of tested strains showed sensitivity simultaneously to all 13 antibiotics

tested. There was no strain isolated tested antibiotics against multi-drug resistant.

Pseudomonas aeruginosa isolated from pleural fluid (12 strains) (H-3), 8.33% were simultaneously resistant to the three antibiotics and antibiotic 9.

The 12 strains of *Pseudomonas aeruginosa* isolated from sputum (H-4), 7.69% were simultaneously resistant: 11 to antibiotics; 23.07% strains to 10 antibiotics; 15.34% vs. 9 antibiotics, 15.34% against 2 strains antibiotics and 7.69% of the strains showed resistance to one antibiotic; 12 strains tested group H-4, 8 strains were resistant to IPM, MEM.

Out of the 15 strains of *Pseudomonas aeruginosa* isolated from urine samples (H-5), 20% of the strains had at the same time resistance to 11 antibiotics, 13.33% compared with 10 strains of antibiotics; 33.33% of the strains showed resistance to one antibiotic.

Out of 25 strains of *Pseudomonas aeruginosa* isolated from ear secretion from human patients (H-6), 4% were simultaneously strains resistant to antibiotics 10 and 8 respectively.

Pseudomonas aeruginosa strains isolated in purulent collections (26 strains) (H-7), 3.84% were resistant to 13 antibiotics.

Perianal purulent collections from animals (A-2) were isolated 25 strains of Pseudomonas aeruginosa of which 36% were resistant to 13 antibiotics tested. Of the 8 strains of *Pseudomonas aeruginosa* isolated from blood cultures from animals (A-3), 25% of the strains were resistant to 12 antibiotics, 25% of the strains were resistant to 11 antibiotics, 25% strains were resistant to 10 antibiotics, resistant strains were 12.5% to 9 antibiotic-resistant strains were eight antibiotics. Of the eight tested strains from the group A-3, 7 strains were resistant to PB and ATM, 6 strains were resistant in MEM, 5 strains were resistant to CIP and IPM three strains were resistant to TOB and two strains AK was resistant. All 8 strains tested were resistant to TZP, PIP, FEP, and TZP CASE

The evidence of conjunctival secretions from animals (A-4) were isolated seven strains of Pseudomonas aeruginosa of which 71.42% were resistant to all antibiotics tested. All 7 strains tested were resistant to TZP, IF ANY, FEP, CIP, GN, PB, PIP, ATM.

Using culture medium Briliance ESBL allowed a 68% growth and multiplication of Pseudomonas aeruginosa strains of human and animal strains 100%.

Following test for the detection of synergy between the two tablets lot ESBL strains tested showed no inhibition zones suggestive / relevant for the identification of ESBL strains or growth inhibition zone around the antibiotic β-lactamase inhibitor boosted. By way of combination discs (Oxoid / Becton

Dickinson - "Combination Discs" masks "MAST DD") was shown to inhibit an increase in the diameter of> 5 mm in the presence of clavulanate, which indicates the presence of ESBL.

Imipenem-EDTA combined disk method for detection of β-lactamases Metal, applied only strains that were resistant to imipenem (52 strains of human and animal 37) surprised by the fact that all these strains develop enzymes metallo-beta- lactamases. In order to support the result of the same strain of *Pseudomonas aeruginosa* they were tested with E-test MBL. The results confirmed that the 89 strains of *Pseudomonas aeruginosa* tested for metallo-beta-lactamases were positive in this test.

In Chapter 7 entitled "Epidemiological Surveillance of Pseudomonas aeruginosa strains" are presented data on epidemiological surveillance of Pseudomonas aeruginosa strains obtained by antibiotipare and molecular tests.

Antibiotyping of isolates was performed with the program UPGMA (unweighted Pair Group Method with Arithmetic Mean) / Quantity One Software (Bio - Rad Laboratories, USA), the study group was divided two groups, the first comprising 125 strains of human origin and 57 strains of animal origin . According to these results; 57 strains isolated animal were collected into 4 main groups (A1-A4) based on 70% similarity. On the other hand, 125 human isolates were collected in seven main groups (H1-H7) to 70% similarity. Antibiotyping is a method that identifies a pattern of a total batch analyzed with help you can watch a closed circuit isolates origin (farms, hospitals etc). Antibiotyping can be used as a method of epidemiological surveillance.

Strains analyzed with molecular technique RAPD a high degree of genetic relatedness, which may conclude that stems from the same background and genetic information is passed from one strain to another.

Pseudomonas aeruginosa strains were grouped according to their origin: 125 strains of Pseudomonas aeruginosa of human origin, gathered in 7 groups (H1-H7); 57 strains of *Pseudomonas aeruginosa* animal pooled into 4 groups (A1-A4), isolated: ear discharge, perianal abscesses, septicemia and conjunctival discharge.

Group H-1 (bronchial aspirate) consisted of three clusters, each cluster having different coefficient of relatedness genetics: the highest is assigned to the first cluster, H1 - RD1, H1 - RD2 and H1 - RD3, respectively strains 115 78 and 53, the affinity profiles inside of being 80% - 85% of the 2nd cluster is the coefficient of similarity of 71% H1 - RG1 and H1 - RG2, represented by the stems 59 and 52, similarly to the first clusters the two strains antibiotipare were part of the same cluster; 3 rd cluster has a degree

of genetic relatedness from 75% to 84%, this cluster among the group analyzed. Relationships between 115 and 53 strains were identified and antibiotipare two strains of the same cluster, which confirms their genetic relatedness.

H-2 group (gastric aspirate) included 14 types of genetic profiles of which 4 are unique type: the first cluster comprises three types of profiles degree of genetic relatedness of 84%; predominant group is the the 2nd cluster, kinship coefficient of 75%, the cluster consisting of 7 types of profiles; a high degree of relatedness is the pair of profiles H2 - d 6 and H2 - RF7, involving strains 11:12 p.m.

H-3 group (pleural fluid) included two clusters: the first having similarity coefficient of 77% and consists of two types of profiles; the 2nd cluster, that predominate in the group, has 4 types of profiles, so the group has 10 types of profiles that are unique type 4. Genetic kinship percentage exceeding 90% a meeting between profiles H3 - RF 3 and H3 - RF4, being represented by strains 90 and 87. antibiotipare that the two strains showed a similarity coefficient of 0.78, the similarity is now proven by 90% genetic relatedness.

Group H-4 (sputum) includes 3 clusters and 4 unique genetic profiles by type: first cluster has 2tipuri of genetic profiles, the coefficient of relatedness exceeds the threshold set by 0.70; at the 2-nd cluster is related to a percentage of 72%, consisting of profile H4 - and H4 RE1 - RE2, 98 and 97 strains respectively; last cluster consists of 4 types of genetic profiles, their degree of kinship ranges from 71% to 76% .All strains of this group come from patients with the same pathology background that patients diagnosed with cystic fibrosis.

Group H-5 (urine) included three clusters and three unique types of genetic profiles: first cluster consists of two types of genetic profiles having kinship coefficient of 79%. the 2-nd cluster, the percentage of relatedness superior first, 82% consists of 2 types of profiles, H5 - RE1 and H5 - RE2, involving strains 114 and 22 isolates from patients with pathologies background different having common area of origin; the last cluster, predominantly in this group consists of seven types of profiles with different percentages of similarity, from 74% to 92%. The highest percentage of relatedness is the pair of profiles H5 - and H5 RF1 - RF2, represented by strains 51 and 32, these isolates come from different patients with different pathologies.

Group H-6 (discharge auticulare) included 4 clusters with 20 different genetic profiles and two unique genetic profiles: first cluster is represented by two profiles that have kinship coefficient of 0.78. Similar to the first cluster, the

2 nd and 3 rd cluster include two types of genetic profiling by having lower coefficient of relatedness first respectively 0.71 and 0.75, their kinship is significant because it exceeds the threshold of 0.70 set by formula math. 4th cluster includes 12 types of genetic profiles with different percentages of genetic relatedness, from 74% to 90%. H6 - RF11 and H6 - RF12 represented by strains 112 and 103, 105, 121 which have the same genetic profiles of 90%, they have been isolated from different patients, hospitalized in different units responsible for pathologies were not similar.

H-7 group (postoperative purulent collections) includes 3 clusters with 21 types of genetic profiling and 7 profiles unique type: the first cluster has kinship coefficient of 0.71, in the interior of reaching up to 92% genetic relatedness . The 2nd cluster consists of five types of profiles, the coefficient of relatedness ranges from 0.74 to 0.90. H7 - RI1 and H7 - RI2, represented by strains 38 and 26 have cognate genetic profile 90% isolates derived from different patients with different pathologies background. 3rd analyzed cluster consists of 6 different genetic profiles îrudite percentage of 73% to 93%. Profile H7 - RJ5 and H7 - RJ6 represented by strains 107 and 99, have kinship coefficient of 0.93. Isolates from patients come from the same social background, same age but with different pathologies background of chronic type.

Group A-1 (ear swabs) included two clusters 9 types of genetic profiles: first cluster comprises three types of profiles kinship coefficient of 0.73; the 2-nd cluster is made up of six types of profiles, the genetic îrudirea ranges from 72% to 94%. Profiles A1 - A1 and RB5 - RB6, represented by 140 and 131 strains have higher degree of genetic relatedness.

Group A-2 (perianal purulent collections) includes 3 clusters with 18 types of genetic profiling and 5 unique type profiles: first cluster consists of two profiles with genetic relatedness coefficient of 0.72. The 2nd cluster totaling 3 types of profiles with variations of the coefficient of kinship from 0.71 to 0.83. 3rd cluster in this group of otherwise predominantly comprises 7 types of genetic profiles similar to the 2nd-cluster here meet changes in the coefficient of kinship, from 0.72 to 0.86. Strains representing the genetic profiles of the last cluster share the results of the antibiotic, where we meet a resistance of about 100%.

Group A-3 (BCs) included a cluster with 5 different genetic profiles and unique one profile type. The cluster has kinship coefficient of 0.74. Inside his profile A3 - RB3, represented by strains 149, 150, 151 and 156 and A3 profile - RB4 represented by 139 strain shows 88% genetic relatedness.

Group A-4 (conjunctival discharge) included a cluster with 3 types of genetic profiles and profile type one single coefficient of kinship up to 79%. A4

- RB2 and A4 - RB3. Strains present 79% degree of kinship and resistance profile is almost identical to those strains, originating from different species (rabbit, cat, and dog).

Total cluster analysis performed on each profile representative strains revealed a single cluster and 6 unique type profiles.

The cluster consists of 5 different genetic profiles, each profile is the one isolated from different groups: TRG1 - H1 (bronchial aspirate taken from humans), TRG2 - A4 (runny ear isolated from pets), TRG3 - A3 (septicemia from animals), TRG4 - H7 (postoperative purulent secretions isolated from humans), TRG5 - A2 (perianal abscess isolates of animal origin).

The 6 profiles unique type are: TRA - H4 (isolated from sputum), TRB - H6 (isolated from runny ear), TRC - H3 (isolated from liquid plural), TRD - A1 (isolated from runny ear), TRE - H5 (isolated from urine samples), TRF - H2 (isolated from gastric aspirates).

The genetic relatedness within the cluster percentages ranging from 73% to 91%. Profile with the highest percentage of consanguinity or 91%, consisting of TRG4 - H7 and TRG5 - A2, it was represented by strains isolated from humans secretions postoperative isolates of abscess perianal from animals that have 91% the percentage of genetic relatedness.

In chapter 8 entitled "Final conclusions and Recommendations", are structured 9 main conclusions and 9 recommendations, drawn from the investigations.