OPEN ACCESS IOURNAL

BIOINTERFACE RESEARCH IN APPLIED CHEMISTRY

ORIGINAL ARTICLE

www.BiointerfaceResearch.com

ISSN 2069-5837

Volume 3, Issue 2, 2013, 551-558

Received: 10.03.2013 / Accepted: 14.03.2013 / Published on-line: 15.03.2013

Molecular characterization of virulence patterns in *Pseudomonas aeruginosa* strains isolated from respiratory and wound samples

Ani Ioana Cotar[1,2], Mariana Carmen Chifiriuc[2,*], Otilia Banu[3], Veronica Lazar[1]

ABSTRACT

The versatile human opportunistic pathogen, *Pseudomonas aeruginosa* is responsible for a large spectrum of infections due to its impressive array of virulence factors, whose coordinated expression is regulated by many virulence regulatory systems. Among them an important role is playing by two well interrelated (las and rhl) quorum-sensing (QS) systems. The mutations occurred in either of the QS genes are associated with low virulence and a poor infective potential. The purpose of this study was to perform the molecular characterization of QS systems and of eleven extracellular virulence factors, whose expression is regulated by QS systems, in 49 *P. aeruginosa* strains isolated from respiratory tract secretions and wound secretions samples from patients with cardiovascular surgery associated infections in order to establish the correlations between certain virulence patterns and the clinical origin of bacterial strains. The results of this study showed that all analyzed strains possess QS genes, and five of eleven virulence genes regulated by QS systems. The protease IV and ExoU genes were specifically associated with the origin of the clinical strains, proving that these virulence genes could represent useful diagnosis markers for differentiating clinical *P. aeruginosa* strains.

Keywords: *Pseudomonas aeruginosa, respiratory tract infections, wound infections, virulence patterns, quorum-sensing.*

1. INTRODUCTION

Pseudomonas aeruginosa is an important opportunistic pathogen responsible for a wide range of acute and chronic infections, particularly in patients with severe burns and in cancer and AIDS patients who are immunosuppressed [1]. Intensive care units (ICUs) worldwide are encountering the highest density of nosocomial infections and the spread of antibiotic-resistant pathogens responsible for emerging infection problems in the hospital [2,3]. A European survey showed that *P. aeruginosa* is one of the most frequent pathogens isolated from ICU-acquired infections [4,5]. The hospital environment, particularly moist sites, are known reservoirs of *P. aeruginosa* strains, often multidrug resistant (MDR) due to intrinsic and acquired determinants [6]. Although one possible explanation of the spread of antibiotic-resistant strains in ICUs is the selection exerted by extensive use of antibiotics, increased spread of MDR *P. aeruginosa* may be due to transmissions of resistant clones between patients [7]. In our country the incidence of this opportunist pathogen in hospitals is high, especially in ICU [8]. The high prevalence of infections in ICU is determined by the immunological status of the patients, which are immunocompromised, mechanically ventilated, and due to multidrug resistance. The respiratory system is the most frequent body site infected by this bacterium, often presenting as acute ventilator-associated pneumonia in nosocomial settings or chronic destructive lung

[1]Department of Microbiology, Faculty of Biology, University of Bucharest, Aleea Portocalelor 1-3, Bucharest ,Romania;
*Corresponding author e-mail address: ***carmen_balotescu@yahoo.com***
[2] National Institute for Research and Development in Microbiology and Immunology "Cantacuzino", Spl. Independentei 103, Bucharest, Romania
[3] Emergency Institute for Cardiovascular Emergencies Prof. C.C. Iliescu, Bucharest, Romania

disease in patients suffering from cystic fibrosis (CF). Lower respiratory tract infections with *P aeruginosa* occur almost exclusively in persons with compromised respiratory systems, especially patients with CF. Also, *P. aeruginosa* is one of the most frequently pathogen involved in wound infections. Postoperative wound infection or surgical site infection is an important cause of health care associated infections among surgical patients. Patients who develop wound infections have longer hospital stays, more expensive hospitalizations, and increased mortality [9]. The development of wound infections depends on the integrity and protective functions of the skin. *P. aeruginosa* contributes substantially to wound-related morbidity and mortality worldwide.

The pathogenesis of *P. aeruginosa* infections is multifactorial, due to the large number of cell-associated and extracellular virulence determinants possessed by the bacterium. Two well-defined, interrelated QS systems, las and rhl are responsible for control of coordinated expression of different extracellular virulence determinants [10,11]. These QS systems exert their action by small diffusible signal molecules called *N*-acylhomoserine lactones (AHLs) which, besides regulation of the coordinated production of virulence factors, regulate different bacterial activities, including: metabolic ways dependent on cellular density, the ability to form biofilms, and motility. Each QS system consists of two components, the autoinducer synthases (LasI and RhlI, respectively) and their cognate transcriptional regulators (LasR and RhlR, respectively). LasI is the synthase for the autoinducer N-(3-oxododecanoyl) homoserine lactone ($3OC_{12}$-HSL), while RhlI synthesizes the autoinducer N-butyryl homoserine lactone (C4-HSL). At high cell density, 3OC12-HSL and C4-HSL reach critical levels and activate their regulators, which in turn enhance the transcription of different virulence genes [12]. The *las* system has been shown to modulate expression of *lasI* itself, *lasB* (elastase), *lasA* (Staphylolytic protease), *apr* (alkaline protease), the *xcp* secretion pathway, twitching motility and *rhlR*[13,14,15,16] whereas the *rhl* system modulates expression of *rhlI* itself, *rhlAB* (rhamnolipid biosynthesis), *lasB* (elastase), *apr* (alkaline protease), twitching motility, *rpoS* expression (encoding stationary-phase sigma factor) and production of secondary metabolites (pyocianin and hydrogen cyanide) [16]. Recent studies showed that QS also regulates the expression of LPS, haemolysin, siderophores and polysaccharides [17]. Nearly 5% of the *P. aeruginosa* total genome (over 300 genes) is under the regulation of QS, out of which, functions of many genes is still not understood well [18].

Importance of QS to establish a successful infection has been shown in different types of infection model studies such as acute pulmonary infection, burn wound infection, microbial keratitis, chronic lung infection and urinary tract infections by employing QS deficient strains [19]. In these studies, inability of QS deficient strains to induce successful infection was proposed to be linked with decreased production of protease, elastase and rhamnolipid [17,20]. Few clinical studies have shown that QS are fully functional during infections within infected tissues like sputum from CF patients and renal tissue in UTI [20].

In our country the evaluation of the virulence potential of *P. aeruginosa* strains isolated from these two types of infections is lacking. In this study we aim to perform the molecular characterization of QS systems and of eleven extracellular virulence factors in 49 *P. aeruginosa* strains isolated from wound secretions and respiratory tract secretions from patients with cardiovascular surgery associated infections. The expression of these virulence factors were previously established as being regulated by QS systems, and the purpose of this study was to find out a specific relationship between the pattern of virulence determinants and the clinical origin of bacterial strains.

2. EXPERIMENTAL SECTION

2.1. Clinical strains. The study was performed on 49 P. aeruginosa strains isolated from patients hospitalized in the Emergency Institute for Cardiovascular Diseases, Bucharest. The analyzed samples were isolated from 21 wound secretions, and 28 respiratory tract secretions (bronchial secretions, tracheal secretions, sputum) collected from consecutive patients who underwent cardiothoracic and vascular surgery at the Institute for Cardiovascular Emergencies Prof. C.C. Iliescu. The patients mean age was 62 (min. 32, max. 81), sex ratio female:male 1:6 (having in view the profile of the hospital and the fact that the masculin sex is a risk factor for cardiovascular

diseases, this unbalanced sex ratio was somehow expected). 50% of the patients from whom P. aeruginosa was isolated exhibited at least one cause of immunodepression, the most frequent being diabetes type II and obesity. The strains identification was performed in the institute with the help of VITEK 2 automatic system and API 20 NE microtests. We also, included in the study the P. aeruginosa strain ATCC 27853. The molecular characterization of eleven extracellular virulence factors involved in toxigenesis and systemic dissemination produced by these bacteria, and QS genetic support was performed using PCR based methods.

2.2. PCR assays. For the molecular characterization of the genetic support of QS and of eleven extracellular virulence factors we extracted chromosomal DNA from the analyzed strains and from P. aeruginosa reference strain ATCC 27853. One colony of each strain cultured on solid medium was inoculated into 5 mL of BHI (Broth Heart Infusion) and grown overnight at 37°C with shaking. From these strain cultures, DNA extraction was performed by using Wizard DNA Genomic Purification kit (Promega, U.S.) according to the manufacturer′s recommendations.

Table 1: The primer sequences used in PCR assays for QS and virulence genes detection in *P. aeruginosa* strains

Gene	Primer	Nucleotide sequence	Amplicon size (bp)
lasI	forward reverse	5′-ATGATCGTACAAATTGGTCGGC-3′ 5’-GTCATGAAACCGCCAGTCG-3′	605
lasR	forward reverse	5′-ATGGCCTTGGTTGACGGTT-3′ 5’GCAAGATCAGAGAGTAATAAGACCCA-3′	725
rhlI	forward reverse	5′-CTTGGTCATGATCGAATTGCTC-3′ 5′-ACGGCTGACGACCTCACAC-3′	625
rhlR	forward reverse	5′-CAATGAGGAATGACGGAGGC-3′ 5′-GCTTCAGATGAGGCCCAGC-3′	730
lasB	forward reverse	5′-TTCTACCCGAAGGACTGATAC-3′ 5′-AACACCCATGATCGCAAC-3′	153
aprA	forward reverse	5′-ACCCTGTCCTATTCGTTCC-3′ 5′-GATTGCAGCGACAACTTGG-3′	140
rhlAB	forward reverse	5′-TCATGGAATTGTCACAACCGC-3′ 5′-ATACGGCAAAATCATGGCAAC-3′	151
protease IV	TCF TCR	5′-TATTTCGCCGACTCCCTGTA-3′ 5′-GAATAGACGCCGCTGAAATC-3	752
plcH	forward reverse	5′- GAAGCCATGGGCTACTTCAA-3′ 5′-AGAGTGACGAGGAGCGGTAG-3′	307
plcN	forward reverse	5′-GTTATCGCAACCAGCCCTAC-3′ 5′-AGGTCGAACACCTGGAACAC-3′	466
exoA	Eta1B Eta2	5′-AACCAGCTCAGCCACATGTC -3' 5′-CGCTGGCCCATTCGCTCCAGCGCT-3'	207
exoS	ExoSf2 ExoSr2	5′- ATC GCTTCAGCAGAGTCCGTC-3' 5′- CAGGCCAGATCAAGGCCGCGC-3'	1352
exoT	forward reverse	5′- AATCGCCGTCCAACTGCATGCG-3' 5′- TGTTCGCCGAGGTACTGCTC-3'	152
exoU	forward reverse	5′- CCGTTGTGGTGCCGTTGAAG-3' 5′- CCAGATGTTCACCGACTC G-3'	134
pvdA	pvdA-F pvdA-R	5′-GACTCAGGCAACTGCAAC-3' 5′-TTCAGGTGCTGGTACAGG-3'	1281

Chromosomal DNAs obtained were used as templates for all PCR experiments. The PCR reactions were carried out in an Applied Biosystems 2700 Thermal Cycler. We selected a set of oligonucleotide primers that allowed us to amplify each whole QS gene (lasI, lasR, rhlI and rhlR) [21]. Also, PCR assays were used to detect the extracellular virulence genes encoding elastase (lasB), alkaline protease (aprA), protease IV, rhamnolipid (rhlAB), haemolytic phospholipase C

(plcH), non-haemolytic phospholipase C (plcN), exotoxin A (exoA), exoenzyme S (exoS), exoenzyme T (exoT), exoenzyme U (exoU) and pyoverdine A (pvdA) in the analyzed strains. The sequences of specific primers used in PCR reactions for QS and virulence genes detection and the molecular weight of the obtained amplicons are presented in table 1 [22,23,24]. The parameters for the amplification cycles used in each PCR experiment are presented in table 2. Each gene was amplified separately using necessary components provided by Fermentas. PCR products were separated in a 1.5% agarose gel for 1 h at 100 V, stained with ethidium bromide and detected by UV transillumination.

Table 2: The conditions used for the PCR amplification of genes encoding QS factors, proteases, phospholipases and exoenzymes in *P. aeruginosa* strains

Gene	The amplification program					
	Initial denaturation	**No. of cycles**	**Denaturation in each cycle**	**Annealing**	**Primers extension**	**Final extension**
lasI	95°C, 2 min.	35	95°C, 30 sec.	50°C, 30 sec.	72°C, 30 sec.	72°C, 7 min
lasR						
rhlI						
rhlR						
lasB		30	95°C, 30 sec.	52°C, 30 sec.	72°C, 30 sec.	72°C, 5 min
aprA						
rhlA B						
protease IV		30		60°C, 30 sec	72°C, 1 min.	72°C, 7 min
plcH		30		55°C, 30 sec.	72°C, 30 sec.	72°C, 5 min
plcN		30				
exoT		36		58°C, 30 sec.	72°C, 30 sec.	72°C, 5 min
exoA		35		65°C, 30 sec.	72°C, 30 sec.	72°C, 7 min
exoS		35				
exoU		36		59°C, 30 sec.	72°C, 30 sec.	72°C, 5 min
pvdA		30		59°C, 30 sec.	72°C, 30 sec.	72°C, 7 min

3. RESULTS SECTION

The results of PCR assays for QS genes showed that each analyzed strain have lasI, lasR, rhlI and rhlR genes. The primers used for detection of QS genes allowed us to amplify the whole QS genes. Thus, the results obtained show that all analyzed strains do not have mutations in QS genes, and have their ability to cause infections is not influenced.

The results of PCR assays concerning the presence of genes encoding three proteases showed that all P. aeruginosa strains do possess genes lasB and aprA, encoding LasB elastase and alkaline protease, whereas the gene encoding protease IV was present in 57.14 % of strains isolated from respiratory tract secretions and in 47.61% of wound secretions, respectively (table 3). The results of PCR analysis showed that all analysed P. aeruginosa strains possess rhlAB gene, encoding the rhamnolipid (table 3). Concerning the presence of two phospholipases C, the results of PCR analysis showed that all P. aeruginosa strains possess plcN gene, whereas plcH gene is present in the majority of analyzed strains (table 2). For genes encoding three analyzed exoenzymes, the results of PCR assays show the prevalence of exoT gene, which was present in all strains, being followed by exoS gene present only in 64.87% from the analyzed strains. Finally, the gene encoding for exoenzyme U was present in more than 50% of strains isolated from respiratory secretions and wound secretions (table 3). The results of PCR analysis showed that all P. aeruginosa strains possess exoA gene, encoding the exotoxin A, a major extracellular virulence determinant (table 3). The last examined virulence factor was type I pyoverdine (PvdI), a fluorescent siderophore. *P. aeruginosa* strains produce several Pvd proteins, which can be classified into three types (PvdI to PvdIII) and can be

distinguished by their peptide amino acid sequences [25]. We investigated the presence of pvdA gene, that encodes an enzyme that catalyses synthesis of N^5-hydroxyornithine, which is present in the type I pyoverdine (pyoverdine$_{PAO}$) made by *P. aeruginosa* PAO1 [26]. The results of PCR analysis concerning the presence of pvdA gene showed its presence with a similar, reduced percentage in strains belonging to the two clinical specimens types (table 3). The reference strain possess lasB, rhlAB, aprA, plcH, plcN, and exoT genes, and is lacking exoA, exoS, protease IV, and pvdA genes.

Table 3: The distribution of virulence genes among *P. aeruginosa* strains (specific markers are marked in grey)

Source of strain isolation	The percent (%) of positive strains for genes encoding different virulence factors										
	lasB	***aprA***	***protease IV***	***rhlAB***	***plcH***	***plcN***	***exoA***	***exoS***	***exoT***	***exoU***	***pvdA***
respiratory tract secretions	100	100	57.14	100	92.85	100	100	67.85	100	60.71	50
wound secretions	100	100	47.61	100	95.23	100	100	61.90	100	52.38	57.14

The combination of virulence factors expressed by each *P. aeruginosa* strain tends to determine the specific syndromes accompanying an infection. However, in the clinical cases, it is often difficult to distinguish between simple colonization and infection, and no diagnostic tool is available to assess the virulence potential of a given isolate [27].

Taking into account that there are poor data in the literature concerning the patterns of virulence factors possessed by *P. aeruginosa* strains isolated from patients with localized infections associated with cardiovascular surgery or cardiovascular devices our study aims to establish one or more virulence markers specific associated with the strains isolated from a particular clinical specimen.

The mucosa membranes and cutaneous tissue are doors for the entrance of infectious agents, especially of those opportunistic. The alteration of the physical barriers, especially in ICU hospitalized patients (indwelling medical devices, immunocompromised status) favors the colonization and dissemination of *P. aeruginosa*.

The ability of *P. aeruginosa* to invade tissues depends upon production of extracellular enzymes and toxins that break down physical barriers by disrupting host cell membranes and annihilating the host, as well as resistance to phagocytosis and host immune defence mechanisms. The tissue invasion by *P. aeruginosa* is promoted by the production of proteases, hemolysins and cytotoxins. *P. aeruginosa* can synthesize four proteases (LasB elastase, LasA staphylolytic protease, alkaline protease, and protease IV), that are extracellular virulence factors, playing a major role during acute infection. Thus, elastase (LasB) breaks down elastin, fibrin, and collagen, which are critical for the mechanical properties of connective tissue. It has also shown to degrade host immunological factors IgG, IgA, IFNγ, TNFα [19]. Alkaline protease has the ability to degrade fibrin, complement molecules C1q, and C3, and in conjunction with LasB, cytokines IFNγ and TNFα. Protease IV degrades important host immunological proteins such as complement and IgG [28]. Protease IV also compromises the integrity of structural proteins such as elastin, therefore causing tissue damage and facilitating bacterial infection. Also, protease IV degrades the iron binding proteins lactoferrin and transferrin which enables *P. aeruginosa* to scavenge iron from the host [29].

We investigated the presence of genes encoding three of these proteases in analyzed strains. The results of our PCR assay are showing the presence of genes encoding for elastase and alkaline protease in all analyzed strains, which confirms the results from other studies which showed a presence of these proteases in more than 90% of clinical *P. aeruginosa* strains [30].

On the contrary, gene encoding protease IV was present in 56% from analysed strains, which means that this virulence factor could represent a useful diagnosis marker for the investigated types of clinical *P. aeruginosa* strains. We don't know if the lower prevalence of this protease in the analyzed strains is associated with a lower virulence potential comparatively with the strains that possess this protease. The role of protease IV in

P. aeruginosa infections was investigated, and is known especially in keratitis of the cornea. Also, this gene was absent in the reference strain. Future studies that will be performed on the strains from patients with these type of infection could confirm or not this hypothesis.

Rhamnolipid, is an important extracellular virulence factor, being a rhamnose-containing glycolipid biosurfactant, which has a detergent-like structure and is considered to solubilize the phospholipids of lung surfactant, making them more accessible to cleavage by phospholipase C [31]. The resulting loss of lung surfactant may be responsible for the atelectasis associated with chronic and acute *P. aeruginosa* lung infection. Rhamnolipid also inhibits the mucociliary transport and ciliary function of human respiratory epithelium. Our PCR results have shown that all *P. aeruginosa* strains isolated from all types of clinical samples possess *rhlAB* gene, encoding the rhamnolipid, suggesting that this virulence factor is ubiquitos among clinical strains.

Also, the analysis of PCR results showed that *plcN* gene, encoding phospholipase C non-hemolytic (PLC-N) is detected in all analyzed strains, which is in agreement with the results provided by different experimental studies that showed that this thermic labile hemolysin is produced by all *P. aeruginosa* strains [32].

Exotoxin A (ExoA), secreted into the extracellular space through a type II secretion system is an ADP-ribosyl transferase inhibiting elongation factor-2 (EF-2) thereby inhibiting protein synthesis and leading to cell death. This extracellular toxin has a major role in *P. aeruginosa* virulence, although to a lesser extent than effector proteins secreted by type III secretion system (T3SS). The *exoA* gene encoding this toxin was present in all analyzed strains. These results are in accordance with the results reported by other authors that showed the presence of this important toxin in high percentage among *P. aeruginosa* clinical strains.

The most important toxins, variably expressed in different strains, injected into host cells by *P. aeruginosa* through the T3SS are: ExoS, ExoT, and ExoU. All these effector proteins participate, at varying levels, in the cytotoxicity of *P. aeruginosa* leading to tissue invasion and dissemination of *P. aeruginosa*. ExoS induces apoptosis, inhibits cell migration, disrupts tight junctions, and disrupts the actin cytoskeleton in epithelial cells (and probably endothelial cells [21]. ExoT inhibits cell division and cell migration, disrupts focal adhesions, and can induce cell death. Both toxins inhibit bacterial uptake into epithelial and phagocytic cells, and also may inhibit neutrophil and macrophage function. ExoU is a potent cytotoxin with phospholipase A2 activity that causes rapid necrotic death in many cell types. ExoU has recently been shown to inhibit caspase-1-mediated pro-inflammatory cytokine production. The extensive tissue destruction induced by ExoU combined with the modulation of the host inflammatory response, particularly its ability to induce localized immunosuppression, probably explains its prominent role in the pathogenesis of severe acute *P. aeruginosa* infections [33]. Our PCR results showed the presence of genes encoding ExoT at all analyzed strains, whereas the gene encoding ExoS was present in 64.87% from strains. ExoU could be a marker of virulence for strains isolated from respiratory tract secretions and wound secretions, because the gene *exoU* was present in only in 56.54% from analyzed strains. Type I pyoverdine, the last examined virulence factor, regulates the secretion of other *P. aeruginosa* virulence factors, exotoxin A and an endoprotease and its own secretion [34]. The results of PCR analysis showed the presence of *pvdA* gene, encoding this virulcne factor in 53.57 % from analyzed strains.

4. CONCLUSIONS

The results of PCR assays showed that all *P. aeruginosa* strains isolated from wound secretions and respiratory tract secretions from patient with cardiovascular surgery associated infections have genes encoding QS systems, exhibiting the potential to induce successful infections. Furthermore PCR assays showed that all analyzed strains have the genes encoding five from eleven virulence determinants (elastase, alkaline protease, rhamnolipid, non-haemolytic phospholipase C and exotoxin A), which show that these virulence determinants cannot be specific linked with particular strains isolated from a specific clinical specimen. The protease IV and ExoU genes were specifically associated with the origin of the clinical strains, proving that these virulence genes could represent useful diagnosis markers for differentiating clinical *P. aeruginosa* strains. Future studies should be carry out on a representative number of strains isolated from each type of clinical specimens in order to demonstrate with confidence these observations.

5. ACKNOWLEDGMENT

The results presented in this study were supported by the Human Resources 135/2010 grant (Contract no. 76/29.07.2010), IDEAS (Contract no. 154/2011) and the strategic grant POSDRU/89/1.5/S/58852, Project ″Postdoctoral program for training scientific researchers″ co-financed by the European Social Fund within the "Sectorial Operational Program Human Resources Development 2007–2013".

6. REFERENCES

[1] Gooderham J.W., and Hancock R.E.W., Regulation of virulence and antibiotic resistance by two-component regulatory systems in *Pseudomonas aeruginosa*, *FEMS Microbiology Reviews*, 33, 279–294, **2009**.

[2] Burke J.P., Infection control– a problem for patient safety, *The New England Journal of Medicine*, 348, 651–656, **2003**.

[3] Grundmann H., Barwolff S., Tami A., Behnke M., Schwab F., Geffers C., Halle E., Gobel U.B., Schiller R., Jonas D., Klare I., Weist K., Witte W., Beck- Beilecke K., Schumacher M., Ruden H., Gastmeier P., How many infections are caused by patient-to-patient transmission in intensive care units? *Critical Care Medicine*, 33, 946–951, **2005**.

[4] Vincent J.L., Bihari D.J., Suter P.M., Bruining H.A., White J., Nicolas- Chanoin M.H., Wolff M., Spencer R.C., Hemmer M. The prevalence of nosocomial infection in intensive care units in Europe. Results of the European prevalence of infection in intensive care (EPIC) study. EPIC International Advisory Committee. JAMA, 274, 639–644, **1995**.

[5] Vincent J.L., Microbial resistance: lessons from the EPIC study. European prevalence of infection. *Intensive Care Medicine*, 26, Suppl 1, S3–S8, **2000**.

[6] Deplano A., Denis O., Poirel L., Hocquet D., Nonhoff C., Byl B., Nordmann P., Vincent J.L., Struelens M.J., Molecular characterization of an epidemic clone of panantibiotic-resistant *Pseudomonas aeruginosa*, *Journal of Clinical Microbiology*, 43, 1198–1204, **2005**.

[7] Meyer E., Jonas D., Schwab F., Rueden H., Gastmeier P., Daschner F.D., Design of a surveillance system of antibiotic use and bacterial resistance in German intensive care units (SARI), *Infection*, 31, 208–215, **2003**.

[8] Borcan E., Ghita C.M., Chifiriuc M.C., Mãrutescu L., Isar C., Lazar V., Antibiotic resistance of gram negative Bacilli strains isolated from the intensive care unit in Fundeni Clinical Institute, Bucharest, Romania. *Roumanian Archives of Microbiology and Immunology*, 68, 4, 228-34, **2009**.

[9] Kirkland K.B., Briggs J.P., Trivette S.L., Wilkinson W.E., Sexton D.J., The impact of surgical-site infections in the 1990s: attributable mortality, excess length of hospitalization, and extra costs, *Infection Control and Hospital Epidemiology*, 20, 725-30, **1999**.

[10] Cotar A.I., Chifiriuc M.C., Dinu S., Bucur M., Iordache C., Banu O., Dracea O., Larion C., Lazar V., Screening of molecular virulence markers in *Staphylococcus aureus* and *Pseudomonas aeruginosa* strains isolated from clinical infections, *International Journal of Molecular Science*, 11, 12, 5273-5291, **2010**.

[11] Cotar A.I., Chifiriuc M.C., Dinu S., Pelinescu D., Banu O., Lazãr V., Quantitative Real-Time PCR study of the influence of probiotic culture soluble fraction on the expression of *Pseudomonas aeruginosa* quorum sensing genes, *Romanian Archives of Microbiology and Immunology*, 69, 4, 213-223, **2010**.

[12] Hooi D., Barrie S.W., Bycroft W., Chhabra S.R., Williams P., Pritchard D.I. Differential immune modulatory activity of *Pseudomonas aeruginosa* quorum-sensing signal molecules, *Infection and Immunity*, 72, 11, 6463–6470, **2004**.

[13] Chapon-Herve V., Akrim M., Latifi A., Williams P. Lazdunski A., Bally M., Regulation of the xcp secretion pathway by multiple quorum-sensing modulons in *Pseudomonas aeruginosa*, *Molecular Microbiology*, 24, 1169-1178, **1997**.

[14] Glessner A., Smith R., Iglewski B., Robinson J., Roles of Pseudomonas aeruginosa las and rhl quorum-sensing systems in control of twitching motility, *Journal of Bacteriology*, 181, 1623-1629, 1999.

[15] Latifi A., Foglino M., Tanaka K., Williams P., Lazdunski A., A hierarchical quorum-sensing cascade in Pseudomonas aeruginosa links the transcriptional activators LasR and RhIR (VsmR) to expression of the stationary-phase sigma factor RpoS, *Molecular Microbiology*, 21, 1137-1146, **1996**.

[16] Pesci E.C., Pearson J.P., Seed P.C., Iglewski B.H., Regulation of las and rhl quorum sensing *in Pseudomonas aeruginosa*, *Journal of Bacteriology*, 179, 3127-3132, **1997**.

[17] Gupta R.K., Setia S., Harjai K., Expression of quorum sensing and virulence factors are interlinked in *Pseudomonas aeruginosa:* an *in vitro* approach, *American Journal of Biomedical Science*, 3, 2, 116-125, **2011**.
[18] Wagner V.E., Li L.L., Isabella V.M., Iglewski B.H., Analysis of the hierarchy of quorum-sensing regulation in *Pseudomonas aeruginosa*, *Analytical and Bioanalytical Chemistry*, 387, 469–479, **2007**.
[19] Gupta S.K., Masinick S.A., Hobden J.A., Berk R.S., Hazlett L.D., Bacterial proteases and adherence of *Pseudomonas aeruginosa* to mouse cornea, *Experimental Eye Research*, 62, 641–50, **1996**.
[20] Kumar R., Chhibber S., Harjai K., Quorum sensing is necessary for the virulence of *Pseudomonas aeruginosa* during urinary tract infection, *Kidney International*, 76, 286–292, **2009**.
[21] Shaver C.M., Hauser A.R., Relative contributions of *Pseudomonas aeruginosa* ExoU, ExoS, and ExoT to virulence in the lung, *Infection and Immunity*, 72, 6969–6977, **2004**.
[22] Craig W., Kaye S.B., Neal T.J., Chilton H.J., Miksch S., Hart A.C., Genotypic and phenotypic characteristics of *Pseudomonas aeruginosa* isolates associated with ulcerative keratitis, *Journal of Medical Microbiology*, 54, 519–526, **2005**.
[23] Lanotte P., Watt S., Mereghetti L., Dartiguelongue N., Rastegar-Lari A., Goudeau A., Quentin R., Genetic features of *Pseudomonas aeruginosa* isolates from cystic fibrosis patients compared with those of isolates from other origins, *Journal of Medical Microbiology*, 53, 73–81, **2004**.
[24] Smith L., Rose B., Tingpej P., Zhu H., Conibear T., Manos J., Bye P., Elkins M., Willcox M., Bell S., Wainwright C., Harbour C., Protease IV production in *Pseudomonas aeruginosa* from the lungs of adults with cystic fibrosis, *Journal of Medical Microbiology*, 55, 1641–1644, **2006**.
[25] Meyer J.M., Stintzi A., Vos D.D., Cornelis P., Tappe R., Taraz K., Budzikiewicz H., Use of siderophores to type pseudomonads: the three *Pseudomonas aeruginosa* pyoverdine systems, *Microbiology*, 143, 35–43, **1997**.
[26] McMorran B.J., Kumara H.M.C.S., Sullivan K., Lamont I.L., Involvement of a transformylase enzyme in siderophore synthesis in *Pseudomonas aeruginosa*, *Microbiology*, 147, 1517–1524, **2001**.
[27] Sadikot R.T., Blackwell T.S., Christman J.W., Prince A.S., Pathogen–host interactions in *Pseudomonas aeruginosa* pneumonia, *American Journal of Respiratory and Critical Care Medicine*, 171, 1209–1223, **2005**.
[28] Engel L.S., Hill J.M., Caballero A.R., Green L.C., O'Callaghan R.J., Protease IV, a unique extracellular protease and virulence factor from *Pseudomonas aeruginosa*, *The Journal of Biological Chemistry*, 273, 16792–7, **1998**.
[29] Wilderman P.J., Vasil A.I., Johnson Z., Wilson M.J., Cunliffe H.E., Lamont I.L., Vasil M.L., Characterization of an endoprotease (PrpL) encoded by a PvdS-regulated gene in *Pseudomonas aeruginosa*, *Infection and Immunity*, 69, 5385–94, **2001**.
[30] Nathan E., Yu H., Cross sectional analysis of clinical factors and environmental isolates of *Pseudomonas aeruginosa:* biofilm formation, virulence and Genome biodiversity, *Infection and Immunity*, 72, 1, 133–144, **2004**.
[31] Van Delden C., Iglewski B.H., Cell-to-cell signaling and *Pseudomonas aeruginosa* infections, *Emerging Infectious Diseases*, 4, 4, 551-560, **1998**.
[32] Abusriwil H., Stockley R.A., The interaction of host and pathogen factors in chronic obstructive pulmonary disease exacerbations and their role in tissue damage, *Proceedings of the American Thoracic Society*, 4, 611–617, **2007**.
[33] Diaz M.H., Shaver C.M., King J.D., Musunuri S., Kazzaz J.A., Hauser A.R., *Pseudomonas aeruginosa* induces localized immunosuppression during pneumonia, *Infection and Immunity*, 76, 4414-4421, **2008**.
[34] Lamont I.L., Beare P.A., Ochsner U., Vasil A.I., Vasil M.L., Siderophore mediated signaling regulates virulence factor production in *Pseudomonas aeruginosa*, *Proceedings of the National Academy of Science-USA*, 99, 7072–7, **2002**.