academicJournals

Vol. 7(27), pp. 3453-3460, 5 July, 2013
DOI: 10.5897/AJMR11.1482
ISSN 1996-0808 ©2013 Academic Journals
http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Variation of virulence profiles in some *Staphylococcus aureus* and *Pseudomonas aeruginosa* stains isolated from different clinical patients

Alina-Maria Holban[1], Ani Ioana Cotar[1], Mariana Carmen Chifiriuc[1]*, Coralia Bleotu[2], Otilia Banu[3] and Veronica Lazar[1]

[1]Department of Microbiology and Immunology, Faculty of Biology, University of Bucharest, Bucharest, Romania.
[2]S. Nicolau Institute of Virology, Bucharest, Romania.
[3]Institute of Cardiovascular Diseases Prof. C.C. Iliescu, Bucharest, Romania.

Accepted 3 July, 2013

Considering the great diversity and prevalence of opportunistic pathogens as well as the medical consequences arising from increasingly frequent cases of infections with resistant pathogens, the aim of this study was to analyze and compare the virulence profiles of some recent isolates of the most frequently encountered human pathogens, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. We screened for the production of seven soluble virulence factors (hemolysins, caseinases, amylases, DNA-se, gelatinase, lipase and lecithinase) in 181 clinical isolates, using a method of culturing on specific substrates. Adherence to host cells was analyzed using Cravioto's adapted method, and invasive potentials were established by an antibiotic protection assay. The results demonstrated that these pathogens may act differently when they are the protagonists of infections and may occur in different clinical condition. We revealed that the analyzed strains display different patterns of soluble virulence factors and different invasive capacity, according to the isolation source; many of the observed differences are statistically significant. By investigating the development of infectious process in various clinical conditions, our studies provide a high contribution to the development of personalized anti-infectious therapies.

Key words: Opportunistic pathogens, soluble virulence factors, adherence and invasion.

INTRODUCTION

During the evolutionary process, a series of relationships and interactions have been established between bacteria and their hosts, either beneficial or detrimental. It has been demonstrated that interactions between bacteria and host play a decisive role in several key processes, such as: survival, growth, health/disease, virulence factors expression and production of secondary metabolites (Aguilar et al., 2003; Tarkka et al., 2009). The pathogenesis of bacteria is multifactorial, as suggested by the large number of cell-associated and extracellular virulence determinants possessed by those bacteria. Some of these determinants help in colonization, whereas others facilitate bacterial invasion. The pathogenesis of bacteria is in part due to secreted molecules that alter the host cell function to facilitate the pathogenic relationship (Holban et al., 2013). The extent of the host tissue injury caused by different bacterial strains is dependent on the host immunity, the size of the

*Corresponding author. E-mail: carmen_balotescu@yahoo.com. Tel: 0040766728315.

Table 1. Isolation sources of the analysed *S. aureus* and *P. aerugiosa* strains. Values represent number of strains isolated from the mentioned anatomic source.

Species	Wound secretions	Blood cultures	Tracheo-bronchial secretions	Genitourinary infections
S. aureus	41	16	28	18
P. aeruginosa	17	14	35	12

bacterial inoculum and on the production of specific bacterial exoproducts (Holban et al., 2013), some of them being toxic (Kudoh et al., 1994). Many successful pathogens have developed the ability to adhere to and invade animal tissues. Recent experimental evidence suggests that many opportunistic pathogens, generally perceived as extracellular pathogens, can also invade and, in some cases, multiply within host cells (Bayles et al., 1998; Shin et al., 2000; Zaas et al., 2005). The variation of virulence profiles reflects the adaptive ability of bacterial species. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are two model bacterial species frequently used in host-pathogens studies, interspecies communication and virulence modulation, due to their cosmopolite abundance and adaptive ability. The published literature on invasive *S. aureus* disease is heavily skewed towards industrialized temperate countries (Nickerson et al., 2009), where it represents a major cause of community-and hospital-acquired infection (Saviuc et al., 2011a). *S. aureus* is a Gram-positive opportunistic pathogen often carried asymptomatically on the human body, colonizing cavities and mucosa (Golubchik et al., 2013). *P. aeruginosa* is a versatile Gram-negative opportunistic pathogen that causes a wide range of acute and chronic infections, including sepsis, wound and pulmonary infections. This organism is known to produce a variety of virulence factors, such as pigment, proteases, exotoxins and the synthesis and coordinated expression of these factors is regulated by an ubiquitous bacterial cell-to-cell signaling mechanism referred to as quorum sensing and response (mediated by autoinducer molecules) (Van Delden et al., 1998; Tateda et al., 2003) or by the host signaling molecules (hormones) (Sperandio et al., 2003; Hughes et al., 2008; Holban and Lazar, 2011). Bearing in mind that the expression of virulence factors directly contributes to bacterial colonization and dissemination, our aim was to analyze and compare the phenotypic virulence profiles of some strains of the most frequently encountered opportunistic pathogens, *S. aureus* and *P. aeruginosa*, recently isolated from different clinical specimens.

MATERIALS AND METHODS

Strains and growth conditions

The study was performed on 181 bacterial strains (103 *S. aureus* strains, 78 *P. aeruginosa* strains), randomly isolated from different infected patients hospitalized in the National Institute for Cardiovascular Diseases, Prof. C.C. Iliescu of Bucharest, during October 2010-March 2011. The isolation sources for the analyzed strains are shown in Table 1.

Isolates were identified using automatic Vitek II system and the API microtest systems (BioMerieux). Subsequently, strains were maintained as -80°C glycerol stocks in the “Microbial Culture Collection of Microbiology Laboratory” of the Faculty of Biology, Bucharest. For further experiments, bacteria from frozen stocks were streaked on Luria agar (Difco, BD) and incubated over night at 37°C, in order to obtain isolated colonies. One colony was used to inoculate 5 ml Luria Broth (LB) and vials were incubated at 37°C for 16 to 18 h, 200 rpm shaking. This culture was used as further inoculum for all the experiments, diluted or not as mentioned in each section.

Adherence to HeLa cells

Bacterial adherence to different natural (skin, mucosa) or artificial (catheters, implants) substrata is a prerequisite in the pathogenesis of microbial infections.

For the adherence assay, Cravioto’s adapted method was used (Cravioto et al., 1979; Holban et al., 2012). Briefly, the HeLa cell monolayers (70 to 80% confluence) grown in 6 well plates (Nunc) were washed 3 times with phosphate buffer saline (PBS) and 1 ml of fresh DMEM (Dulbecco's Modified Eagle's Medium, Sigma) medium without antibiotics was added to each well. *P. aeruginosa* and *S. aureus* LB cultures were diluted to an optical density of 0.5 McFarland (~10^8 CFU/ml) and 1 ml was used to inoculate each well. The inoculated plates were incubated for 2 h at 37°C (Saviuc et al., 2011b). After incubation, the monolayers were washed 3 times with PBS, briefly fixed in cold methanol (3 min) and stained with Giemsa solution (1:10, neutral pH) for 20 min (Holban et al., 2012). Stained samples were washed, dried at room temperature overnight, and examined in optic microscopy using immersion oil (×2500 magnification), in order to evaluate the adherence indexes and patterns. Three distinct adherence patterns were identified: localized adherence, when bacteria attach to host cells and form microcolonies in distinct regions of the cell surface; diffuse adherence, described by bacteria which adhere evenly to the whole cell surface, and aggregative adherence, when clustered bacteria attach to the cell in a stacked-brick arrangement.

The adherence indexes were expressed as the ratio between the number of the eukaryotic cells with adhered bacteria and 100 eukaryotic cells counted on the microscopic field using an Axiolab (Zeiss) microscope. Three microscopic fields were analyzed for each sample.

Soluble virulence factors production

Bacterial strains grown for 16 to 18 h in LB were evaluated for seven enzymatic virulence factors (Saviuc et al., 2011c) (pore forming toxins: lecithinase, lipase, hemolysins; exoenzymes:

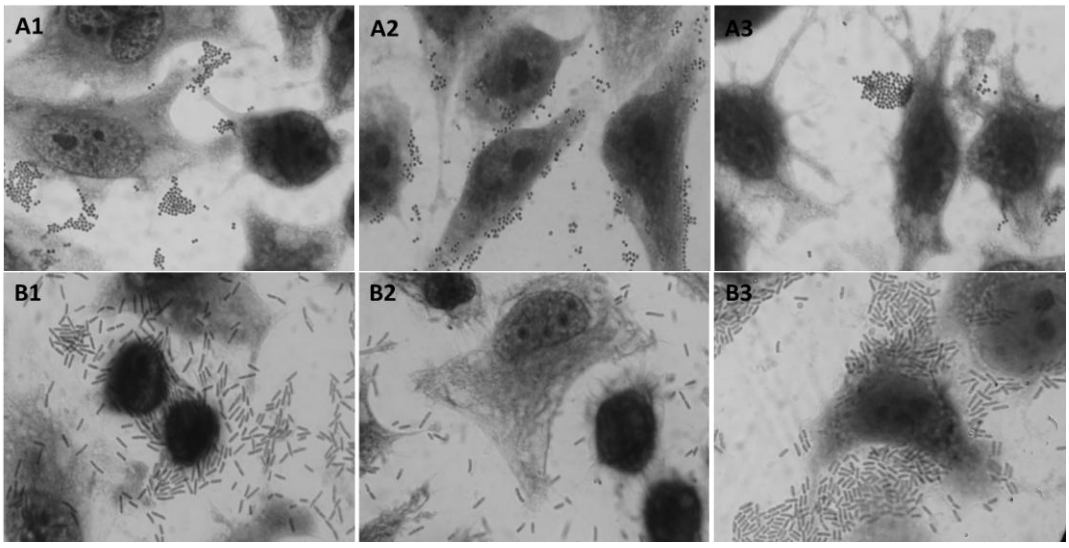

Figure 1. Adherence patterns observed in *S. aureus* (A1-A3) and *P. aeruginosa* (B1-B3) tested strains. A1 and B1 = aggregative adherence, A2 and B = diffuse adherence, A3 and B3 = localized adherence. Giemsa staining, ×1000, immersion oil.

gelatinase, amylase, caseinase, DN-ase) by cultivating the strains in available media containing specific substrate for enzymes activity detection (Holban et al., 2012). For detection of hemolysins, the strains were streaked on blood agar plates containing 5% (vol/vol) sheep blood in order to obtain isolated colonies. After incubation at 37°C for 24 h, the clear zone (total lysis of red blood cells) around the colonies was registered as positive reaction. The caseinase and amylase activity was determined using two different media as substrate: 15% soluble casein and starch (final concentration 1% in nutritive gelose). The strains were spotted and after incubation for 24 h at 37°C, precipitation surrounding the growth area indicated casein/starch proteolysis (caseinase/amylase presence). DNA-se production was studied using DNA agar medium. The strains were spotted and after incubation for 24 h at 37°C, a drop of HCl/1N solution was added to the spotted cultures; a clearing zone around the culture was registered as a positive reaction. For lecithinase, lipase and gelatinase production, the cultures were spotted onto 2.5% yolk agar, on Tween 80 agar, and respectively on agar with gelatin with a substrate at a final concentration of 1%, and thereafter the plates were incubated at 37°C up to 7 days. An opaque (precipitation) zone surrounding the spot showed the lecithinase, lipase, respectively, in gelatinase production (Holban et al., 2012).

Invasion assay

To determine the invasion ability, we used a modified antibiotic survival assay (Fleiszig et al., 1996; Zaas et al., 2005). Diluted bacterial suspensions were used to inoculate HeLa cells grown in 6-well plates and incubated at 37°C for two hours. The infection assay was performed in the same manner as the adherence assay, except that each bacterial strain was inoculated in two different wells from two different plates. After the incubation period, one sample for each strain was treated with 500 µl/well of 100 µg/ml gentamicin solution prepared in PBS, in order to kill extracellular bacteria. Additionally, 500 µl/well of 40 µg/ml vancomycin for *S. aureus* strains, or 70 µg/ml amikacin for *P. Aeruginosa* strains were added. The plates were further incubated for another hour in the same conditions. The other set of samples was maintained in standard conditions (in the initial culture medium). After incubation, plates were washed 3 times with PBS and permeabilized with 0.1% Triton X-100 (Sigma) for 5 min at 37°C. Serial dilutions of suspended cells harvested from the plate wells were seeded on solid media (three technical replicates/dilution) in order to establish the invasion indexes (CFU/ml).

Statistical analysis

One way analysis of variance (ANOVA) and unpaired t test were used to analyze the data (*GraphPad In Stat* software). *P* values < 0.05 were considered significant.

RESULTS

The qualitative assay of the bacterial adherence to the cellular substrate demonstrated that all tested strains adhered to HeLa cells, expressing all classical adherence patterns (Figure 1). *S. aureus* strains exhibited mainly aggregative adherence patterns (52%) and adherence rates ranging from 15 to 100%, approximately 55% of tested strains revealing adherence indexes exceeding 80%. *P. aeruginosa* strains exhibited preferentially diffuse (49%) or aggregative (35%) adherence patterns and 25 to 100% adherence rates, approximately 60% of studied strains exposing adherence indexes exceeding 80%. *P. aeruginosa* isolate obtained from tracheo-bronchial and genitourinary infections were the most adherent strains, while in *S. aureus*, the adherence peaks were observed in genitourinary isolates. In many situations, intracellular bacteria were observed, and sometimes we noticed

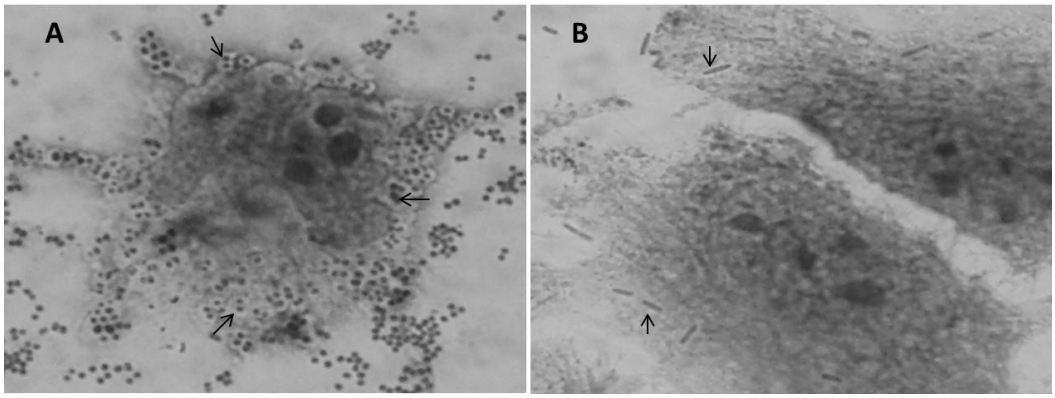

Figure 2. Micrographs revealing *S. aureus* (A) and *P. aeruginosa* (B) bacterial cells invading HeLa cultured cells. HeLa cells morphology alterations after invasion are highly visible. Arrows indicate invasive bacteria. Giemsa staining, ×2500 magnification, immersion oil.

changes in morphology of the host cell. Frequently, modifications were observed in the nucleus (condensation, fragmentation and budding) or cytoplasm alterations which are similar to those that occur in apoptotic cells (Figure 2).

Determination of soluble enzymatic virulence factors production demonstrate that tested strains release a wide range of virulence determinants and they seem to cluster to specific patterns depending on bacteria species and isolation source. *P. aeruginosa* strains preferentially express the exoenzymes amylase, caseinase and the pore forming toxin lecithinase, while only few isolates produce hemolysins, DN-ases and gelatinases. Some differences could be observed in the virulence profiles of strains isolated from different clinical sources (Figure 3B). Wound secretion isolated strains did not express gelatinases, while the *P. aeruginosa* strains isolated from the urinary tract infections (UTI) did not express *DN-ases.* In *S. aureus,* soluble virulence factors profiles indicate that staphylococci was expressed in high proportion with regards to hemolysins and *DN-ases* and in a lower extent with regards to lipases, lecithinases and gelatinases (Figure 3A).

Regarding differences that occurred between tested groups; the data revealed that *S. aureus* strains isolated from genitourinary and tracheo-bronchial secretions are significantly less virulent when compared with other isolates (Figure 4). In *P. aeruginosa*, blood culture and tracheo-bronchial isolates are the most virulent, while strains obtained from genitourinary infections produce significantly less soluble virulence determinants as comparing to all other isolates (Figure 4).

When considered classical extracellular pathogenic bacteria, the quantitative assay of the invasion ability showed that both *S. aureus* and *P. aeruginosa* strains possess the ability to invade the epithelial, non-phagocytic HeLa cells. Keeping in mind the large amount of tested strains and the heterogeneity of the results, we clustered the analyzed strains in four invasion groups according to the invasive potential expressed of each strain (Table 2). Strains that expressed an invasive potential that could not be clearly included in any of the invasion groups have been removed.

In *P. aeruginosa*, the invasion assay revealed that a high percentage of investigated strains were invasive, about 62% were moderate and highly invasive, while only about 15% of tested strains were non-invasive. Significant differences regarding their invasive potentials were noticed among *P. aeruginosa* clinical isolates, depending on their isolation source. Isolates from blood cultures revealed the greatest invasiveness, as compared to all other clinical sources, the differences being statistically significant (Figure 5). *P. aeruginosa* strains obtained from genitourinary infections revealed the lowest invasive potential, most of the isolates being low or moderate invasive.

S. aureus isolates also demonstrated different invasion abilities, depending on the isolation source. The most invasive *S. aureus* strains were obtained from blood cultures and tracheo-bronchial secretions, while isolates from genitourinary infections exhibited the lowest invasive potential (Figure 5). Even though *S. aureus* and *P. aeruginosa* isolates seem to develop similar invasion patterns depending on the isolation source, *P. aeruginosa* tested strains demonstrated significantly higher invasion potential as compared to *S. aureus* isolates (Figure 5).

DISCUSSION

Considering the great diversity and prevalence of opportunistic pathogens as well as the medical consequences arising from increasingly frequent cases of

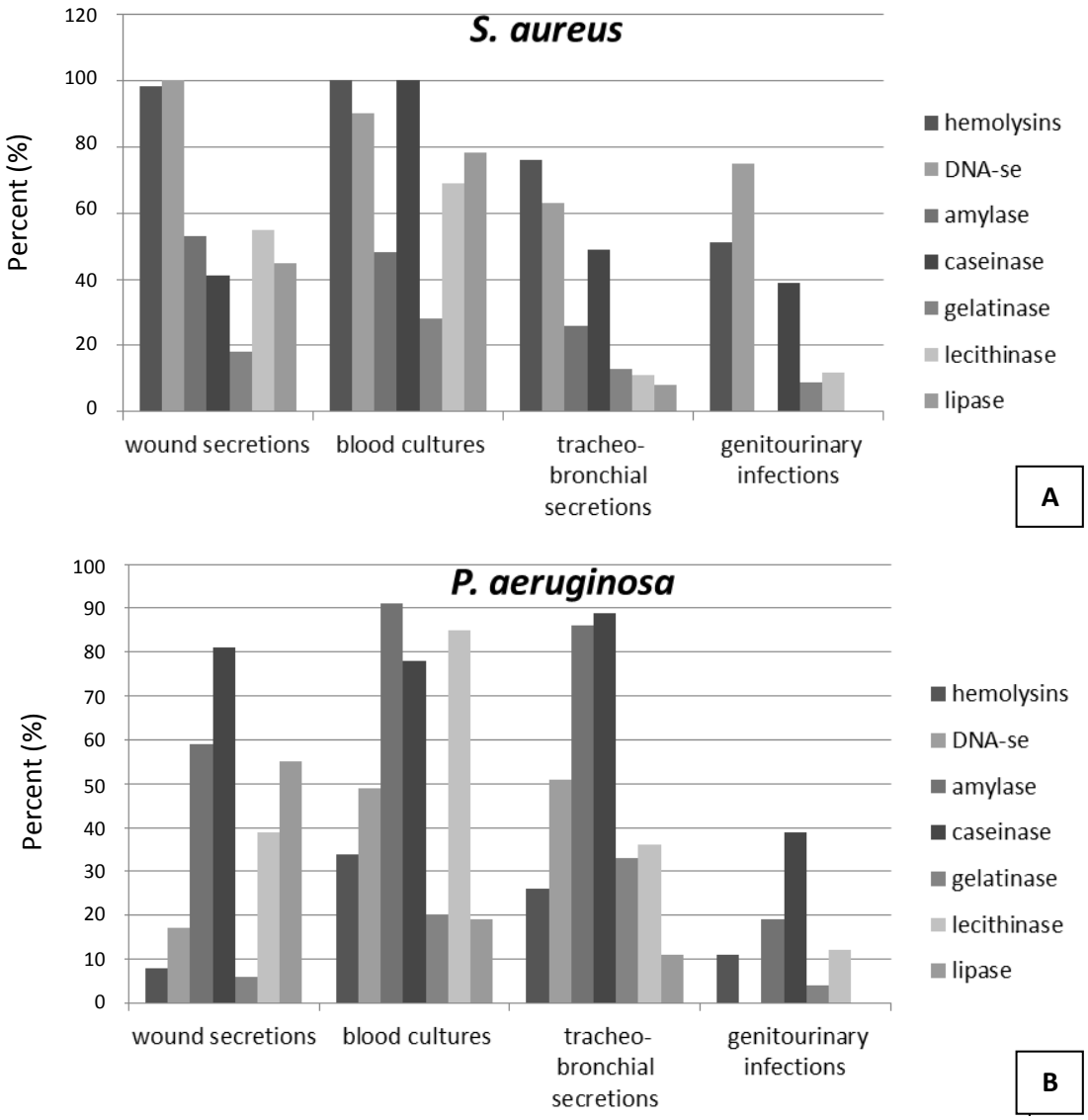

Figure 3. Graphic arrangement of soluble enzymatic virulence factors patterns observed in *S. aureus* (A) and *P. aeruginosa* (B) tested strains.

infections with resistant pathogens, studies that aim to investigate the development of infectious process in different clinical contexts are of great benefit. This study demonstrates that bacteria behave differently within host, modulating their virulence profiles. This observation can be explained by the fact that bacteria are able to sense and respond to different molecular cocktails and density for a better adaptation and survival (Karavolos et al., 2013).

We showed that all tested strains may specifically adhere to epithelial HeLa cells, exhibiting different adhesion patterns, which indirectly suggests that they express specific adhesins, extremely important molecules for the initiation of the infectious process. Qualitative assay of the soluble enzymatic virulence factors indicated that distinct strains manifest different virulence patterns according to the clinical source of isolation. The expression of virulence factors directly contributes to bacterial colonization and dissemination, which may determine the course and outcome of the disease in infected individuals. *S. aureus* strains seem to produce mainly DN-ases and exotoxins as hemolysins, while *P. aeruginosa* isolates produce many proteases as caseinase and other degrading enzymes as lecithinases and amylases, reflecting the great potential of this opportunistic pathogen to adapt to many natural and host related environments. The fact that genitourinary isolates express less soluble virulence factors but also a great

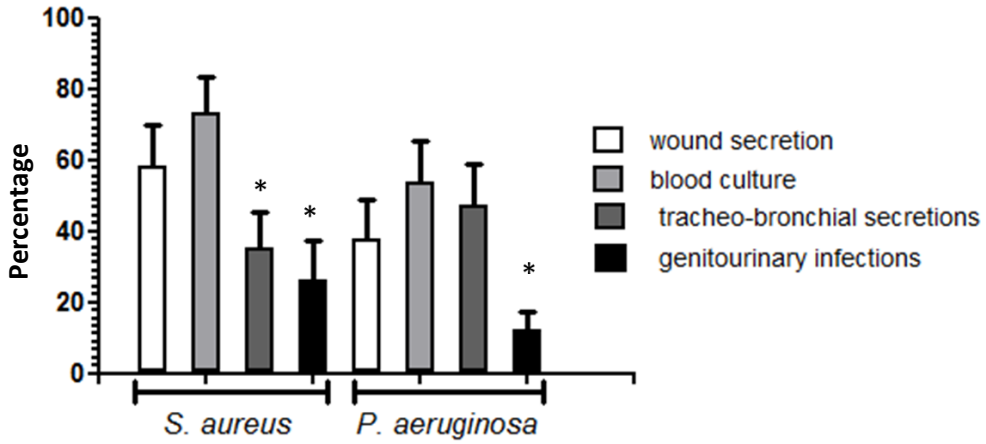

Figure 4. Statistical comparison between *S. aureus* and *P. aeruginosa* clinical isolates virulence, clustered by their isolation source. On X axis are represented *S. aureus* and *P. aeruginosa* clinical strains according to their isolation source, while Y axis represent the percent of strains positive for the production of tested virulence factors. *=p< 0.05.

Table 2. Invasion groups: 4 invasion clusters were assessed according to the number of CFU/ml obtained for each strain.

Non-invasive	Poorly invasive	Moderate invasive	Highly invasive
0-10 CFU/mL	10^2-10^3 CFU/mL	10^4 CFU/mL	over 10^5 CFU/mL

adherence ability to host cells demonstrate that the anatomical site can dictate bacteria behavior. Genitourinary strains mainly need to attach to the host epithelium in order to initialize an infection and subsequently invade the tissue; therefore producing a wide arsenal of virulence factors is not a requisite at least for initial steps of genitourinary infections. Another example stating this idea is the fact that *P. aeruginosa* tracheo-bronchial isolates are amongst the most virulent strains tested, producing also less common virulence determinants for this species. The high adherence ability (the first step for biofilms formation) of tracheo-bronchial isolates explains the great biofilm associated *P. aeruginosa* infections in the airways and lungs (LaSarre and Federle, 2013). DN-ase production by these strains may indicate the fact that Pseudomonas needs to handle thick mucus, frequently found in tracheo-bronchial infection, especially in cystic fibrosis patients, which is comprised mainly by chromatin and may act as a microbial trap (Young et al., 2011).

The results of earlier studies suggested the ability of *S. aureus* and *P. aeruginosa* to invade and survive inside the host cell (Edwards et al., 2010; Bianconi et al., 2011) but studies on the extent of invasive potential of different strains found in distinct clinical contexts have not been achieved to date. Our results demonstrated that bacteria may develop various levels of invasiveness, according to their species or clinical source. Our results showed that *P. aeruginosa* strains possess a higher invasive potential when compared with *S. aureus* strains, and isolates from blood cultures are certainly the most invasive in both cases. Differences related to the invasiveness potential between *S. aureus* and *P. aeruginosa* species, could be explained, among others, by the adherence capacity and adherence patterns. *P. aeruginosa* strains which adhere often in a diffuse manner will be likely more invasive than *S. aureus* strains, which exhibited a higher inter-bacterial aggregative potential, knowing that a big aggregate is more difficult to be internalized by the host cell.

Conclusions

This paper reports the virulence behavior of the most studied Gram-positive and negative human pathogens models, which infect different anatomical sites. The present data demonstrate that versatile pathogens like *P. aeruginosa* and *S. aureus* may act differently when they are the protagonists of infections that occur in different clinical contexts. Our studies support the idea that the relationship between pathogens and their hosts, as well as the influences of each entity on the other play an important role in the dynamics of infectious process.

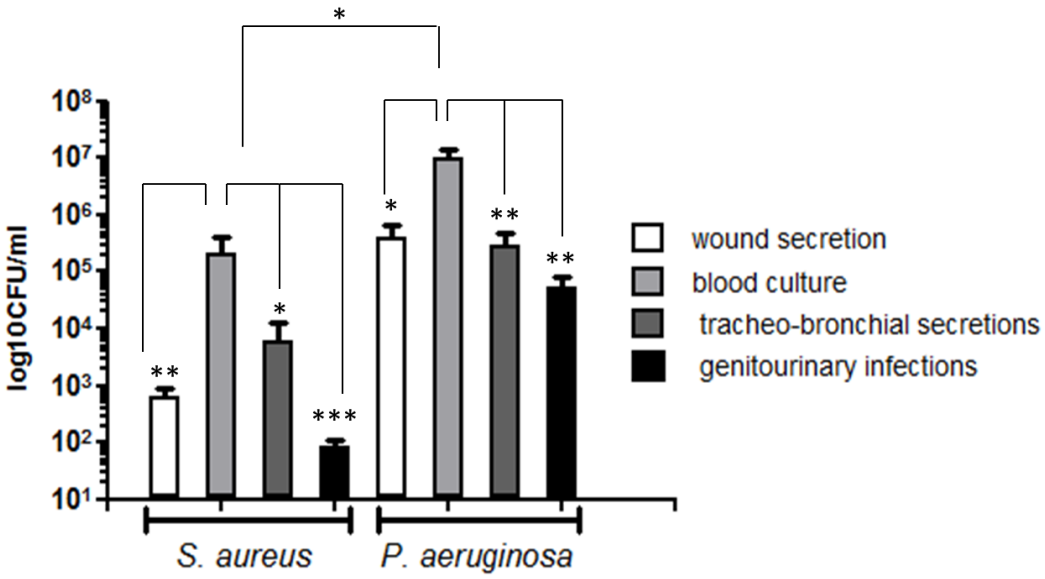

Figure 5. Invasion potentials of *S. aureus* and *P. aeruginosa* tested strains, clustered according to their isolation source. On the X-axis are figured clustered strains according to their isolation source. Y axis represents the invasive abilities of tested strains, referred to as log_{10}CFU/ml. *=P<0.05; **=P<0.01; ***=P<0.001.

Variations in virulence profiles reflect the adaptive ability of bacterial species and the great adaptability of pathogens enables them to evolve quickly; therefore deep knowledge of the infectious process, according to the clinical context and features of the incriminated strain represents a key aspect for setting up efficient procedures for prevention or for personalized treatment of infections.

ACKNOWLEDGEMENTS

This research was partially financed by the European Funding Program POSDRU 107/1.5/S/80765 and Human Resources 135 (Contract. No 76/2010).

REFERENCES

Aguilar C, Friscina A, Devescovi G, Kojic M, Venturi V (2003). Identification of quorum-sensing regulated genes of *Burkholderia cepacia*. J. Bacteriol. 185:6456-6462.

Bayles KW, Wesson C, Liou LE, Fox LK, Bohach GA, Trumble WR (1998). Intracellular *Staphylococcus aureus* escapes the endosome and induces apoptosis in epithelial cells. Infect. Immun. 66:336-342.

Bianconi I, Milani A, Cigana C, Paroni M, Levesque RC (2011). Positive Signature-Tagged Mutagenesis in Pseudomonas aeruginosa: Tracking Patho-Adaptive Mutations Promoting Airways Chronic Infection. PLoS Pathog. 7:e1001270.

Cravioto A, Gross RJ, Scotland SM, Rowe B (1979). An adhesive factor found in strains of *Escherichia coli* belonging to the traditional infantile enteropathogenic serotypes. Curr. Microbiol. 3:95-99.

Edwards AM, Potts JR, Josefsson E, Massey RC (2010). *Staphylococcus aureus* host cell invasion and virulence in sepsis is facilitated by the multiple repeats within FnBPA. PLoS Pathog. 6:e1000964.

Fleiszig SMJ, Zaidi TS, Preston MJ, Grout M, Evans DJ, Pier GB (1996). The relationship between cytotoxicity and epithelial cell invasion by corneal isolates of *Pseudomonas aeruginosa*. Infect. Immun. 64:2288-2294.

Golubchik T, Batty EM, Miller RR, Farr H, Young BC (2013). Within-Host Evolution of *Staphylococcus aureus* during Asymptomatic Carriage. PLoS ONE 8(5):e61319.

Holban AM, Chifiriuc MC, Lazăr V (2013). Host cells response in *Pseudomonas aeruginosa* infections - role of quorum sensing molecules. Afr. J. Microbiol. Res. 7(21):2420-2429.

Holban AM, Lazar V (2011). Inter-kingdom cross-talk: the example of prokaryotes - eukaryotes communication. Biointerface Res. App. Chem. 1:95-110.

Holban AM, Saviuc C, Grumezescu AM, Chifiriuc MC, Banu O, Lazăr V (2012). Phenotipic investigation of virulence profiles in some *Candida spp.* strains isolated from different clinical specimens. Lett. App. NanoBioSci. 1(3):72-76.

Hughes DT, Sperandio V (2008). Inter-kingdom signalling: communication between bacteria and their hosts. Nat. Rev. Microbiol. 6:111-120.

Karavolos MH, Winzer K, Williams P, Khan CM (2013). Pathogen espionage: multiple bacterial adrenergic sensors eavesdrop on host communication systems. Mol. Microbiol. 87(3):455-65.

Kudoh I, Wiener-Kronish JP, Hashimoto S, Pittet JF, Frank D (1994). Exoproduct secretions of *P. aeruginosa* strains influence severity of alveolar epithelial injury. Am. J. Physiol. 267:551-556.

LaSarre B, Federle MJ (2013). Exploiting Quorum Sensing To Confuse Bacterial Pathogens. Microbiol. Mol. Biol. Rev. 77(1):73-111.

Nickerson EK, Hongsuwan M, Limmathurotsakul D, Wuthiekanun V, Shah KR (2009). *Staphylococcus aureus* Bacteraemia in a Tropical Setting: Patient Outcome and Impact of Antibiotic Resistance. PLoS ONE 4:e4308.

Saviuc C, Grumezescu AM, Oprea E, Radulescu V, Dascalu L, Chifiriuc MC, Bucur M, Banu O, Lazar V (2011c). Antifungal activity of some vegetal extracts on *Candida* biofilms developed on inert

substratum. Biointerface Res. App. Chem. 1:15-23.
Saviuc C, Grumezescu AM, Holban A, Bleotu C, Chifiriuc C, Balaure P, Lazar V (2011a). Phenotypical studies of raw and nanosystem embedded *Eugenia carryophyllata* buds essential oil antibacterial activity on *Pseudomonas aeruginosa* and *Staphylococcus aureus* strains. Biointerface Res. App. Chem. 1:111-118.
Saviuc C, Grumezescu M, Holban A, Chifiriuc C, Mihaiescu D, Lazar V (2011b). Hybrid nanostructurated material for biomedical applications, Biointerface Res. App. Chem. 1:64-71.
Shin JS, Gao Z, Abraham SN (2000). Involvement of cellular caveolae in bacterial entry into mast cells. Science 289:785-788.
Sperandio V, Torres AG, Jarvis B, Nataro JP, Kaper JB (2003). Bacteria–host communication: The language of hormones. PNAS 100:8951-8956.
Tarkka MT, Sarniguet A, Frey-Klett P (2009). Inter-kingdom encounters: recent advances in molecular bacterium–fungus interactions. Curr. Genet. 55:233-243.
Tateda K, Ishii Y, Horikawa M, Matsumoto T, Miyairi S, Pechere JC, Standiford TJ, Ishiguro M, Yamaguchi K (2003). The *Pseudomonas aeruginosa* autoinducer *n*-3-oxododecanoyl homoserine lactone accelerates apoptosis in macrophages and neutrophils. Infect. Immun. 71:5785-5793.
Van Delden C, Iglewski BH (1998). Cell-to-cell signaling and *Pseudomonas aeruginosa* infections. Emerg. Infect. Dis. 4:551-560.
Young RL, Malcolm KC, Kret JE, Caceres SM, Poch KR (2011). Neutrophil Extracellular Trap (NET)-Mediated Killing of *Pseudomonas aeruginosa*: Evidence of Acquired Resistance within the CF Airway, Independent of CFTR. PLoS ONE. 6(9):e23637.
Zaas DW, Duncan MJ, Li G, Wright JR, Abraham SN (2005). *Pseudomonas* Invasion of Type I Pneumocytes Is Dependent on the Expression and Phosphorylation of Caveolin-2. J. Biol. Chem. 280:4864-4872.