The Pseudomonads as a versatile opportunistic pathogen in the environment

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Members of the genus *Pseudomonas* are aerobic, cytochrome oxidase-positive, Gram-negative bacilli widely found as free-living organisms in soils, fresh water, marine environments, and in many other natural habitats. They can also be found in close association with plants and animals as normal flora or as agents of disease. *Pseudomonas* is highly resistance to major classes of antibiotics and this resistance has been attributed to the efflux system. Thus, efflux pump inhibitors are thought to be useful in limiting the invasiveness and antimicrobial resistance of *P. aeruginosa* and may be a useful tool as new anti-infectious agents. Thus, the ecological nature of pseudomonads in the ecosystem has been a subject of attention.

Keywords: *Pseudomonas* species; Commensal; Resistance determinants; Public health

1. Introduction

The Pseudomonads are ubiquitous Gram-negative, motile and oxidase positive bacteria that flourishes and inhabits a diversity of environments [1], including soil and surface water [2]. Members of the genus *Pseudomonas* have meager nutritional requirements and are able to grow well under normal conditions in diverse populations with other types of microorganisms [1]. The Pseudomonads have metabolic capabilities that enable them to make use of a wide range of organic compounds [3, 4], and occupy a significant position and ecological niche in the carbon cycle. The capability of *Pseudomonas* species to colonize these diverse ecological niches is attributed to their ability to synthesize a large number of different virulence factors such as alginate, pili and lipopolysaccharides, collectively with toxin, proteases and haemolysins [5]. They also play significant role as pathogens of insect, animals, plants tissues and the rhizosphere [2, 6-10]. Some *Pseudomonas* species have become valuable for use in biological control due to their ability to exhibit plant growth-promoting and pathogen-suppressing functions [11, 12].

Different species of *Pseudomonas* has been described however, *Pseudomonas aeruginosa* is the most common pseudomonal specie, other significant species in the genus include, *Pseudomonas fluorescens*, *Pseudomonas putida*, *Pseudomonas stutzeri*, *Pseudomonas mendocina*, *Pseudomonas alcaligenes*, *Pseudomonas pseudoalcaligenes*, *Pseudomonas* species CDC group, among others [13, 14]. *Pseudomonas aeruginosa* is the most commonly encountered Gram-negative organisms and type specie of the genus. Although *P. aeruginosa* is regarded as a saprophyte, it rarely causes disease in healthy persons, but may become opportunistic pathogen in immunocompromised host [15].

Different species of *Pseudomonas* demonstrate some degree of variability in their ability to thrive in diverse plant environments, with *Pseudomonas syringae* known to be well adapted for growth on leaf surfaces and in plant tissues, while *P. fluorescens, P. aeruginosa,* and *P. putida* showing greater abilities to thrive in the rhizosphere [7, 10, 16-17].

Strains of *Pseudomonas* have emerged as a major nosocomial pathogen in immuno-compromised and debilitated persons as well as cyctic fibrosis host [15]. They are considered to be difficult targets for antimicrobial chemotherapy, either intrinsically (due to the constitutive expression of β-lactamases and efflux pumps, together with low permeability of the outer-membrane) or antibiotic resistance genes acquisition (as genes for β-lactamases or modifying their target or enzymes inactivating aminoglycosides), decreased expression of porins, over-expression of efflux pumps, or mutations in quinolone target [18-21]. However, *Pseudomonas aeruginosa* is increasingly recognized as an opportunistic pathogen of clinical significance. A number of different epidemiological studies are usually employed to track its occurrence as a nosocomial pathogen. In public health this opportunistic pathogen has gained attention by affecting primarily immuno-compromised individuals as well as possession of high resistance against antibiotics [22]. Thus, the ecological nature of pseudomonads in the ecosystem has been a subject of attention.

2. The genus *Pseudomonas*

The *Pseudomonas* (Gamma-Proteobacteria subclass, Pseudomonadales order, Pseudomonadaceae family) are non-sporulating rods, motile (one or several polar flagella), with Gram-negative reaction and GC content of 58–69% [23], and is classified as the most diverse class of Proteobacteria [24-25]. They are chemo-organotrophic and catalase positive, with a strict respiratory metabolism. Within the *Pseudomonas sensu stricto*, which corresponds to the rRNA

group I (Palleroni, 2008), the fluorescent pseudomonads include all *Pseudomonas* species that possess the ability to produce fluorescent pyoverdine siderophores, example of such are *P. aeruginosa*, *P. syringae*, *P. putida* and *P. fluorescens* [26]. The genus has undergone subsequent reviews and its phylogenetic associations with related groups or species that were determined previously as *Pseudomonas* was reclassified based on 16S rDNA sequence [27]. The similarities of rRNA sequence between *Pseudomonas* species were determined originally by hybridization of DNA to ribosomal rRNA. The outcome of the findings of the genus *Pseudomonas* was subdivided into five distinct rRNA homology group as shown in Table 1.

Table 1 rRNA homology group based on DNA-rRNA hybridization.

rRNA Group[a]	Subclasses of Proteobacteria[b]	Reclassification into new genus[c]
I	γ (Gamma)	*Pseudomonas*
II	β (Beta)	*Burkholderia, Ralstonia*
III	β (Beta)	*Comamonas, Acidovorax, Hydrogenophage*
IV	α (Alpha)	*Brevundimonas*
V	γ-β (Gamma-Beta)	*Stenotrophomonas*
Unknown	β (Beta)	*Telluria*
Unknown	α (Alpha)	*Aminobacter*
Unknown	α (Alpha)	*Oligotropha*
Unknown	α (Alpha)	*Zavarzinia*
Unknown	α (Alpha)	*Sphingomonas*
Unknown	α (Alpha)	*Devosia*
Unknown	γ (Gamma)	*Chryseomonas*
Unknown	γ (Gamma)	*Flavimonas*

[a]Subclasses of Proteobacteria according to Woese [28] and Stackebrandt *et al*. [29]
[b]rRNA group according to Palleroni *et al*. [30] and Palleroni [24]
[c]Adapted from Kerster *et al*. [27]

3. Taxonomy and Classification of the *Pseudomonas* species

The genus *Pseudomonas*, described by Migula in 1894 as an aerobic, Gram-negative, rod-shaped, non-spore forming microorganisms [23-24], has been regularly undergoing taxonomic reclassification [1, 3]. The phenotypic and chemotaxonomic diversity of the genus is reflected in a fairly wide DNA guanine plus cytosine (G+C) content ranging from 58 to70 mol% (Palleroni, 1984). DNA-RNA hybridization procedures have revealed five rRNA groups of pseudomonads (rRNA groups 1 to V) [1, 23, 24, 30]. This broad degree of heterogeneity amid the old genus of *Pseudomonas* is revealed by the presence of distantly related species which have since been placed in existing or newly defined genera. *Pseudomonas* species previously belonging to rRNA groups II to V have been reclassified to genera including *Ralstonia*, *Comamonas*, *Acidovorax*, *Brevundimonas*, *Burkholderia*, *Sphingomonas*, *Hydrogenophaga*, and *Stenotrophomonas* [3, 27, 31]. These analyses and taxonomical reorganization identified the rRNA group I into several intrageneric clusters, which include *P. syringae, P. putida, P. aeruginosa, P. fluorescens, P. pertucinogena, P. stutzeri,* and *P. chlororaphis* groups as members of a phylogenetically homogeneous group referred to as *Pseudomonas* (*sensu stricto*) [2, 32-35]. The phylogenetic distance in between these rRNA groups was more precisely determined by the inclusion of a wide array of Proteobacteria in rRNA:DNA hybridization studies [36-38]. The main outstanding outcome of these rRNA studies was the placement of *Pseudomonas* species in Beta (β) Alpha (α) and Gamma (γ) subclasses of the Proteobacteria and the demonstration that some *Pseudomonas* species were closely related to other genera (e.g., *Escherichia, Xanthomonas, Halomonas,* etc.). Molecular organization based on 16S rRNA sequences places the genus *Pseudomonas*, previously referred to as the type I or fluorescent group of pseudomonads, in the group called *Pseudomonas* and relatives, along with the subgroups of *Acinetobacter* and *Teredinibacter* [39] Lately, the taxonomic status of *P. chlororaphis* was reorganized to create three subspecies within *P. chlororaphis*: *P. chlororaphis* subsp. *chlororaphis*, *P. chlororaphis* subsp. *aureofaciens* and *P. chlororaphis* subsp. *aurantiaca* [40].

4. The *Pseudomonas* species

The genus *Pseudomonas* includes species of environmental, health-related and other economic importance. Pseudomonads are well renowned as widespread bacteria that have been isolated from a diversity of natural sources including soil, plants, mineral waters, surface waters, wastewater treatment plants and from clinical specimens, and they are characterized by a high level of metabolic diversity [10, 33, 41-44,114]. Some *Pseudomonas* species live in commensalism with plants, thereby utilizing nutrients exuded from plant surfaces and surviving environmental stress by occupying protected sites provided by the plant's architecture. These commensal species exhibit profound effects on plants by suppressing pests, enhancing access to vital nutrients, alteration of physiological processes or degradation of environmental pollutants. Also, *Pseudomonas* species have exceptional capability to produce a wide variety of secondary metabolites, including antibiotics that are toxic to plant pathogens [45-46].

Pseudomonas is highly resistance to major classes of antibiotics and this resistance has been attributed to the efflux system. Thus, efflux pump inhibitors are thought to be useful in limiting the invasiveness and antimicrobial resistance of *Pseudomonas* and may be useful tool as new anti-infectious agents. Pseudomonad infection has been described to occur in three stages which are: bacterial attachment and colonization, followed by local invasion and thirdly, dissemination and systemic disease [15]. *Pseudomonas* produces proteases among other extracellular substances, which may play a role in pathogenesis. They can cause a variety of infections which include bacterial meningitis, otitis media, chronic pulmonary colonisation and pneumonia, endocarditis, urinary tract infections and osteomyelitis [47]. First line of treatment of most *Pseudomonas* infections involves the use of aminoglycosides, either alone or in combination with β-lactams. *Pseudomonas* are known to have an innate resistance to several antibiotics due to the presence of lipopolysaccharides in the outer membrane, but relentless administration of antimicrobial agents, has resulted in the emergence of multi-resistant strains of *Pseudomonas* [48-49].

4.1 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a gram-negative aerobic motile rod having ability to grow and survive in almost any environment but known primarily dominant in freshwater sources, soil, sewage and vegetation. It has been found on and may occasionally be pathogenic for plants and vegetables. *P. aeruginosa* is an opportunistic pathogen characterized by an innate resistance to multiple classes of antimicrobials [10, 43-44, 50]. *P. aeruginosa* is capable of causing both acute and chronic infections. It has been estimated as the third-leading cause of nosocomial infections and is the predominant pathogen associated with morbidity and mortality of cystic fibrosis patients [51]. However, despite tremendous opportunities for spread, *P. aeruginosa* seldom causes community-acquired infections in immuno-competent individuals. As a result, they are regarded as opportunistic pathogen [52-53].

The ubiquitous occurrence of *Pseudomonas* in diverse environments is as a result of several factors, including its abilities to colonize multiple environmental niches and to utilize numerous environmental compounds as energy sources [54]. *P. aeruginosa* is regarded as unusual pathogen in that it is endowed with an outstandingly large genome containing genes for several different virulence factors and regulatory mechanisms allowing it to adapt to hostile environment [53, 55]. The associations of virulence factors are the same for both plant and animal infections [6, 56]. The development of multidrug resistance by *P. aeruginosa* strains is as a result of a number of genetic events including acquisition of different mutation and / or horizontal transfer of antibiotics resistance genes

Within hospital settings, *P. aeruginosa* has been found from equipment that contain or use water (i.e., sink drains, toilets, showers, bathroom fixtures, sanitary plumbing, air humidifiers), with incidence rate of up to 10^9 organisms per ml after undisturbed overnight growth. In an aqueous environment, the organism can colonize different kinds of surfaces including silicon rubber, plastic, chlorinated polyvinyl chloride (CPVC) pipe, and stainless steel. *P*seudomonas *aeruginosa* is capable of elaborating an impressive array of virulence factors, which are divided into specific groups dependent upon their mode of action or method of delivery to the host cell. *P. aeruginosa* virulence factors are described as belonging to adhesins and other secreted toxins. Exotoxins are either passively secreted from the cell or actively secreted via the type I secretion system (T1SS), the type II secretion system (T2SS) or the type III secretion system (T3SS) [57].

4.2 *Pseudomonas chlororaphis*

Pseudomonas chlororaphis is a heterotrophic, soil bacteria that can be found in plant rhizosphere, phyllosphere and sediments [58-59]. They are mesophilic with growth temperature range between 20°C-28°C [59-62], and grow best around a pH of 6.3-7.5 [59, 63]. *P. chlororaphis* is employed as a soil inoculant in agriculture and horticulture [64]. It can also act as a biocontrol agent against some plant pathogens through production of phenazine type antibiotics [64]. The characteristics nature of *P. chloroaphis* includes pyoverdin secretion, or fluorescent [65]. While *P. chlororaphis* is an efficient biocontroller of certain fungi, it is generally considered a non-pathongenic bacterium. A report from the Environmental Protection Agency states that "*P. chlororaphis* has shown no toxicity or pathogenicity to humans, wildlife, or the environment" [66]. Based on 16S rRNA analysis, *P. chlororaphis* has been placed in the *P. chlororaphis* group. The other components of the *P. chlororaphis* subgroup are *P. fragi, P. aurantica*, *P. aureofaciens, P. lundensis* and *P. taetrolens* [34].

4.3 *Pseudomonas fluorescens*

Pseudomonas fluorescens encompasses non-pathogenic saprophytes group of *Pseudomonas* that colonize water, soil and plant surface environments [23]. They live in commensal relationship with plants, allowing plants to acquire vital nutrients, degradation of chemical and biological pollutants, and suppression of pathogens by means of antibiotic productions [67]. In spite of their commensal nature, *P. fluorescens* are non-pathogenic and is deficient of virulence potentials of other plant pathogens [67]. They are well adapted to survival in soil environment and colonization of plant roots system [68], and this applies also to the particular case of biocontrol agents from this species. *P. fluorescens* uncommonly cause disease in humans, and usually affects patients with compromised immune systems [69]. Strain *P.*

fluorescens Pf-5 is a plant commensals noteworthy as a biological control organism, for its rhizosphere competence and the spectrum of antimicrobial and other secondary metabolites that it produces. *P. fluorescens* Pf-5 live in the rhizosphere of a variety of plants and suppresses plant diseases caused by soil borne plant pathogens [70-72]. However, the application of *P. fluorescens* strains for use in release and survival of bacteria in the soil are being investigated extensively. Among these are bioremediation of a variety of organic compounds, and biocontrol of pathogens in agricultural environments.

4.4 *Pseudomonas putida*

Pseudomonas putida is a paradigm of a class of varied opportunistic bacteria found in aquatic and terrestrial environments; they can use a vast range of substrates for their growth [73]. The occurrence of *P. putida* in freshwater and wastewater environments has been documented Igbinosa *et al.* [43]. Also, *Pseudomonas putida* has been found to be dominant in plant root, cultivated soil and plant rhizosphere; hence, seasons and different sample sources may play a role in the distribution of commensal Pseudomonas in the environment [10]. Garbeva *et al.* [74] revealed how different agricultural regimes strongly influence the structure of Pseudomonas population dynamic in soil. *P. putida* is a unique soil microorganism that has the potential to help clean up organic pollutants which can resist the adverse effects of organic solvents [73]. The rhizobacterium *Pseudomonas putida* GR12-2 is becoming a strong candidate for development as a soil inoculant to improve crop yields. The inoculation of canola, tomato, and other agriculturally important plants with this strain has resulted in substantial promotion of seedling root growth [75-76]. *P. putida* is usually non-pathogenic; other pseudomonads are able to cause disease. *P. putida* is known as opportunistic pathogen that rarely cause human infection [77], making it to be considered as a low-grade pathogen [78-79]. Recently, multi-drug resistant *P. putida* have been found in connection with difficult to treat infections [80-81]. The presence of cytotoxin, alkaline protease, phenazine and blaVIM-2 in environmental strains of *P. putida* has been documented [55]. Although *P. putida* is not common in human infection, they can act as reservoir for antibiotic resistance and virulence genes determinant in the environment.

4.5 *Pseudomonas syringae*

Pseudomonas syringae is mainly associated with plants, lives in the phyllosphere and it is extensively known as a plant pathogen [82]. *P. syringae* are mostly psychrophiles with optimum growth temperature of 30 °C. *P. syringae* is a phytopathogenic bacterial species currently divided into more than fifty pathovars and nine genomospecies [83-85], that are pathogenic to over 180 plant species, including fruit plants, vegetables, plant leaves, ornamentals and other annual and perennial species [83-84]. It can also prevent fungal decomposition of fruit crops after harvest [86]. Several *P. syringae* pathovars that are known to cause these diseases can be clearly differentiated. Firstly, *P. syringae* pv. *syringae* (*Pss*), belonging to the genomospecies 1 as defined by Gardan, *et al.* [85], is highly pathogenic to all its hosts and many other hosts. On the other hand, other *P. syringae* pathovars usually have a much narrower host range [83]. Secondly, *P. syringae* pv. *morsprunorum* (*Psm*), with race 1 [87], (Wormald, 1932) belonging to genomospecies 2, pathogenic to some fruits crops like cherry, plum and apricot, and race 2 [88], belonging to genomospecies 3 is pathogenic to cherry. Thirdly, *P. syringae* pv. *avii*, belonging to genomospecies 3, pathogenic to wild cherry trees that are cultivated for wood production [89]. Lastly, *P. syringae* pv. *persicae*, belonging to genomospecies 3, pathogenic to nectarine, Japanase plum and peach [90-91].

5. Pathogenicity and Virulence Factors

Several bacteria pathogens have life cycles that involve survival and reproduction in non-host environmental habitats. In the case of human pathogens, numerous studies have revealed how adaptation to environmental habitats is linked to the evolution of their pathogenicity and emergence of pathogens (Morris, *et al.,* 2010). For plant pathogens (*Pseudomonas syringae*), the connection between adaptation to non-host habitats and pathogenicity has been investigated by Morris, *et al.* [86]. The biology of pathogens in their non-host environmental reservoirs can have key effect on their ability to cause disease in their hosts. This has been demonstrated for some human pathogens, for example *Vibrio cholerae*, with a marked capacity to survive and flourish in environmental reservoirs [92-93]. Other examples consist of *Legionella pneumophila* [94], *Pseudomonas aeruginosa* [95], the *Burkholderia cepacia* complex [96], and *Cryptococcus neoformans* [97]. Environmental reservoirs play three key roles in the evolutionary ecology of these pathogens [98]. First is their ability to survive and proliferate in environmental reservoirs which ensures a competitive advantage in the absence of the host. Secondly, these reservoirs promote acquisition of genes for virulence factors. Thirdly, some virulence factors also play roles in adaptation to the conditions of the environmental reservoirs and are thereby favourable selected in the reservoirs in the absence of interaction with the primary host [96].

Various *P. aeruginosa* virulence factors contribute significantly to pathogenesis of their infections. Rahme *et al.* [6], demonstrated the occurrence of virulence factors of *P. aeruginosa* contributing to pathogenesis in both burn and wound infection of rodents and leaf destruction in the plant *Aradopsis thaliana*. *P. aeruginosa* cause infection in an immunocompromised host defense, yet different potential virulence factors have contributed to its pathogenicity in the

immunocompromised individual. A summary of virulence factors of *P. aeruginosa* is described in Table 2. *P. aeruginosa* infections symbolize those of a pathogen with various potential virulence factors that allows it to colonize and infect basically any mammalian tissue. The organism demonstrates a multitude of factors that support adherence to host cells and mucins, elicit inflammation, damage host tissue and disrupt defense mechanisms. Regardless of the ubiquitous nature of this organism, and the occurrence with which it is encountered, most human hosts counteract the infectious process effectively through the innate immune system [52].

Table 2 Pathogenesis and virulence factor of *Pseudomonas aeruginosa.*

Bacterial cell surface virulence factors	**Biological action**
Lipopolysaccharide (LPS)	Dominant antigenic determinant on cell surface; loss of sugar unit side chains during chronic infection creates "rough" LPS and serum sensitivity
Pili or fimbriae	Adherence to epithelium
Flagella	Tethering and adhering to epithelial cells
Alginate (mucoid exopolysaccharide)	Adherence to epithelium; barrier to phagocytes and antibiotics; inhibits antibody and complement binding
Secreted virulence factor	
Protease enzymes	Tissue damage; epithelial cell tight junction separation; degrade fibronectin; cleave antibodies creating non-functional blocking antibodies; inactivate α_1-antiproteinase, complement components and cytokines; cleave C3b receptors from neutrophils
Exotoxin A	Cytotoxic by inhibiting protein synthesis; toxic to macrophages; T cell mitogen; inhibits granulocyte and macrophage progenitor cell proliferation
Phospholipase C	Haemolysis; tissue damage; surfactant inactivation
Pigments (pyocyanin, and pyoverdine)	Inhibit ciliary beat; siderophores; toxic to other bacterial species and human cells; enhance oxidative metabolism of neutrophils; inhibit lymphocyte proliferation
Rhamnolipid	Haemolysis; inhibit ciliary beat; stimulate mucus secretion, affect ion transport across epithelium
Lipase	Tissue damage
Histamine	Impair epithelial integrity
Exoenzyme S	Adherence to epithelium; cytotoxic
Leukocidin	Cytotoxic to neutrophils and lymphocytes

Source: Adapted from Wilson and Dowling [99].

Type III secretion systems are common in *Pseudomonas* species as a means of injecting toxins directly into the host cells. The type III secretion system (TTSS) of *P. aeruginosa* is a complex pilus-like structure that permits the translocation of effector proteins from the microorganism, across the bacterial membranes and into the eukaryotic cytoplasm through a needle-like appendage forming a pore in the eukaryotic membrane [100]. There are four known toxins that are variably expressed in different *Pseudomonas* strains and isolates, injected into host cells by *P. aeruginosa* through the TTSS: ExoY, ExoS, ExoT and ExoU (Table 3).

Table 3 Type III secretion systems in *Pseudomonas aeruginosa.*

Toxins	**Biological action**	**References**
ExoS	Bifunctional cytotoxin; disruption of normal cytoskeletal organization; modulate host immune and inflammatory response	[101-104]
ExoT	Effects on the eukaryotic cytoskeleton; cytotoxin	[102, 105]
ExoY	An adenylate cyclase; host cytosolic by TTSS and increase cAMP enhanced by a eukaryotic cofactor; cytosolic cAMP lead to increased pulmonary microvascular intercellular gap formation	[106-107]
ExoU	Major cytotoxin; Possess phospholipase / lysophospholipase activity; disrupting eukaryotic cell membrane	[108 -111]

6. Antibiotic Resistance in *Pseudomonas* species

Generally, *P. aeruginosa* strains involved in infections are both invasive and toxigenic due to the production of surface virulence factors (e.g., bacterial attachment, invasion and colonisation) and secretion of virulence factors (damage tissue or trigger the production of cytokines) [53]. *Pseudomonas aeruginosa* is intrinsically resistant to a number of antibiotics as a result of the low permeability of its outer-membrane, the constitutive reflection of various efflux pumps, and the production of antibiotic-inactivating enzymes (e.g., cephalosporinases) [18]. Furthermore, it also has a noteworthy capability to develop or acquire new mechanisms of resistance to antibiotics. The huge size and the versatility of its genome play a key role in its resistance to antibiotics, and to its distribution in aquatic environment, which may constitute a reservoir for bacteria carrying other resistance genes [19]. The most important resistance mechanisms in clinical isolates for the three main classes of anti-pseudomonal agents currently in use (β-lactams, aminoglycosides and fluoroquinolones) has been described by some authors McGowan [21] and Thomson and Bonomo [20]. These mechanisms are frequently present simultaneously, conferring multi-resistance to many *Pseudomonas* strains [20-21].

7. Antibiotic Resistance Control and Management

Control measures to limit the incidence and spread of highly resistant clones appear to be crucial. At the level of clinical service, these should include the stringent implementation of infection control measures aimed at controlling and preventing cross-contamination among patients, within and across units/wards, and even among hospitals, and the strict isolation and control of transfer of infected or colonised individuals with multi-resistant *P. aeruginosa* infection [112]. At the laboratory level, *in-vitro* studies, which include quantitative data (MIC determinations), should be carried out on a regular basis in order to monitor the resistance patterns of the clones present in a particular hospital. This knowledge is essential in guidance of choice of the most appropriate antibiotics for empirical treatment. Studies aimed at deciphering the means of transmission of these clones would also be of interest when formulating strategies for better control of their spread [113].

The therapeutic level improvement of antibiotic use is a highly efficient strategy for decreasing rates of resistance [41]. Two lines of action are necessary to be followed. First, programmes and strategies aiming at reducing antibiotic use in general and at restriction in the administration of certain specific drugs would be beneficial [115-116]. Second, optimization of antibiotic dosage regimens, based on the pharmacokinetic / pharmacodynamic attributes of the drugs used should be considered essential for appropriate treatment of pseudomonal infections [117]. There is a strong link between antibiotic consumption and resistance rates for *P. aeruginosa* [118], (and for many other pathogenic bacteria. Antibiotic rotation (to avoid continuous exposure to the same drugs) is been proposed, but no data is available that support its benefit for resistance control.

Acknowledgments This work was supported by grant from The World Academy of Science (TWAS), Research Grant No.: 14-091 RG/BIO/AF/AC_1-UNESCO FR: 324028575.

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