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Pseudomonas aeruginosa transition from environmental generalist to human pathogen

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Pseudomonas aeruginosa transición de generalista ambiental a patógeno humano

Abstract

Opportunistic bacteria *Pseudomonas aeruginosa* is a major concern as an etiological agent of nosocomial infections in humans. Many virulence factors used to colonize the human body are the same as those used by *P. aeruginosa* to thrive in the environment, such as membrane transport, biofilm formation, oxidation/reduction reaction, and others. The origin of *P. aeruginosa* is mainly from the environment; the adaptation to mammalian tissues may follow a source-sink evolution model. The environment is the source of many lineages, some of them capable of adaptation to the human body. Some lineages may adapt to humans and go through reductive evolution in which some genes are lost. The understanding of this process may be critical in order to implement better methods of controlling outbreaks in hospitals.

Keywords: bacteria, host adaptation, evolution, opportunistic,

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Resumen

La bacteria oportunista *Pseudomonas aeruginosa* es una de las principales preocupaciones por su rol como agente etiológico de infecciones nosocomiales humanas. En muchos casos, los mismos factores de virulencia que le permiten a *P. aeruginosa* causar infecciones en humanos pueden ser empleados para prosperar en el ambiente, como los sistemas de transporte de membranas, la formación de biopelículas, las reacciones de oxidación / reducción y otros. El origen de *P. aeruginosa* es principalmente el ambiente; mas su adaptación a los tejidos de los mamíferos puede seguir un modelo de evolución del tipo fuente-sumidero. El ambiente es la fuente de muchos linajes de esta bacteria, donde algunos de ellos son capaces de adaptarse al cuerpo humano. Algunos linajes pueden adaptarse a los humanos y pasar por una evolución reductiva en la que se pierden algunos genes. La comprensión de este proceso puede ser fundamental para implementar mejores métodos de control de brotes en los hospitales.

Palabras clave: bacteria, adaptación al hospedador, evolución, oportunista

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INTRODUCTION

Pseudomonas aeruginosa is a Gram-negative aerobic rod bacteria, ubiquitous in the environment, and an opportunistic human pathogen [1]. It accounts for 9-19% prevalence of bacterial nosocomial infections and 7% of community-acquired pneumonia cases [2, 3]. *Pseudomonasa eruginosa* infections occur in the respiratory tract [4,5], eyes [6], ears, skin wounds [7], bloodstream [1], or surgical site infections [8]. *Pseudomonas aeruginosa* can colonize human intestines and skin, and it can take advantage of any host's immunodeficiency to produce acute or chronic systemic infections [4, 9]. Moreover, it is argued that *P. aeruginosa* infections are acquired from the bacterial population that colonizes the proximal environment of the host [10].

Pseudomonas aeruginosa prospers in soil, tap water, plants, intestinal contents, food, puddles, and swimming pools; it has some remarkable abilities to survive in the presence of heavy metals and chlorine [11]. *Pseudomonas aeruginosa* lives surrounded by predatory amoeba, which may have selected bacterial cells with the ability to survive phagocytosis [12]. Living outside the host, *P. aeruginosa* adapts to changes (physical and chemical) and competes with other environmental microorganisms. This bacterium is a generalist and heterotrophic, possessing an arsenal of enzymes to oxidize many organic carbon sources, mostly decaying organic matter (and even xenobiotic compounds), to obtain energy [13–19].

One of the main problems associated with *P. aeruginosa* is the occurrence of nosocomial outbreaks where the source of the bacteria is unknown; many times hospitals have resorted to extreme measures such as plumbing system replacements to stop *P. aeruginosa* dissemination [20, 21]. Also, multidrug resistance *P. aeruginosa* is one of the most critical concerns of hospital-acquired infections [8].

In this review, we describe the complexity of the evolutionary processes involved in the adaptation of environmental *P. aeruginosa* to human tissues. The sporadic infections by environmental strains and infections by strains adapted to humans are examined. We will not address antibiotic resistance which is comprehensively covered by other manuscripts [22–24].

SPORADIC INFECTIONS BY ENVIRONMENTAL STRAINS

Pseudomonas aeruginosa infections with environmental strains involve the migration of bacteria from a heterogeneous ecosystem (environment) to a more homogeneous and restrictive ecosystem (human tissues). This movement is also known as a source-sink dynamic. The *source* is the environment to which the bacteria are adapted, and the *sink* is the human tissue that is often a harsher milieu. The bacterial growth rate in the sink may not compensate for the death rate; therefore, the bacterial population in the sink is maintained by a constant introduction of bacteria from the source (environment outside the host) [25].

The adaptation of *P. aeruginosa* to different environments implies multiple mechanisms of DNA modifications such as inheritance of mutations, homologous recombination,

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horizontal gene transfer (acquisition of accessory genome), and gene deletion [26, 27]. Environmental strains of *Pseudomonas* obtain nutrients from human tissues and neutralize immune responses using the same genes that are useful for dealing with environmental challenges [14, 28, 29]. For example, a type 3 secretion system (T3SS) is a translocation apparatus enabling the bacteria to export effector proteins from the bacterial cell to a eukaryotic cell without an extracellular step. Effector proteins can cause different consequences in the eukaryotic cell; exoenzyme (Exo) U is a phospholipase that induces cell death of predatory amoebas [28, 30] whereas ExoS, another T3SS-exported effector protein, is involved in anti-predatory responses against free-living amoeba [31]. The same T3SS and its ExoU phospholipase also kill macrophages [32]; the effector protein, ExoS, has two domains that act in ADP-ribosylation and GTPase activities in host cell proteins [32] and also activates Toll-like receptors in phagocytes [33].

In the environment outside the host, stress caused by toxic levels of metal ions like copper, iron, and zinc induces the synthesis of pyoverdine, superoxide dismutase, fumarate hydratase, metal cation efflux transporter CzrA, ATP-binding cassette transporters (ABC transporters), copper resistance oxidase proteins, and others [34]. These molecules protect *P. aeruginosa* from oxidative insults and increase the availability of iron. In the host, the same molecules protect bacteria against oxidative reactions (from neutrophils or macrophages) and help to capture iron [35], as iron is sequestered in the host's proteins [36]. Iron associates and inactivates Fur-like transcriptional repressors, which downregulate many genes required to scavenge iron from animal tissues [37].

The range of niches in different environments outside of humans is reflected in vast *P. aeruginosa* diversity [38]; this diversity makes it challenging to identify the environmental source of clinical strains, and some researchers indicate that all environmental *P. aeruginosa* could cause human disease [39]. In the last years, whole-genome sequencing (WGS) provided irrefutable evidence of the environmental origin of clinical *P. aeruginosa* [26, 27, 40, 41]. Some environmental clones are genetically indistinguishable from clinical isolates [39]; recently, whole-genome sequence comparison of geographically related strains (belonging to ST-1146, Mallorca, Spain) obtained from the environment and a clinical case showed no genetic differences, although the clinical isolate had a mutation in the *oprD* gene (causing carbapenem resistance) [42].

Nevertheless, recent evidence suggests also that not all environmental *P. aeruginosa* strains may be able to cause human infections; apparently some phenotypes are required to become human pathogens [43, 44]. Some evolutionary steps enabling *P. aeruginosa* strains to colonize human tissues may have occurred in the environment outside the host [27]; selective forces in niches outside the host may simulate some conditions in host tissues. For example, the abundance of environmental amoeba may select bacterial lineages able to survive macrophage attack. Nucleotide polymorphisms along the genome of clinical isolates (including epidemic and non-epidemic CF strains) have signs of positive selection (high rates of non-synonymous mutations, dN/dS > 1) [26, 45]; among the genes under positive selection are those involved in LPS biosynthesis, flagella, secretion systems, iron scavenging, and iron uptake [26]. Analysis of *P. aeruginosa* genomes also shows that strains causing clinical infections have suffered higher levels of homologous recombination in genes similar to those showing signs of positive selection: membrane transport structures, biofilm formation, oxide/reduction,

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and other cell wall functions [27]. The recombination in the genome regions where positive selection is observed and the coincidence of recombinations in the same genes in many clinical isolates suggest that natural selection is affecting these strains.

INFECTIONS BY STRAINS ADAPTED TO HUMANS

Recent evidence suggests that human-to-human transmission of *P. aeruginosa* may be associated with the ability of these strains to colonize intestines; patients suffering from *P. aeruginosa* infections were colonized by the same clones [46].

However, some *P. aeruginosa* may become adapted to humans and transmitted from person to person as happens among pulmonary strains of cystic fibrosis patients [47]. Human-adapted *P. aeruginosa* strains have suffered similar evolutionary pressures as strains causing sporadic infections; however, they have acquired additional adaptations during chronic infections, such as cystic fibrosis (CF) (Fig. 1). *Pseudomonas* strains, causing CF infections, tend to form a different group of *P. aeruginosa* populations from strains causing other types of infections in hospitals [27]. CF lung may select strains with additional aptitudes [26, 27] that may have been acquired in inhospitable niches outside the host before adaptation to human tissues [26, 27, 48, 49]. Some of these CF strains become epidemic strains (found in different continents and transferred from person to person) and show positive selection in core genome genes involved in oxidation/reduction, membrane transport, and type III secretion systems, among others [26].

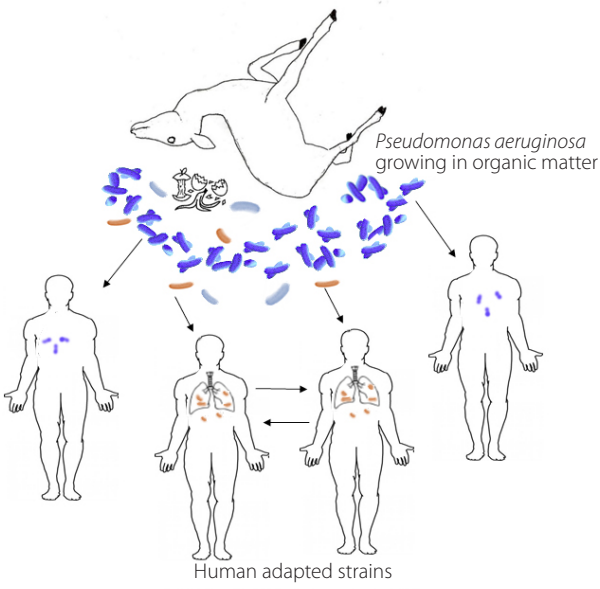

Figure 1. Diverse *Pseudomonas aeruginosa* strains (blue bacteria) thrive in the environment degrading organic matter. Some strains have improved aptitude to grow in human tissue (brown bacteria); a subset of them have evolved additional adaptations and can be transmitted from human to human.

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Going back to source-sink dynamics, if the sink conditions are not too harsh, the adaptation may occur especially if there is a constant migration rate [50] or in the presence of temporal less restrictive conditions [51]; chronic infections in CF patients may represent this. When an environmental generalist (such as *P. aeruginosa*) with a large genome (many genes required to thrive in an environment outside the host) [52–55] enters human tissue, a reductive evolution is prone to occur [18, 52–55]. In this scenario, evolution from environmental to pathogen involves gene loss due to small effective population size and lack of purifying selection; this gene loss is especially intense if these genes are unnecessary in the new niche (Fig. 1) [53, 56].

Longitudinal studies of cystic fibrosis *P. aeruginosa* strains have improved our understanding of evolutionary changes during chronic infections. Strains from these chronic infections show more profound evolutionary changes which probably occurred during infection of CF patients; paradoxically, some of these changes involve the loss of some apparent virulence factors. Evidence of reductive evolution may be apparent by the selection of amino acid auxotrophs in some strains [57, 58], loss of twitching motility [44], or reduction of type IV pili [59]; chronic infections of cystic fibrosis lungs may select patho-adaptive phenotypes [60]. Lungs from cystic fibrosis patients go through oxidative stress by the accumulation of hemoglobin, ferrous iron, and transferrin [61]. Pyoverdine (a siderophore involved in iron scavenging in human tissue) no longer seems a critical factor to obtain iron which may select pyoverdine mutants in genes such as *pvd*S (a sigma factor involved in pyoverdine transcription) and the pyoverdine gene and PrrF (an RNA that reduces pyoverdine expression and increases expression of HemO) [60]. An alternative iron-acquisition mechanism may take over in these mutants, allowing iron to be taken from the heme molecules; these strains may require heme oxygenase (HemO), which breaks the heme and releases biliverdin, carbon dioxide, and iron [62].

Also, the long-term colonization of the lungs selects variants of unusual biofilm formation. In *P. aeruginosa,* biofilm-mucoid phenotypes show increased expression of the exopolysaccharide Psl and alginate. Psl induction occurs by at least six different mutations in operons which occur when the infection lingers for a long time [63]. These strains also show mutations in the *las*R gene, a transcriptional regulator of biofilm formation and other virulence genes [64].

Other *P. aeruginosa* adaptations to human hosts include higher mutation rates [65, 66], increased synthesis of multidrug-efflux pumps, higher antibiotic resistance, mucoid phenotypes, increased biofilm formation, higher tendency to form micro-colonies in tissue [67, 68], upregulation of metalloproteinases, lipid A modifications, reduced fucosyltransferase 2 expression, and better adhesion to tissues [27]. These host-adapted phenotypes have not been found outside the human host even in the same environment where infected patients reside [43, 44].

Some genotypes (LESA and ESB) could be found in CF patients on different continents [26, 27], suggesting human-to-human transmission. Evidence of human-to-human transmission has also been observed in some non-CF lineages (ST-111, ST-235, and ST-175) that caused infections in five hospitals in France during four years [47]—some of these were described in other countries [69]—and in the strain O12, a clone which caused a different type of non-CF infection in different countries during different years [39]. These observations may indicate that non-CF *P. aeruginosa* strains could also adapt to human tissues.

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Additionally, adaptive evolution of bacteria to a new niche may involve antagonistic pleiotropy [70]; a mutation that is adaptive in the new environment could be detrimental in the original environment [71] and may account for many additional variations found in these strains. *In vitro* studies indicate that *P. aeruginosa* derived from CF patients are outcompeted by environmental *P. aeruginosa* [56, 72], which means that selective pressures during the host colonization make *P. aeruginosa* less capable of competing in the environment outside the host and that these strains are possibly transmitted from person to person [27].

CONCLUSIONS

The environment outside the host may contain a variety of ecosystems with characteristics that allow the selection of bacterial lineages to colonize human tissue and evade the immune attack. The environment outside the host is also an endless source of opportunistic pathogens, which can infect immunocompromised patients. Controlling and preventing *P. aeruginosa* infections in hospitals are very complex endeavors that may require an understanding of *P. aeruginosa* population genetics of the strain causing the outbreak and its relationship with the environment. It is critical to recognize the physiological diversity of the different strains of *P. aeruginosa* causing infections. Current information suggests that the routes of transmission of the opportunistic pathogen *P. aeruginosa* are diverse: inanimate objects (such as plumbing and fixtures), human carriers (intestinal or respiratory tract), plants, animals, rivers, etc. It is imperative to use molecular tools to establish whether isolates obtained from different patients or during different timeframes are clonal; genomic information may allow exploring the presence of this pathogen in human carriers, inanimate objects, water sources, etc. Control and preventive measures should be diverse and in agreement with the strain's origin. Sometimes these measures may require the use of disinfectants or antibiotic treatment of carriers; in other cases, they may require the removal of plumbing or fixtures or other sources of organic matter or water sources.

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AUTHOR CONTRIBUTIONS

Both authors wrote and reviewed the manuscript.

CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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