Infection Prevention in Practice 6 (2024) 100331

Available online at www.sciencedirect.com

Infection Prevention in Practice

journal homepage: www.elsevier.com/locate/ipip

Short Report

Endogenous origin of *Pseudomonas aeruginosa* infecting hospitalized patients in Ecuador

Gabriela Vasco [a,b,*], Mishell Achig [b], Belén Prado-Vivar [a,e,f], Maritza Páez [c], Franklin Espinosa [d], Evelyn Espinoza [c], Danny Quinancela [b], Paul Cardenas [a], Gabriel Trueba [a]

[a] *Instituto de Microbiología, Universidad San Francisco de Quito USFQ, Quito, Ecuador*
[b] *Escuela de Medicina, Facultad de Ciencias Médicas, Universidad Central del Ecuador, Quito, Ecuador*
[c] *Laboratorio Clínico-Microbiología Hospital General Docente de Calderón, Quito, Ecuador*
[d] *Laboratorio Clínico, Hospital "Padre Carollo Un Canto Para la Vida", Quito, Ecuador*
[e] *The Walter and Eliza Hall Institute of Medical Research, 1G Royal Parade, Parkville, VIC, 3052, Australia*
[f] *Department of Medical Biology, The University of Melbourne, Melbourne, VIC, 3052, Australia*

ARTICLE INFO

Article history:
Received 5 October 2023
Accepted 21 November 2023
Available online 11 December 2023

Keywords:
Pseudomonas aeruginosa
Nosocomial
Colonization
Acute infection
Whole genome sequencing

SUMMARY

Recent evidence suggests that *Pseudomonas aeruginosa*, a bacterium that has the ability to cause deadly infections in hospitalized patients, could originate in the patient's own flora. We employed the Oxford Nanopore platform to obtain whole genome sequences (WGS) from clinical and rectal screen *P. aeruginosa* strains belonging to 15 patients from two hospitals. Our study found evidence that clinical and rectal isolates were clonal, with some evidence suggesting that the infecting strain was present in the patient's intestine at the time of admission, ruling out hospital acquisition. The use of WGS analysis is crucial to detect alternative sources of *P. aeruginosa* to develop new preventive measures against these serious infections.

Introduction

Pseudomonas aeruginosa is a priority pathogen by the World Health Organization [1] as part of the ESKAPE pathogens by the Infectious Diseases Society of America, due to their role in nosocomial infections and resistance to last-resort antimicrobials [2]. It is generally accepted that *P. aeruginosa* infections are primarily contracted within hospitals through contact with inanimate objects, such as fixtures and wet surfaces [3]. However, many studies have revealed that in some cases, there is no clonal relationship between the infecting strains in the same hospital, even in infections from temporally related patients [4]. Other studies show that strains causing infections are constantly changing over time within the same hospital [5]. Moreover, recent research suggests that these

* Corresponding author. Address: Universidad San Francisco de Quito, Campus Cumbayá, Diego de Robles s/n, Quito 170901, Ecuador.
E-mail address: gpvasco@uce.edu.ec (G. Vasco).

https://doi.org/10.1016/j.infpip.2023.100331

Table I
Information on patients, origin and type of infection, and discrepancies between clinical and rectal samples

Patient	Hospital	Isolate ST	Sample origin	Infection environment	Days staying in a previous hospital	Days between admission to sample collection	Days between clinical and rectal samples
P1	2P	274	Abdominal wound	**Hospital**	**1**	**10**	**4**
		274	Rectal			**14**	
		274	Abdominal discharge	**Hospital**		**21***	**2**
		274	Rectal			**23***	
P2	2P	253	Tracheal discharge	**Hospital**	**29**	**1**	**11**
		253	Rectal			**11**	
P3	1C	253	Sputum	**Hospital**	**-**	**55**	**3**
		253	Rectal			**58**	
P4	1C	n1	Sputum	**Hospital (?)**	**-**	**25**	**0**
		n1	Rectal			**25**	
P5	1C	n2	Tracheostome secretion	**Hospital (?)**	**-**	**6**	**3**
		n2	Rectal			**9**	
P6	1C	17	Blood	**Hospital (?)**	**-**	**12**	**3**
		17	Rectal			**15**	
P7	1C	389	Wound	**Community**	**-**	**6**	**-6**
		389	Rectal			**0****	
P8	2P	n3	Tracheal discharge	**Community**	**-**	**1**	**3**
		n3	Rectal			**4**	
P9	1C	253	Tracheal discharge	**Hospital**	**-**	**17**	**4**
		253	Rectal			**21**	
P10	1C	n4	Urine	**Hospital (?)**	**-**	**18**	**3**
		n4	Rectal			**21**	
P11	2P	253	Blood	**Hospital**	**20**	**13**	**1**
		253	Rectal			**14**	
P12	1C	309	Wound	**Hospital (?)**	**-**	**20**	
		253	Wound	**Hospital**	**-**	**53**	**17**
		253	Urine	**Hospital**	**-**	**70***	**0**
		253	Rectal			**70**	
P13	1C	n5	Acetabulum	**Hospital (?)**	**-**	**64**	**0**
		n5	Femur	**Hospital (?)**	**-**	**64**	
		n5	Wound	**Hospital (?)**	**-**	**64**	
		n5	Rectal			**64**	
P14	1C	3142	Urine	**Community**	**-**	**0**	**1**
		3142	Wound	**Community**	**-**	**1**	
		3142	Rectal			**1**	
P15	1C	2433	Wound	**Community**	**-**	**0**	**0**
		2433	Rectal			**0****	

"n" represents a new ST, not listed in PubMLST. Single asterisks represent recurrence isolates; double asterisks represent rectal samples obtained on admission to the hospital. Dash indicates no previous hospitalization. Question marks indicate the most likely environment.

infections may originate from *P. aeruginosa* colonizing the patient's intestine before the infection episode [6].

Enhanced genotyping methods are necessary to reliably identify the sources of *P. aeruginosa*. In this study, we employed phylogenetic analysis of whole genome sequences (WGS), core genome Single Nucleotide Polymorphisms (SNPs), and Average Nucleotide Identity (ANI) to investigate the relationship between intestinal and clinical *P. aeruginosa* isolates from 15 hospitalized patients in Quito, Ecuador.

Materials and methods

For one year, we recruited adult patients suffering from *P. aeruginosa* infection at two general hospitals (Hospital General Docente de Calderón-Hospital_1C and Hospital Padre Carollo Un Canto Para la Vida-Hospital_2P) in Quito City, Ecuador. All participants provided informed consent approved by the Comité de Ética de Investigación en Seres Humanos de la Universidad San Francisco de Quito USFQ (CEISH-USFQ). We recruited the patients after a *P. aeruginosa* infection was confirmed by the hospital's clinical laboratory, except for 2 patients for whom faecal samples were obtained (and submitted to culture) at the time of admission as part of the clinical procedures. Clinical samples were identified from the hospital's clinical laboratory, and rectal swab samples were collected from each patient using Stuart transport media (BD). Samples were streaked on Cetrimide agar plates (Difco, BD) to obtain up to 5 green-blue colonies, and after

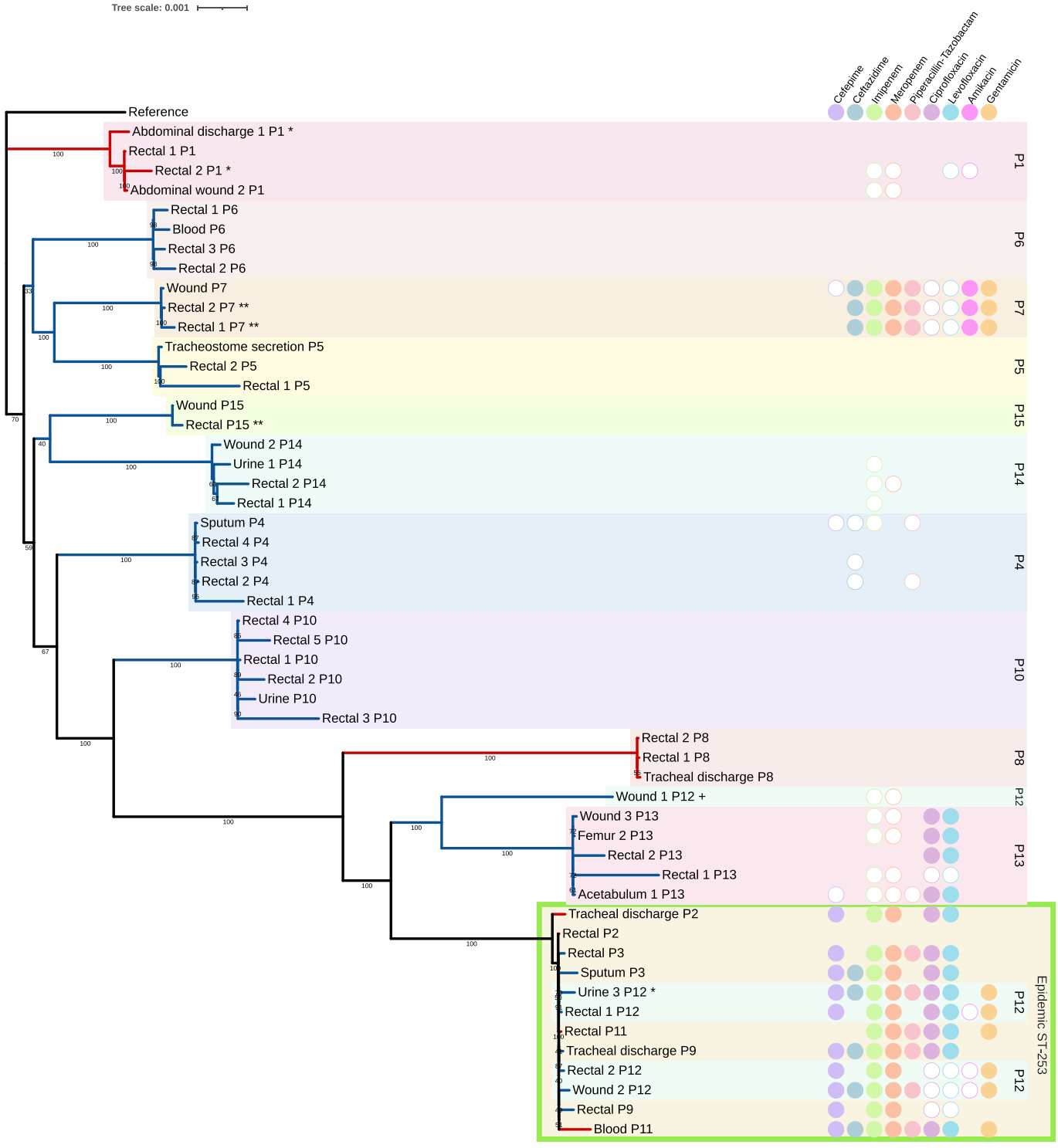

Figure 1. Phylogenetic tree using IQTREE software (maximum likelihood) of 5.29 Mbps length alignment of 53 *P. aeruginosa* isolates (including the reference sequence). Blue branches represent isolates from Hospital_1C. Red branches represent isolates from Hospital_2P. Shaded boxes represent each patient's isolates. The yellow shade shows the ST-253 epidemic clade. The numbers in nodes indicate bootstrap values using 1000 pseudo-replicates; the bootstrap number is shown as a percentage (%). Resistance phenotypes are shown on the right. Resistant strains with at least one genetic marker statistically significantly related to its phenotype are drawn in filled circles, and the ones with the resistant phenotype but not the genetic feature are in empty circles. Susceptible isolates are not illustrated. A single asterisk denotes the recurrence isolates. Double asterisks denote rectal isolates collected on the admission date.

biochemical testing, colonies were confirmed as *P. aeruginosa* isolates.

We extracted DNA from samples using the Wizard® Genomic DNA Purification Kit. For some isolates, DNA quality did not meet the sequencing requirements, and we used the Qiagen DNeasy Blood & Tissue Kit columns (both DNA extraction kits included RNAse treatment). To reduce sequencing costs, we used nucleotide sequences of two genes to detect possible clonal strains; we PCR amplified, and Sanger sequenced (Macrogene, South Korea) the *acsA* and *aroE* genes according to PubMLST.org *P. aeruginosa* protocols of all the isolates. *Isolates with identical acsA* and *aroE* sequences from the same patient were selected for whole-genome sequencing.

To obtain draft bacterial genomes, we used Oxford Nanopore long-read sequencing. The library preparation was performed using the Rapid Barcoding Kit 96 SQK-RBK110.96, following the manufacturer's protocol. Whole Genome Sequencing was conducted using R9.4.1 flow cells (FLO-MIN106) on a GridION Mk1 platform from Oxford Nanopore Technologies.

We obtained the WGS assemblies of all the isolates and used them for the phylogenetic analysis. We built a WGS tree, a core SNPs tree, an SNPs matrix, and a whole genome ANI matrix. We also obtained the sequence types (STs) from the *P. aeruginosa* website on PubMLST.org. Bioinformatic analysis was conducted using the programs and workflows summarized in Table S1. We used the AE004091.2 *P. aeruginosa* PAO1 complete genome reference GenBank accession GCA_000006765.1 (size 6,26 Mbps) as the reference genome. For quality purposes, one isolate (Rectal_P2) underwent duplicate sequencing.

We obtained the phenotypic resistances of the strains using the Kirby-Bauer method, according to Clinical and Laboratory Standards Institute M-100 2022 standards. We tested the β-lactams cefepime, ceftazidime, imipenem, meropenem, and piperacillin-tazobactam, the fluoroquinolones ciprofloxacin and levofloxacin, and the aminoglycosides amikacin and gentamicin. We used the genomic assemblies of the isolates to perform an analysis of the presence of antibiotic-resistance genes or markers according to three genotype resistance databases (Table S1).

Statistical analysis was performed in JASP software version 0.16.4.0.

Results

We enrolled 40 patients during the study between November 2021 and October 2022. We obtained rectal samples from 22 patients and found the presence of *P. aeruginosa* in 16 of them.

We obtained genomic information from isolates (N=52) from 15 patients, of which 21 were clinical and 31 were rectal isolates (Table I, Table S2 metadata).

The alignment information for the phylogenetic tree construction covered 5.29 million base pairs (Mbps), which represents 77.9% of the P. aeruginosa reference strain genome size (range from 5.5 to 7 Mbps). The phylogenetic analysis of draft genomes showed clustering of isolates (clinical and rectal) from each patient (Figure 1). However, 12 isolates from 5 patients (P3, P9, and P12 from Hospital_1C and P2, and P11 from Hospital_2P) showed genetic similarity (WGS phylogeny, core SNPs, and ANI, Figure 1, Figure S1, and S2) and belonged to the clone ST-253. These patients were not hospitalized during the same period, however, their persistence and abundance among the strains may be compatible with transmission within the hospital (Figure S3).

The isolates within the same clade (clinical and faecal isolates from the same patient) had an SNPs average difference of 0.053% (ranging from 0.0077% to 0.1%). The average difference across all genomes was 0.71%, thirteen times higher than the clade SNPs differences. Additionally, the 6.02 Mbps alignment of the two sequences from the same isolate (quality control) showed 0.0058% SNPs of difference, comparable to those observed in isolates (faecal and clinical) from the same patient (clade) (Figure S4).

Due to bioethics considerations, we obtained the rectal samples, for most cases, after the P. aeruginosa infection. However, in patients P7 and P15, we obtained the rectal samples on the first day (due to high-risk assessment hospital policies at admission). In P7, the clinical isolate was obtained six days after hospital admission (Figure 1, double asterisks**). There were *P. aeruginosa* infection recurrences in two cases: patients P1 and P12; in both cases, the isolates from the recurrent infection were genetically related to the other isolated from the same patient (Figure 1, asterisks*).

The clinical and rectal isolates from the same patient had comparable phenotypic resistances within them in most cases (Figure 1, circles). We utilized three genotypic resistance analysis databases; each detected or lost some genotypic resistances (Table S3). Our findings showed that $bla_{\text{PDC-34}}$ and $bla_{\text{OXA-488}}$ were associated with resistance to cefepime, imipenem, meropenem, piperacillin-tazobactam, and ST-253 strains (Fisher's exact tests $P<0.05$). Additionally, $bla_{\text{VIM-2}}$ was associated with ceftazidime, imipenem, meropenem, piperacillin-tazobactam resistances, and ST-389 strains (Fisher's exact tests $P<0.05$). We found a significant association of *gyrA*_T83I mutation with fluoroquinolone's ciprofloxacin and levofloxacin resistances. Lastly, for aminoglycosides amikacin and gentamicin resistances, we found an association with genes *aac*(6′)-II, *ant*(2″)-Ia, and *aph*(3′)-VIa, and for gentamicin only, the *aadA6* gene. Other genes were statistically associated with phenotypic resistance to different antibiotic classes, including the *sul1*, *qacE*Δ1, ArmM-MexR, and OprM efflux pump encoding genes (Table S4).

Discussion

We present genetic evidence through a comprehensive approach involving WGS (>5 Mbps), core SNPs, and ANI that intestinal and clinical isolates from the same patient have a clonal relationship. Moreover, in two patients, P. aeruginosa-positive rectal samples were obtained on the same day of hospital admission, which rules out the possibility of nosocomial transmission. These results suggest that the patient's gastrointestinal tract was the source of many *P. aeruginosa* infections. Previous studies have found that intestinal carriage of *P. aeruginosa* increases the risk of intensive care infection by this bacterium [6,7], and in most cases, the intestinal and the clinical strains share the same PFGE genotype [6].

However, we also found evidence that ST-253 strains may be transmitted within the hospital, even when these cases were not temporally related. We hypothesize that a hospital facility or intestinal colonization (in patients or personnel) may be the source of these strains.

In most cases, the phenotypic resistance was the same between intestinal and clinical isolates (Figure 1). Also, genomic data revealed that antimicrobial resistance genes

were not always associated with phenotypic resistance to the respective antimicrobial and vice versa. These discrepancies may be related to mutations that affect the resistance genes or other epistatic events that cause resistance [8].

Some studies of environmental, intestinal, and clinical *P. aeruginosa* isolates in hospitals have used single-gene sequencing, Multilocus Sequence Typing (MLST) with seven genes, and Pulsed Field Gel Electrophoresis (PFGE), which analysed around 30 restriction sites. These techniques produce reliable results only when used to find clonal relationships in *P. aeruginosa* isolates from outbreaks within the same hospital but show spurious associations when used to study clones from different timelines or between hospitals [9].

Even though we present an analysis of draft genomes (as opposed to closed genomes), the accuracy of our sequences was assessed by sequencing twice the same strain (Rectal_P2), and the results were within the parameters described previously [10]. Another limitation of our study was the low number of isolates. Nevertheless, our results provide evidence of the endogenous nature of these P. aeruginosa infections. Our study underlines the need for investigations with a larger number of patients and using WGS to determine the prevalence of *endogenous* P. aeruginosa transmission. High frequency of endogenous infections may warrant profound changes in P. aeruginosa prevention protocols, such as faecal-carriage screening for patients upon hospital admission and strain-specific decolonization, which could include bacteriophage therapy for individuals identified as high-risk cases. The origin of *P. aeruginosa* acquired outside the hospitals remains unknown. Human-to-human transmission, particularly of high-risk clones, and environmental sources need to be addressed in future studies. Finally, this study shows the utility of Oxford Nanopore (a low-cost sequencing technology) to study phylogenetic relations between bacterial isolates in countries with limited resources.

Acknowledgements

We thank hospitals General Docente de Calderón and Padre Carollo Un Canto Para la Vida for their invaluable assistance in providing clinical isolates and granting patient access. The authors also thank Oriana Culbert for her valuable suggestions.

Conflict of interest statement

All authors have no conflicts of interest to declare.

Funding statement

This study was funded by the "Fondos para Proyectos de Investigación Científica para Doctorantes Convocatoria 2016" from the Universidad Central del Ecuador and by the Instituto de Microbiología from Universidad San Francisco de Quito USFQ, Quito, Ecuador.

Credit author statement

Gabriela Vasco: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Writing-Reviewing, and Editing. Mishell Achig, Belén Prado-Vivar, Maritza Páez, Franklin Espinosa and, Evelyn Espinoza: Investigation, Resources. Danny Quinancela: Investigation, Data Curation. Paul Cardenas: Validation, Supervision. Gabriel Trueba: Conceptualization, Supervision, Visualization, Writing-Reviewing, and Editing.

Ethics approval and consent to participate

The study was approved by the Comité de Ética de Investigación en Seres Humanos de la Universidad San Francisco de Quito USFQ (CEISH-USFQ) reference 2021-090M.

Data availability

Whole genome sequences from this study are available in the NCBI repository under BioProject PRJNA946810.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.infpip.2023.100331.

References

[1] World Health Organization. Prioritization of pathogens to guide discovery, research and development of new antibiotics for drug-resistant bacterial infections, including tuberculosis. 2017. Geneva.

[2] Rice LB. Federal Funding for the Study of Antimicrobial Resistance in Nosocomial Pathogens: No ESKAPE. J Infect Dis 2008;197:1079—81. https://doi.org/10.1086/533452.

[3] Juan C, Peña C, Oliver A. Host and Pathogen Biomarkers for Severe Pseudomonas aeruginosa Infections. J Infect Dis 2017;215:S44—51. https://doi.org/10.1093/infdis/jiw299.

[4] Berthelot P, Grattard F, Mahul P, Pain P, Jospé R, Venet C, et al. Prospective study of nosocomial colonization and infection due to Pseudomonas aeruginosa in mechanically ventilated patients. Intensive Care Med 2001;27:503—12. https://doi.org/10.1007/s001340100870.

[5] Bonten MJM, Bergmans DCJJ, Speijer H, Stobberingh EE. Characteristics of Polyclonal Endemicity of *Pseudomonas aeruginosa* Colonization in Intensive Care Units. Am J Respir Crit Care Med 1999;160:1212—9. https://doi.org/10.1164/ajrccm.160.4.9809031.

[6] Gómez-Zorrilla S, Camoez M, Tubau F, Cañizares R, Periche E, Angeles Dominguez M, et al. Prospective observational study of prior rectal colonization status as a predictor for subsequent development of Pseudomonas aeruginosa clinical infections. 2015. https://doi.org/10.1128/AAC.04636-14.

[7] Venier A-G, Leroyer C, Slekovec C, Talon D, Bertrand X, Parer S, et al. Risk factors for Pseudomonas aeruginosa acquisition in intensive care units: a prospective multicentre study. J Hosp Infect 2014;88:103—8. https://doi.org/10.1016/j.jhin.2014.06.018.

[8] López-Causapé C, Oliver A. Insights into the evolution of the mutational resistome of Pseudomonas aeruginosa in cystic fibrosis. Future Microbiol 2017;12:1445—8. https://doi.org/10.2217/fmb-2017-0197.

[9] Quainoo S, Coolen JPM, van Hijum SAFT, Huynen MA, Melchers WJG, van Schaik W, et al. Whole-Genome Sequencing of Bacterial Pathogens: the Future of Nosocomial Outbreak Analysis. Clin Microbiol Rev 2017;30:1015—63. https://doi.org/10.1128/CMR.00016-17.

[10] Klockgether J, Cramer N, Wiehlmann L, Davenport CF, Tümmler B. Pseudomonas aeruginosa Genomic Structure and Diversity. Front Microbiol 2011;2. https://doi.org/10.3389/fmicb.2011.00150.