Pseudomonas aeruginosa population genomics among adults with bronchiectasis across Germany

Received: 13 March 2023
Accepted: 20 March 2023

To the Editor:

Pseudomonas aeruginosa is a metabolically versatile, aquatic γ-proteobacterium that colonises animate surfaces of plants, animals and humans [1]. During recent decades, this cosmopolitan Gram-negative bacterium has become one of the most frequent causative agents of nosocomial infections, associated with substantial morbidity and mortality [1]. Chronic airway infections with *P. aeruginosa* are a major cause of morbidity in people with cystic fibrosis (CF), COPD or bronchiectasis.

P. aeruginosa is one of the most frequently isolated pathogens in patients with non-CF bronchiectasis [2]. Clinical studies have revealed that chronic airway infection with *P. aeruginosa* is associated with more lung lobe involvement and a higher frequency of exacerbations [3, 4]. *P. aeruginosa*-positive patients had a longer disease course, faster decline of lung function and more severe Bronchiectasis Severity Index stage than those without *P. aeruginosa* [5, 6, 7]. Patient-to-patient transmission of *P. aeruginosa* is known to happen frequently in people with CF [8] but molecular epidemiology studies did not substantiate any nosocomial spread among patients with bronchiectasis [9, 10].

Transmission risk is low within families and within bronchiectasis clinics [8]. Thus, most people with bronchiectasis should have acquired *P. aeruginosa* from other sources, most likely from the inanimate environment. *P. aeruginosa* is ubiquitously present in aquatic habitats [1]. All organisms, including humans, are exposed to this bacterium, albeit usually far below the threshold of an infectious dose and culture-based microbiology diagnostics [1]. Conversely, *P. aeruginosa* is isolated from 20% [6] to 33% [11] of respiratory secretions of people attending a bronchiectasis clinic. Considering the high prevalence of *P. aeruginosa* in people with bronchiectasis, it is clinically relevant whether *P. aeruginosa* is generally prone to take residence in a bronchiectasis lung or whether a subset of peculiarly adapted clonal lineages preferentially conquers this habitat.

To address this issue, the molecular epidemiology of *P. aeruginosa* isolates was examined from patients with non-CF bronchiectasis. 296 isolates were collected from March 2018 to November 2020 at the Adult Bronchiectasis Clinic of Hannover Medical School from all patients who had signed the broad consent of the German Center of Lung Research and whose respiratory specimen (sputum as well as nasal and bronchoalveolar lavage) had been *P. aeruginosa* positive by culture-dependent diagnostics. The cohort consisted of, in total, 110 patients from all over Germany (figure 1a). Overall, 66% of the 110 patients were female (n=72). Mean±SD age was 55±17 years (median 58 years, range 21–83 years), while forced expiratory volume in 1 s was 63±25% of predicted (median 65%, range 17–131% of predicted). The most common aetiologies (in >5% of subjects) were primary ciliary dyskinesia and/or Kartagener syndrome in 32% (n=35), followed by "idiopathic" in 28% (n=31), asthma/allergic bronchopulmonary aspergillosis in 16% (n=17), "other" in 14% (n=15) and nontuberculous mycobacterial pulmonary as well as autoimmune disease in 6% of patients (n=6) each. The 296 *P. aeruginosa* isolates were analysed by shotgun whole-genome sequencing. The multilocus sequence type (ST) [12] was determined using a mapping-based approach leveraging Illumina short reads against the *P. aeruginosa* PA14 reference genome. If serial isolates had been collected from one patient, one strain per clone was selected for further analysis. As shown in the phylogram (figure 1b), this set of 130 independent isolates demonstrated a genomically highly diverse *P. aeruginosa* population at our Adult Bronchiectasis Clinic. In total, 73 STs

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Genome sequencing of 130 *Pseudomonas aeruginosa* isolates from 110 bronchiectasis patients identified a few dominant clones common in the global bacterial population and numerous rare clones infrequently seen in the environment or other human infections https://bit.ly/3lIfD2X

Cite this article as: Rosenboom I, Oguz S, Lüdemann IM, *et al*. *Pseudomonas aeruginosa* population genomics among adults with bronchiectasis across Germany. *ERJ Open Res* 2023; 9: 00156-2023 [DOI: 10.1183/23120541.00156-2023].

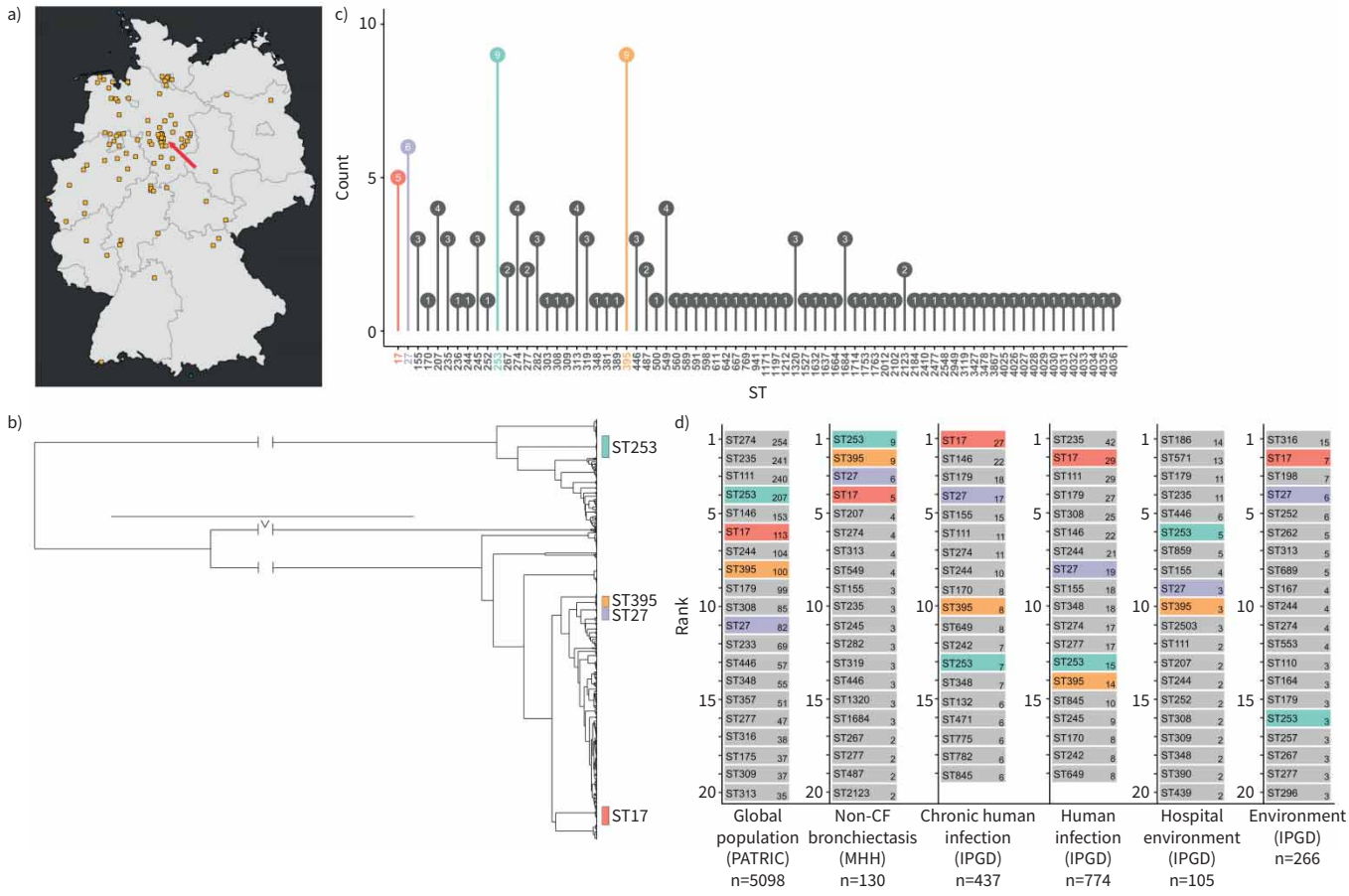

FIGURE 1 Epidemiology of the *Pseudomonas aeruginosa* population in non-cystic fibrosis (CF) bronchiectasis across Germany compared with that of two international *Pseudomonas* genome databases. a) Distribution of patients' place of residence (yellow dot) across Germany, in relation to the Adult Bronchiectasis Clinic at Hannover Medical School (MHH) (red arrow; mean distance 120 km). b) Phylogram of the sequence types (STs) of the 5098 *P. aeruginosa* genomes in the Pathosystems Resource Integration Center (PATRIC) database. Numerical ST and colour indicate the four most frequent STs identified in the bronchiectasis cohort. c) Clonal distribution of in total 130 *P. aeruginosa* isolates from people with non-CF bronchiectasis across Germany. Absolute counts of different STs verified by multilocus sequence typing (MLST) are shown. MLST is based on sequence variants in eight highly conserved housekeeping genes [12]. d) Ranks of the 20 most common STs in people with non-CF bronchiectasis in Germany at MHH compared with the top 20 ranks of STs of *P. aeruginosa* genomes and their absolute numbers (right side in each ST box) deposited in the PATRIC [13, 14], the International *Pseudomonas* Genome Database (IPGD) [15] and the top 20 ranks of database subgroups therein differentiated by habitat of origin. The chosen categories were (from left to right) chronic human infections, human infections, the hospital environment and the inanimate environment untouched by medicinal activity. Entries from the IPGD [15] were curated for independent isolates if metadata were available. Metadata were unavailable for 2333 out of 4264 genomes deposited with a MLST. The four most common *P. aeruginosa* STs in people with bronchiectasis are depicted by the same colours in parts b–d.

were identified in the 130 genomes, 53 STs of which were only observed in a single strain (figure 1c). 12 STs were novel and had not been reported before. New STs were assigned by the Pub-MLST database [12]. Of shared STs, we detected four pairs, eight trios and four quartets. Four STs were represented by five or more strains.

Figure 1d compares the relative abundance of *P. aeruginosa* STs among our bronchiectasis population with that of isolates from chronic and acute human infections, the hospital environment, soil and aquatic habitats in the inanimate environment, and the global population documented in the Pathosystems Resource Integration Center [13, 14] and the International *Pseudomonas* Genome Database [15]. The four most frequent STs found in bronchiectasis airways belonged to the 20 most common clones in the worldwide *P. aeruginosa* population that are widespread in human infections and in the non-human inanimate environment [12, 15]. Of these four most common clonal lineages in the bronchiectasis isolates, ST27 is characterised by extreme genomic homogeneity, *i.e.* >90% of strains in the phylogenetic subgroup belong

to ST27 [13, 14]. ST17 and ST253 represent the global clones C and PA14 that had been detected in a wide range of habitats all over the world. ST395 represented the most frequently detected clone among our bronchiectasis population. This clonal lineage is less frequent in human infections and rare in the inanimate environment. Thus, ST395 was the only clone in our population that potentially may be overrepresented in bronchiectasis compared to other habitats. However, this assumption needs to be validated in independent bronchiectasis cohorts [16].

P. aeruginosa is one of the bacteria belonging to the ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp.) pathogens that are a threat all over the world due to their capacity to become increasingly resistant to all available antibiotics [1]. Driven by this threat, multiple isolates of the multidrug-resistant high-risk clones ST111, ST146, ST235 and ST274 have been sequenced [12, 13, 15]. Thus, their high frequency in the genome databases may be attributable to clinical needs and focus on hospitals, particularly intensive care units. However, in the context of our Adult Bronchiectasis Clinic, it is reassuring that high-risk clones were only identified at comparably very low frequency.

In summary, our findings indicate that the population biology of *P. aeruginosa* in bronchiectasis is characterised by a broad range of rare clones and few dominant clonal lineages, which show worldwide distribution in disease and environment. Neither within-centre nosocomial transmission, epidemic virulent clones nor outgrowth lineages that are specific for other diseases were observed. These findings among people with bronchiectasis are in contrast to the long-standing experience in CF where within-family, within-centre, domestic and international spread of epidemic clones has been frequently reported [8], and support the originality of bronchiectasis as a distinct disease entity [4].

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Provenance: Submitted article, peer reviewed.

Acknowledgments: The authors are grateful to Jan Fuge (Department of Respiratory Medicine, Hannover Medical School, and Biomedical Research in Endstage and Obstructive Lung Disease BREATH, German Center for Lung Research, Hannover, Germany) for data extraction from our clinical database. The authors would also like to acknowledge and thank the sequencing core unit at Hannover Medical School (RCUG) for generating the sequencing data.

Support statement: This study was supported by a grant of the German Centre for Infection Research (DZIF, project: TTU09.911). Funding information for this article has been deposited with the Crossref Funder Registry.

Conflict of interest: I. Rosenboom, S. Oguz, I. M. Lüdemann, J. Rademacher, L. Sedlacek and N. Cramer have nothing to disclose. F.C. Ringshausen declares grants to their institution from the German Center for Lung Research (DZL), the German Center for Infection Research (DZIF), IMI (EU/EFPIA), iABC Consortium (including Alaxia, Basilea, Novartis and Polyphor), Mukoviszidose Institute, Novartis, Insmed Germany, Grifols, Bayer and InfectoPharm; consulting fees from Parion, Grifols, Zambon, Insmed and the Helmholtz-Zentrum für Infektionsforschung; payments or honoraria from I!DE Werbeagentur GmbH, Interkongress GmbH, AstraZeneca, Insmed, Grifols and Universitätsklinikum Frankfurt am Main; payment to their institution for expert testimony at the Social Court Cologne; support for attending meetings from German Kartagener Syndrome and PCD PAG and Mukoviszidose e.V; participation on a data safety monitoring or advisory board for Insmed, Grifols and Shionogi; fees for clinical trial participation paid to their institution by AstraZeneca, Boehringer Ingelheim, Celtaxsys, Corbus, Insmed, Novartis, Parion, University of Dundee, Vertex and Zambon; and honorary roles as Coordinator of the

ERN-LUNG Bronchiectasis Core Network, Chair of the German Bronchiectasis Registry PROGNOSIS, Member of the SteerCo of the European Bronchiectasis Registry EMBARC, Member of the SteerCo of the European NTM Registry EMBARC-NTM, Co-Speaker of the Medical Advisory Board of the German Kartagener Syndrome and PCD PAG, Speaker of the Respiratory Infections and TB group of the German Respiratory Society (DGP), Speaker of the Cystic Fibrosis group of German Respiratory Society (DGP), PI of the DZL, member of the protocol review committee of the PCD-CTN and Member of Physician Association of the German Cystic Fibrosis PAG. B. Tümmler reports grants from the German Centre for Infection Research (DZIF), the German Center for Lung Research (DZL), the German Research Foundation (DFG); and consulting fees from Helmholtz Institute for Infection Research (HZI); payments or honoraria from Vertex Pharmaceuticals (Germany) Inc.

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