MOLECULAR CHARACTERIZATION OF *Pseudomonas aeruginosa* STRAINS ISOLATED FROM PHARMACEUTICAL INDUSTRY FACILITY BY ERIC-PCR AND MLST

VASCONCELLOS, L. [1,2]; SILVA, S.V. [1]; COSTA, L.V. [1]; VILLAS BÔAS, M.H.S. [2]; BRANDÃO, M.L.L.[1]*

[1]Laboratório de Controle Microbiológico, DEQUA; Bio-Manguinhos/Fiocruz; [2]Laboratório de Microbiologia de Alimentos e Saneantes, INCQS/Fiocruz
*Corresponding Author: marcelo.brandao@bio.fiocruz.br

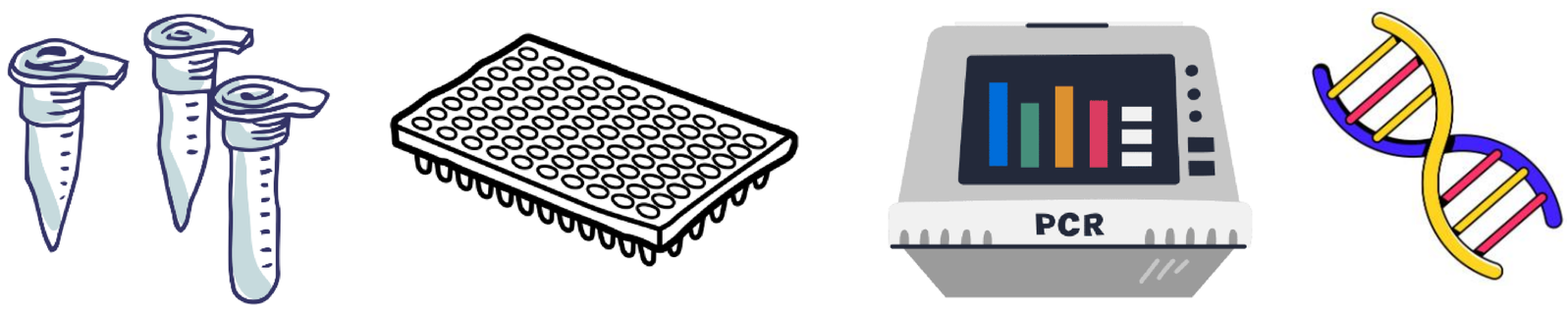
INTRODUCTION

Pseudomonas aeruginosa is a saprophytic microorganism that causes human infections, in particular respiratory tract infections. It's a pathogen that can survive and form biofilms on inert materials surfaces (Zhang et al., 2017). Multidrug resistance of this pathogen was reported, and the World Health Organization has classified it as a critical priority due to its resistance to carbapenems (WHO, 2017). In the pharmaceutical industry, the isolation of *P. aeruginosa* can mean contamination during the stages of the production chain (Anvisa, 2017). In this scenario, molecular characterization techniques, such as the Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR) (Versalovic et al.,1991) and the Multilocus Sequence Typing (MLST), allow an assessment of the genetic diversity and clonal profile of these strains (Maiden et al., 1998, 2013), helping the evaluation of deviation and risk analysis, making decisions regarding immunobiological products.

METHODOLOGY

P. aeruginosa strains (n=18) isolated from a pharmaceutical industry facility from 2015 to 2020.

- Sterility tests of active pharmaceutical ingredients (API, n=13)
- Purified water (PUW, n=3)
- Potable water (POW, n=2).



Enterobacterial Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR) and the Multilocus Sequence Typing (MLST)

Housekeeping genes

BioNumerics

MLST profiles were clustered with the BioNumerics 8.1 software using a categorical coefficient and graphing was assessed using the minimum spanning tree tool and analyzed using the eBURST algorithm for identification of clonal complex (CC) being a single-locus variant (SLV) or double-locus variant (DLV).

RESULTS AND CONCLUSIONS

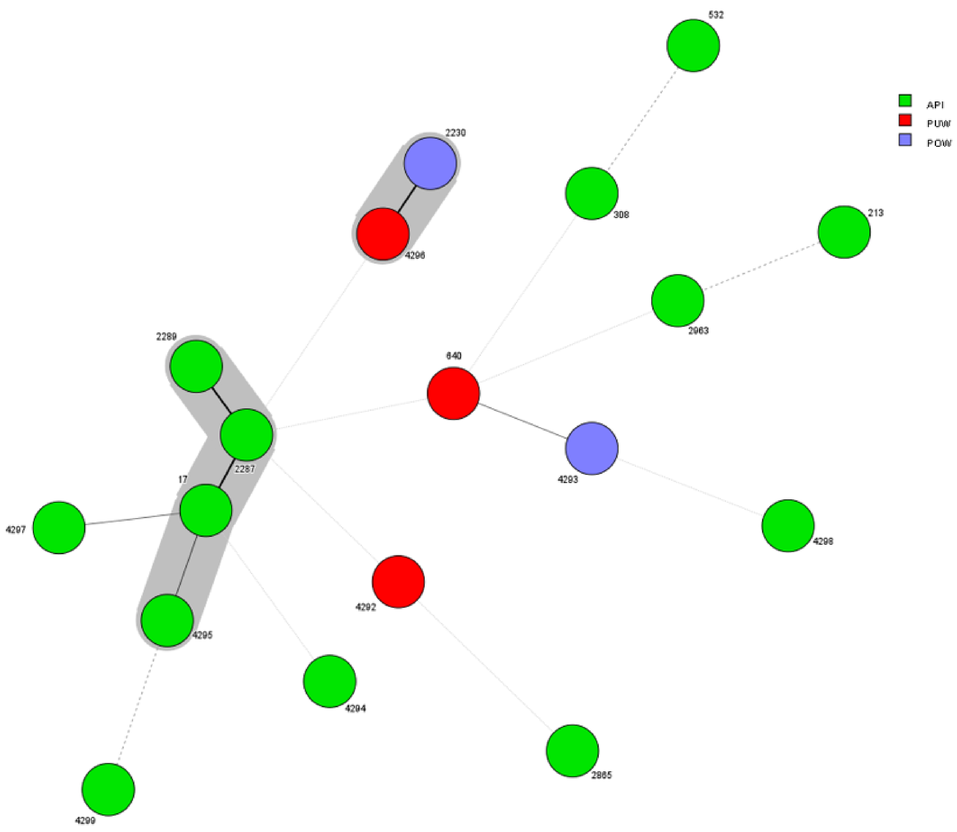


Figure 1: Minimum spanning tree of 18 *Pseudomonas aeruginosa* strains grouped by STs according to the source of isolation.

The 18 strains formed 17 profiles after ERIC-PCR and presented 18 different STs. Ten (50.0%) STs were identified in the database: ST 17, 213, 308, 532, 640, 2230, 2287, 2289, 2865, and 2963. Eight (40.0%) different new STs were identified as ST4292-4299. MLST SI was 1.00 and ERIC-PCR SI was 0.99 among the strains. ST 640 has already been isolated from blood and water samples in the Czech Republic and France and is a SLV from ST 1913. ST 17 possessed 90 strains deposited, isolated from different sources and water, in many countries, forming a CC with 6,437 isolates, divided into 2,398 different STs. STs 17, 308 and 532 belong to the same CC, with isolates of clinical and environmental origin. In this study, these strains were isolated from API samples. ST 4296 (isolated from PUW) is a SLV of ST 2230 (isolated from POW) that was identified from clinical samples (otitis) in the USA. Two strains showed the same cluster in ERIC PCR, but different STs (2289 and 4294) originated from API. No strain originating from a pharmaceutical industry facility had been deposited in the MLST database until the present study. This study can contribute to the scientific literature in monitoring bacterial clones and help other pharmaceutical industries to eradicate strains of *P. aeruginosa* in quality control assays for immunobiological.

BIBLIOGRAFY

AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA (ANVISA). Gerência de Investigações e Prevenção das Infecções e dos Eventos Adversos (Gipea) / Gerência Geral de Tecnologia em Serviços de Saúde (GGTES). **Investigação e Controle de Bactérias Multirresistentes**. 21 p., 2017.

MAIDEN, M. C. et al. Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. **Proc Natl Acad Sci** *USA*; v. 95, n. 6, p. 3140-3145, 1998

MAIDEN, M. C. et al. MLST revisited: the gene-by-gene approach to bacterial genomics. **Nat Rev Microbiol**; v. 11, n. 10, p. 728-736, 2013.

VERSALOVIC, J. et al. Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. ***Nucleic acids research*** v. 19, n. 24, p. 6823-6831, 1991.

ZHANG, Y. et al. Pitfalls associated with evaluating enzymatic quorum quenching activity: the case of MomL and its effect on *Pseudomonas aeruginosa* and *Acinetobacter baumannii* biofilms**. PeerJ**; v. 5, p. 3251, 2017..

WORLD HEALTH ORGANIZATION (WHO). Antibacterial agents in clinical development: an analysis of the antibacterial clinical development pipeline, including tuberculosis. Geneva: World Health Organization; Switzerland.2017. **WHO/EMP/IAU/2017.12**. Licence: CC BY-NC-SA 3.0 IGO.

ACKNOWLEDGMENT

Instituto de Tecnologia em Imunobiológicos Bio-Manguinhos
INCQS
Instituto Nacional de Controle de Qualidade em Saúde
CAPES
FAPERJ Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro
INSTITUTO FEDERAL DE EDUCAÇÃO, CIÊNCIA E TECNOLOGIA RIO DE JANEIRO