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# 82-1 MOLECULAR CHARACTERIZATION OF Pseudomonas aeruginosa STRAINS ISOLATED FROM PHARMACEUTICAL INDUSTRY FACILITY BY ERIC-PCR AND MLST

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**Resumo:**

*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. Multi-drug resistance of this pathogen was reported, and the World Health Organization has classified it as a critical priority due to its resistance to carbapenems. In the pharmaceutical industry, the isolation of *P. aeruginosa* can mean contamination during the stages of the production chain. In this scenario, molecular characterization techniques, such as the Enterobacterial Repetitive Intergenic Consensus-Polymerase Chain Reaction (ERIC-PCR) and the Multi-Locus Sequence Typing (MLST), allow an assessment of the genetic diversity and clonal profile of these strains, helping the evaluation of deviation and risk analysis, making decisions regarding immunobiological products. The aim of this study was to typify *P. aeruginosa* strains (n=18) isolated from a pharmaceutical industry facility from 2015 to 2020 through ERIC-PCR and MLST. The Simpson's index (SI) was applied to calculate the resolution power of both techniques for typing strains. Strains were isolated from sterility tests of active pharmaceutical ingredients (API, n=13), purified water (PUW, n=3), and potable water (POW, n=2). ERIC-PCR was performed using the ERIC-2 primer, and after the amplification reaction, the band patterns were analyzed using the BioNumerics 8.1 software, and the dendrogram was constructed with the Dice index and the Unweighted Pair Group Method with Arithmetic Average. For the determination of sequence types (STs), the seven housekeeping genes were amplified according to PubMLST (https://pubmlst.org/paeruginosa). PCR products were purified and sequenced by the Sanger method. 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). 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.

**Palavras-chave:**

Pseudomonas aeruginosa, MLST, ERIC-PCR, molecular typing

**Agência de fomento:**

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