

**DESAFIOS DE IDENTIFICAÇÃO E CONTROLE DE *Ralstonia pickettii* NA  
INDÚSTRIA FARMACÊUTICA: UMA REVISÃO INTEGRATIVA DA  
LITERATURA**

**CHALLENGES IN THE IDENTIFICATION AND CONTROL OF *Ralstonia pickettii*  
IN THE PHARMACEUTICAL INDUSTRY: AN INTEGRATIVE LITERATURE  
REVIEW**

**Isadora Rodrigues da Silva**

Fundação Oswaldo Cruz, FIOCRUZ, RJ

<https://orcid.org/0000-0002-8033-6035>

[isadora.silva@fiocruz.br](mailto:isadora.silva@fiocruz.br)

**Maria Helena Simões Villas Bôas\***

Fundação Oswaldo Cruz, FIOCRUZ, RJ

<https://orcid.org/0000-0003-1626-7650>

[maria.villas@fiocruz.br](mailto:maria.villas@fiocruz.br)

**Luciana Veloso da Costa**

Fundação Oswaldo Cruz, FIOCRUZ, RJ

<https://orcid.org/0000-0003-1873-4056>

[luciana.costa@bio.fiocruz.br](mailto:luciana.costa@bio.fiocruz.br)

**Marcelo Luiz Lima Brandão**

Fundação Oswaldo Cruz, FIOCRUZ, RJ

<https://orcid.org/0000-0003-1121-7312>

[marcelo.brandao@bio.fiocruz.br](mailto:marcelo.brandao@bio.fiocruz.br)

**\*Autor por correspondência**

ARTIGO CIENTÍFICO

Submetido em: 20.06.2026

Aprovado em: 17.07.2026

## RESUMO

Contaminações microbianas na indústria farmacêutica geram riscos à saúde e prejuízos econômicos. Nesse cenário, *Ralstonia pickettii* é um patógeno relevante em surtos por produtos contaminados. Sua detecção frequente em sistemas hídricos evidencia sua resiliência ambiental. Este estudo discute a relevância de *R. pickettii* em sistemas de água e produtos farmacêuticos, enfatizando a identificação e tipificação na prevenção de desvios de processo. A revisão integrativa no PubMed, SciELO e Google Scholar (2016–2026) incluiu artigos originais sobre surtos nosocomiais, controle de qualidade da água e métodos de identificação e tipagem microbiana; revisões, literatura cinzenta e duplicatas foram excluídas. Dez estudos selecionados revelaram a dispersão global de *R. pickettii*, predominando na Ásia (50%) e Europa (40%). Os principais reservatórios identificados foram soluções salinas (60%), medicamentos (20%), água purificada (10%) e meio de cultura/bulk/torneira (10%), evidenciando sua persistência na fabricação. Na identificação, a espectrometria de massas por dessorção/ionização a laser assistida por matriz - tempo de voo (MALDI-TOF MS) foi a ferramenta central (70%), seguida por sequenciamento do genoma completo (SGC; 40%). Clonalidade e rastreamento de fontes foram avaliados principalmente por eletroforese em gel de campo pulsado (PFGE; 50%) e SGC (40%). A presença de *R. pickettii* estabelece um elo crítico entre falhas no controle de sistemas hídricos e infecções. A caracterização molecular avançada é essencial para direcionar ações corretivas e preventivas e otimizar medidas de controle, como a rotação de desinfetantes e a validação de utilidades, assegurando o cumprimento das boas práticas de fabricação.

**Palavras-chave:** *Ralstonia pickettii*. Indústria farmacêutica. Surto. Controle de qualidade microbiológico.

## ABSTRACT

Microbial contamination in the pharmaceutical industry poses severe health risks and substantial economic losses. Within this scenario, *Ralstonia pickettii* emerges as a highly relevant pathogen, frequently driving outbreaks associated with contaminated products. Its frequent isolation from water utilities highlights its pronounced environmental resilience. This study aims to discuss the relevance of *R. pickettii* in the pharmaceutical industry, emphasizing the importance of identification and typing in preventing process deviations. An integrative literature review was conducted across PubMed, SciELO, and Google Scholar (2016–2026), including original articles on nosocomial outbreaks, water quality control, and microbial identification and typing methods were included, whereas reviews, gray literature, and duplicates were excluded. Ten selected studies revealed the global dispersion of *R. pickettii*, with the highest predominance reported in Asia (50%), followed by Europe (40%). The primary reservoirs identified were saline solutions (60%), medicines (20%), purified water (10%), and culture media/bulk/tap water (10%), underscoring its persistence across manufacturing processes. Regarding identification, matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) emerged as the main method for identification (70%), followed by whole genome sequencing (WGS; 40%). Clonality assessment and source tracking were primarily performed by pulsed-field gel electrophoresis (PFGE; 50%) and WGS (40%). The presence of *R. pickettii* establishes a critical link between water system control failures and clinical infections. Advanced molecular typing is essential to drive corrective and preventive actions and optimize manufacturing controls, such as disinfectant rotation and utility validation, thereby ensuring compliance with good manufacturing practices.

**Keywords:** *Ralstonia pickettii*. Pharmaceutical industry. Outbreak. Microbiological quality control.

## 1 INTRODUCTION

The pharmaceutical and biotechnology industries play a pivotal role in global public health through the production of medicines, vaccines, and immunobiologicals (de la Torre; Albericio, 2025; Perez-Aleman; Ferretti, 2023). Given the critical health risks associated with contaminated products, the rigorous characterization of microorganisms isolated from this industrial ecosystem is of paramount importance (Gaoh *et al.*, 2024; Nunes Ramos *et al.*, 2025). To mitigate these risks, strict adherence to Good Manufacturing Practices (GMP) guidelines is mandatory (EMA, 2022; FDA, 2004; Sharma; Gamta; Luthra, 2023).

Quality assurance in pharmaceutical manufacturing relies heavily on continuous environmental monitoring across classified areas, surfaces, and critical utilities like purified water and water for injection. This proactive approach aims to detect microorganisms early in the process, preventing contaminated products from reaching the end of the production chain (Brasil, 2022; EMA, 2022). When a contaminant is detected, it cannot be treated as an isolated incident; it must immediately trigger a formal deviation investigation that cross-references cleaning and sterilization data with long-term microbial trends (Desai; Njoku; Nimo-Sefah, 2022; FDA, 2004; Gupta; Desai, 2024).

Securing precise microbiological identification is the core element of this process; without it, tracing the contamination source during root-cause investigations or building effective corrective and preventive action (CAPA) plans is nearly impossible (EMA, 2022). This operational challenge is clear in water monitoring. While the Brazilian Pharmacopeia (Brasil, 2022) and the United States Pharmacopeia (USP, 2026) set a strict microbiological limit of  $\leq 100$  colony-forming unit (CFU)/mL for purified water, certain opportunistic pathogens easily bypass standard controls due to their survival in oligotrophic environments, resistance to disinfectants, and capacity for biofilm formation (Ryan; Pembroke; Adley, 2006). Tracking these microbial counts over time to trigger alerts before action limits are breached requires advanced typing methods, which are crucial for uncovering clonal persistence across the facility (Patel *et al.*, 2024). Plant sanitization strategies, including disinfectant rotation, simply lose their efficacy if the specific resistance profile of the resident contaminant remains unknown (Gupta; Desai, 2024). Ultimately, while non-sterile formulations tolerate a controlled, route-dependent microbial load, parenteral drugs leave no room for error and demand absolute sterility (Brasil, 2024; EMA, 2022), as control failures trigger severe operational consequences, batch rejections, and full-scale product recalls (Ghijs; Wynendaele; De Spiegeleer, 2024; Patel *et al.*, 2024).

Notably, data from the Food and Drug Administration (FDA) indicate that between 2012 and 2019, more than 50% of pharmaceutical recalls in the United States of America (USA) were attributed to microbiological deviations (Jimenez *et al.*, 2019). Furthermore, a direct link exists between industrial contamination and healthcare-associated safety. Waterborne or drug-mediated nosocomial outbreaks have been extensively documented, primarily affecting immunocompromised patients or those undergoing invasive therapies (Choudhury *et al.*, 2015; Krone *et al.*, 2024; Saunders *et al.*, 2024). Within the hospital setting, the selective pressure exerted by antimicrobial misuse favors the persistence of non-fermenting Gram-negative bacilli, which act as opportunistic pathogens (Ryan; Pembroke; Adley, 2006; Souza *et al.*, 2024, 2025a,b; Yuan *et al.*, 2024). The bacterium *Ralstonia pickettii* was identified as the second most prevalent agent in non-sterile product contamination within FDA reports (Jimenez *et al.*, 2019).

The taxonomic trajectory of *R. pickettii* is characterized by successive reclassifications. Initially described as *Pseudomonas pickettii* (Ralston; Palleroni; Doudoroff, 1973), it was temporarily transferred to the genus *Burkholderia* (Holmes *et al.*, 1993) until nucleic acid hybridization and lipid profiling validated its assignment to the newly established genus *Ralstonia*, within the *Burkholderiaceae* family (Göker *et al.*, 2025; Yabuuchi *et al.*, 1995). Phenotypically, *R. pickettii* is a small, aerobic, non-fermenting Gram-negative bacillus endowed with metabolic versatility, allowing it to thrive in oligotrophic environments such as soil and drinking water systems (Baker *et al.*, 2022; Ryan; Pembroke; Adley, 2006; Stelzmueller *et al.*, 2006; Yuan *et al.*, 2024). Under nutrient-depleted conditions, *R. pickettii* can reduce its cell size, a physiological adaptation that enables it to traverse the 0.22 µm sterilizing filter membranes standard in industrial manufacturing (Anderson *et al.*, 1985; Sundaram *et al.*, 1999). This capability, compounded by a wide thermal tolerance (15–42°C) and a robust capacity for biofilm formation on pipework and medical devices, severely hinders its eradication by conventional chemical sanitizers (Ryan; Pembroke; Adley, 2006; Schnetzinger *et al.*, 2023; Yuan *et al.*, 2024). Although historically regarded as a low-virulence microorganism, it has consolidated its status as an emerging opportunistic pathogen capable of triggering severe outbreaks of bacteremia, endocarditis, pneumonia, and meningitis (Chen *et al.*, 2017; Orme *et al.*, 2015; Viana-Cárdenas *et al.*, 2024).

Serious infectious episodes have been linked to medical supplies and devices contaminated with *R. pickettii*, including the USA (Baker *et al.*, 2022), Germany (Ross *et al.*, 2014), and India (Choudhury *et al.*, 2015). In Colombia, a nosocomial outbreak tied to contaminated intravenous medication resulted in an 81% mortality rate among patients with severe comorbidities (Viana-Cárdenas *et al.*, 2024). In Brazil, the bacterium has been correlated

with bacteremia outbreaks in neonates and oncological patients in Rio de Janeiro, traced back to contaminated water for injection and dialysis solutions (Jesus *et al.*, 2022; Moreira *et al.*, 2005; Pellegrino *et al.*, 2008). Clinical management is further exacerbated by its intrinsic resistance to carbapenems, rifampicin, and trimethoprim, as well as the expression of active efflux pumps (Basso *et al.*, 2019; Yuan *et al.*, 2024). This challenge is compounded by the absence of species-specific interpretive breakpoints in current Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines, forcing laboratories to adopt generic criteria established for other non-fermenters (CLSI, 2024; EUCAST, 2024; Sheng *et al.*, 2024).

Implementing rapid molecular identification and typing methodologies is a core component of the Contamination Control Strategy (CCS) currently demanded by regulatory agencies for sterile medicinal products manufacturing (EMA, 2022). Elucidating the genetic determinants of environmental persistence of microorganisms is important for development of effective cleaning and disinfection procedures for its elimination (Costa *et al.*, 2026; Sheng *et al.*, 2024; Vasconcellos *et al.*, 2023, 2026).

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) stands out for its speed and accuracy in identifying *R. pickettii* isolates (Fluit *et al.*, 2021; Krone *et al.*, 2024). At the genotypic level, whole genome sequencing (WGS) has revolutionized molecular epidemiology by providing unparalleled analytical resolution, essential for discriminating closely related clonal lineages (Li *et al.*, 2026; Menghwar *et al.*, 2022; Simar; Hanson; Arias, 2021). A traditional curated multilocus sequence typing (MLST) scheme for *R. pickettii* in public repositories, like PubMLST (<https://pubmlst.org/organisms>, access: 06-11-2026), is not available yet. However, core-genome multilocus sequence typing (cgMLST), which screens thousands of core *loci*, was established in the literature as the method of choice for the high-resolution genetic monitoring and epidemiological tracking (Fluit *et al.*, 2021; Krone *et al.*, 2024; Yuan *et al.*, 2024).

The environmental persistence of *R. pickettii* and its associated public health risks underscore the need to map its dissemination dynamics and identify current analytical bottlenecks. To do so, the aim of this study was to perform an integrative review to explore the difficulties in identifying and controlling this pathogen by addressing two central questions: (1) how does the scientific literature characterize the occurrence of this microorganism in contaminated pharmaceutical inputs and products, connecting industrial scenarios to hospital outbreaks? and (2) how can high-resolution diagnostic and typing tools help overcome current identification hurdles and strengthen control strategies to prevent process deviations?"

## 2 METHODOLOGY

An integrative literature review was conducted between March and June 2026 across the PubMed, SciELO Brazil, and Google Scholar databases. In PubMed and SciELO Brazil, the search was executed using the following combination of descriptors and Boolean operators: (*Ralstonia pickettii* OR *R. pickettii*) AND (*pharmaceutical* OR *water* OR *product*) AND (*identification* OR *control* OR *contamination* OR *quality*). To mitigate the high volume of literature initially retrieved via Google Scholar ( $n = 4,180$ ), an adapted search strategy was applied using the specific combination (*Ralstonia pickettii* OR *R. pickettii*) AND (*contamination* OR *pharmaceutical water*) AND (*outbreak* OR *water*), which successfully narrowed the yield to 400 entries.

Inclusion criteria encompassed original scientific articles published between 2016 and 2026 focusing on healthcare-associated outbreaks caused by contaminated pharmaceutical products, alongside the microbiological monitoring and quality control of pharmaceutical water, including the assessment of phenotypic and genotypic methods applied for microbial identification and typing. The exclusion criteria comprised: (i) document types such as reviews, editorials, letters, conference abstracts, thesis, and dissertations; (ii) publications outside the scope of this study; and (iii) duplicate items within or across databases.

The following data were extracted from the selected articles: study setting (clinical or pharmaceutical industry), author(s), publication year, the contaminated matrix associated with *R. pickettii* isolates, identification and typing methods, geographic location, and key findings.

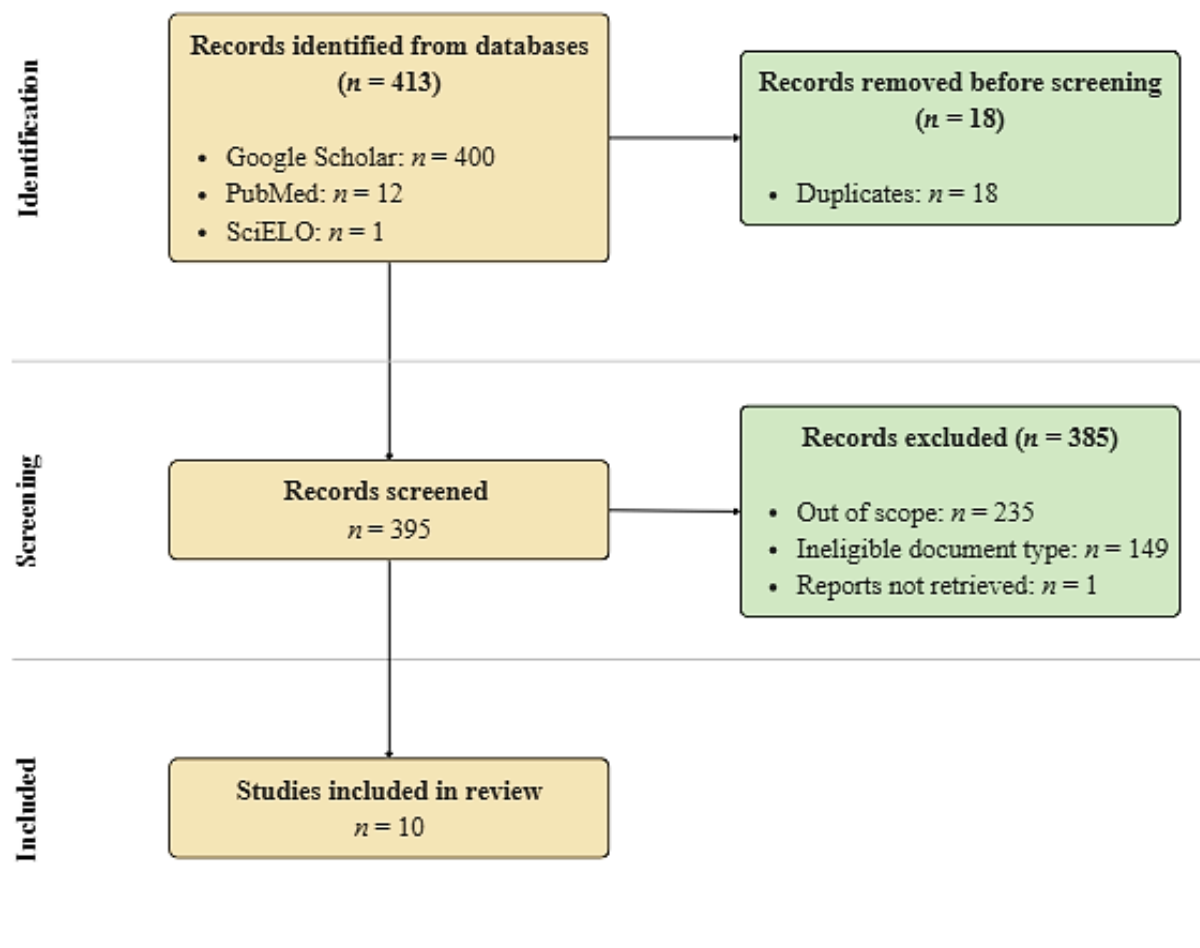
## 3 RESULTS AND DISCUSSION

### 3.1 LITERATURE SEARCH AND STUDY SELECTION FLOW

A total of 413 scientific articles were retrieved across the three databases (Figure 1). While the primary search strategy yielded twelve articles from PubMed and a single record from SciELO, its initial application to Google Scholar generated a high volume of literature ( $n = 4,180$ ). To mitigate this, the adapted search strategy was applied specifically to Google Scholar, successfully narrowing its yield to 400 relevant records. The retrieved items were organized using Microsoft Excel 365 software (Microsoft Corporation, Redmond, WA, USA), where 18 intra- and inter-database duplicates were removed, leaving 395 publications for title and abstract screening. Of these, 235 papers were excluded for lack of alignment with the objective, 149 were rejected as ineligible document types, and one was excluded due to full-

text unavailability. The final selection yielded 10 articles that met all criteria and were included in this review (Table 1).

**Figure 1 – Flowchart of the selection and screening process for studies retrieved from the Google Scholar, PubMed, and SciELO Brazil databases between 2016 and 2026.**



**Source:** Developed by the authors (2026).

**Table 1 – Main characteristics, methodological approaches, and key findings of included studies investigating *R. pickettii* contamination.**

| Reference                     | Database              | Study Source            | Country  | Matrice         | Method                                                    |        | Key findings                                                                                                                                                                                                                                             |
|-------------------------------|-----------------------|-------------------------|----------|-----------------|-----------------------------------------------------------|--------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                               |                       |                         |          |                 | Identification                                            | Typing |                                                                                                                                                                                                                                                          |
| Chen <i>et al.</i> , 2017     | Google Scholar PubMed | Clinical                | Taiwan   | Saline solution | Biochemical test (Vitek®)<br>MALDI-TOF MS                 | PFGE   | Reported a <i>R. pickettii</i> clinical outbreak associated with contaminated saline solutions and documented clonal similarity between clinical and product isolates.                                                                                   |
| Forero <i>et al.</i> , 2021   | Google Scholar PubMed | Clinical                | Colombia | Medicine        | MALDI-TOF MS                                              | N/S    | Identified a bacteremia outbreak associated to <i>R. pickettii</i> contaminated hydromorphone. Strains demonstrated clonal similarity.                                                                                                                   |
| Batarilo <i>et al.</i> , 2022 | Google Scholar        | Pharmaceutical industry | Croatia  | Purified water  | Biochemical tests<br>MALDI-TOF MS<br>Species-specific PCR | PFGE   | Evaluated <i>R. pickettii</i> contamination within water purification systems, demonstrating its capacity to survive routine sanitization protocols. Highlighted pharmaceutical waters as potential environmental reservoirs of resistance determinants. |
| Demirdag <i>et al.</i> , 2022 | Google Scholar        | Clinical                | Turkey   | Saline solution | MALDI-TOF MS                                              | PFGE   | Described a BSI outbreak of <i>R. pickettii</i> caused by contaminated saline solution. Clinical and product strains were confirmed to be the same clone.                                                                                                |

|                                      |                             |          |                   |                    |                              |             |                                                                                                                                                                                                                                                                                                                                                                        |
|--------------------------------------|-----------------------------|----------|-------------------|--------------------|------------------------------|-------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Rajachandran<br><i>et al.</i> , 2022 | Google<br>Scholar           | Clinical | India             | Medicine           | N/S                          | N/R         | Investigated a bacteremia outbreak associated to fentanyl ampoules contaminated with <i>R. pickettii</i> .                                                                                                                                                                                                                                                             |
| Çelen <i>et al.</i> ,<br>2024        | Google<br>Scholar           | Clinical | Turkey            | Saline<br>solution | Biochemical test<br>(Vitek®) | PFGE        | Described a nosocomial outbreak of <i>R. pickettii</i> BSI in a transplant center caused by contaminated saline solutions. Establishment of clonal relationship among clinical and product strains was unsuccessful.                                                                                                                                                   |
| Krone <i>et al.</i> ,<br>2024        | Google<br>Scholar<br>PubMed | Clinical | Germany           | Saline<br>solution | MALDI-TOF MS<br>WGS          | WGS         | Reported a <i>R. pickettii</i> outbreak with high clonal similarity among clinical isolates. Detected <i>R. pickettii</i> in local water and cell culture medium samples but ruled them out as the outbreak source due to significant divergence from the clinical strains. Attributed the contamination to an unidentified, commercially distributed medical product. |
| Saunders <i>et al.</i> , 2024        | Google<br>Scholar<br>PubMed | Clinical | United<br>Kingdom | Saline<br>solution | WGS                          | PFGE<br>WGS | Confirmed a <i>R. pickettii</i> outbreak in the United Kingdom linked to contaminated global saline supplies, showing complete genetic correlation with a previous Australian outbreak in 2023. Contamination root was traced to a leaking safety seal in the manufacturing facility that may have compromised process water and final products.                       |

|                                  |    |                             |                            |         |                                 |                                               |     |                                                                                                                                                                                                                                                                                                                           |
|----------------------------------|----|-----------------------------|----------------------------|---------|---------------------------------|-----------------------------------------------|-----|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Sandfort<br><i>et al.</i> , 2025 | et | Google<br>Scholar<br>PubMed | Clinical                   | Germany | Saline<br>solution              | MALDI-TOF MS<br>WGS                           | WGS | Identified a nationwide <i>R. pickettii</i> outbreak potentially linked to contaminated saline solutions, despite no source was detected; highlighted critical systematic failures in product traceback due to lack of patient-level lot documentation and limitations in 'parametric release' quality control protocols. |
| Li <i>et al.</i> , 2026          |    | Google<br>Scholar           | Pharmaceutical<br>industry | China   | Culture<br>media<br>Bulk<br>Tap | MALDI-TOF MS<br>16S rDNA<br>sequencing<br>WGS | WGS | WGS successfully tracked clonal dispersion and resistome profiles in a GMP plant; <i>R. pickettii</i> isolates were linked to water supply points, highlighting persistent environmental colonization challenges.                                                                                                         |

**Note:** 16S rDNA, 16S ribosomal deoxyribonucleic acid sequencing; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; N/R, not reported; N/S, not specified; BSI, bloodstream infections; PCR, polymerase chain reaction; PFGE, pulsed-field gel electrophoresis; WGS, whole genome sequencing; GMP, good manufacturing practices.

**Source:** Developed by the authors (2026).

### 3.2 GEOGRAPHICAL DISTRIBUTION AND OCCURRENCE CONTEXTS OF *R. pickettii*

*R. pickettii* is a ubiquitous opportunistic pathogen with a global distribution. In this review, the included studies (n=10) demonstrated that its presence was predominantly reported in Asia (50%) and Europe (40%), with only a single study originating from the Americas (Table 1). Turkey and Germany were the countries that published the most scientific articles related to the research topic using the specific keywords. The geographic distribution of published reports should be interpreted cautiously, as it may reflect regional differences in diagnostic infrastructure and surveillance systems rather than the actual global burden of *R. pickettii* (Jones *et al.*, 2008; Ryan; Adley, 2014). This is illustrated by recent hospital outbreaks in Germany (Krone *et al.*, 2024; Sandfort *et al.*, 2025) and the United Kingdom (Saunders *et al.*, 2024), which point to a potential link between patient infections and internationally distributed saline solutions. Because of a highly globalized raw material supply chain, outbreaks reported in one country are often tied back to contaminated products manufactured abroad.

Notably, there is a striking concentration of publications within the healthcare context: 80% of the articles describe hospital outbreaks, whereas only 20% provide an overview of microbiological monitoring within the pharmaceutical industry (Table 1). This higher number of clinical articles in literature do not reflect its day-to-day prevalence, but rather the critical nature of its appearance. The occurrence of severe infections in immunocompromised patients and neonates, coupled with associated mortality, accounts for why *R. pickettii* cases are heavily documented when they do occur (Ryan; Adley, 2014; Stelzmueller *et al.*, 2006; Viana-Cárdenas *et al.*, 2024). In these environments, precise pathogen identification and antimicrobial susceptibility tests are essential for establishing treatment protocols and controlling infection dissemination.

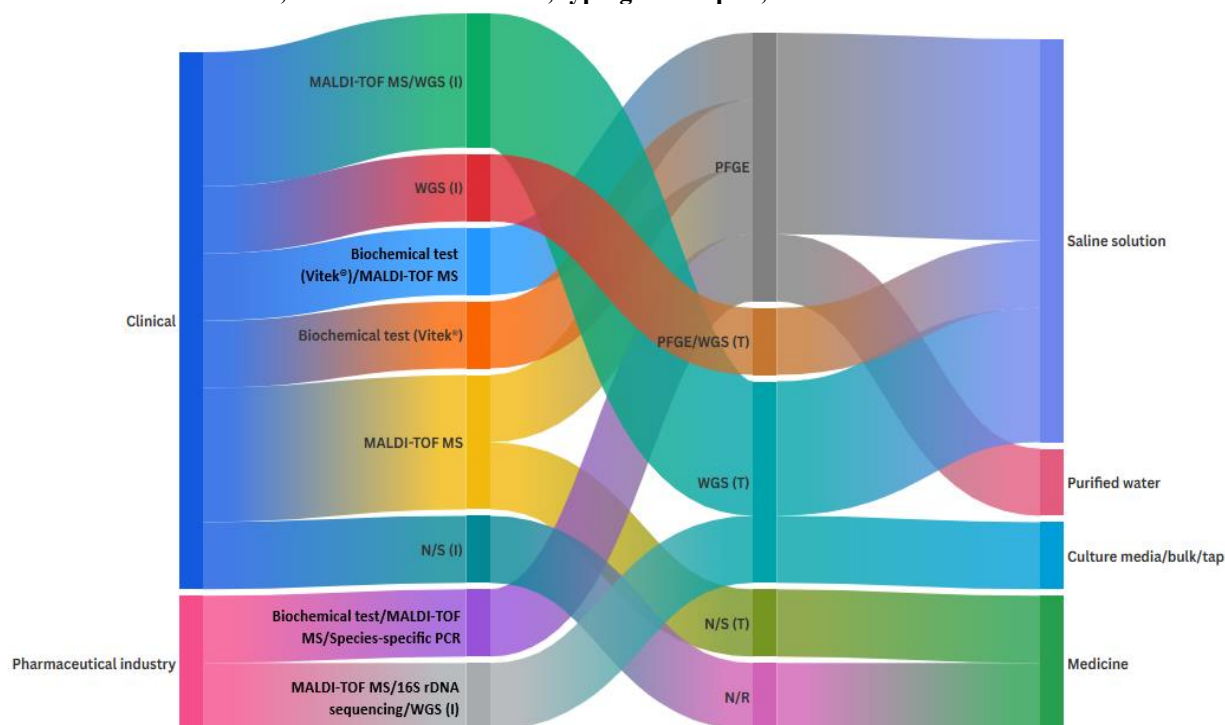
In the industrial environment, the lower number of studies may evidence a probable underreporting or industrial secrecy in routine contamination scenarios, considering that such occurrences can impact the company's reputation and lead to financial losses (Bernaus, 2025). This lack of data underscores the fact that industrial contamination events are traditionally confined to internal investigations and regulatory audits, meaning they rarely make it into published academic literature (Jimenez *et al.*, 2019). This risk is further heightened by the reliance on parametric release, a practice where production batches are cleared based strictly on physical sterilization parameters, eliminating the need for final product sterility testing (Sandfort *et al.*, 2025). This approach can be dangerous if a sterilization failure occurs, because contaminated medicines can easily enter distribution and cause severe, life-threatening infections (Saunders *et al.*, 2024). A tragic illustration of this risk occurred in Argentina, where

50 patients died from infections linked to fentanyl contaminated with both *Klebsiella pneumoniae* and *R. pickettii*, traced back to an Indian manufacturer (Bernaus, 2025).

### 3.3 CONTAMINATED MATRICES AND THE TECHNOLOGICAL SHIFT IN MICROBIAL TYPING

Saline solution emerged as the most widely reported product associated with *R. pickettii* contamination (60%), establishing it as the leading vehicle for clinical contamination over the past decade, followed by pharmaceuticals (20%) (Figure 2). The broad application of saline solutions in both healthcare and everyday contexts (including injections, wound cleansing, eye care, and nebulization) means that any contamination poses a widespread risk. Tracking these affected batches then becomes highly complex (Krone *et al.*, 2024).

**Figure 2 – Sankey diagram illustrating the multivariate flow and distribution of *R. pickettii* isolates across source contexts, identification methods, typing techniques, and contaminated matrices**



Legend: The width of the streams represents the proportion of each specific workflow. **Abbreviations:** 16S rDNA, 16S ribosomal deoxyribonucleic acid sequencing; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; N/R, not reported; N/S (I), not specified for identification; N/S (T), not specified for typing; PCR, polymerase chain reaction; PFGE, pulsed-field gel electrophoresis; WGS (I), whole genome sequencing used for identification; WGS (T), whole genome sequencing used for molecular typing.

**Source:** Developed by the authors (2026).

Data from two studies in pharmaceutical quality control and environmental monitoring demonstrate that *R. pickettii* can be found in different places in industrial systems, utilities,

intermediate and final products, purified water, culture media, bulk solutions, and facility taps. This widespread presence stems directly from the bacterium's biological traits: it is waterborne origin, its ability to survive under oligotrophic (nutrient-poor) conditions, and its capacity to establish persistent biofilms within water distribution networks (Ryan; Adley, 2014). These contaminations can represent vulnerabilities in aseptic processing, particularly regarding filtration integrity, sterilization parameters, and media-fill validations (Bernaus, 2025; Ryan; Adley, 2014). The stakes are incredibly high for patients; if *R. pickettii* compromises batches meant to be sterile, it poses a direct threat to neonates and immunocompromised individuals, frequently triggering severe infections and fatal outcomes.

Accurate microbial identification and typing are essential for tracking contamination sources, halting environmental spread, and implementing immediate corrective actions (Li *et al.*, 2026; Mattoso *et al.*, 2025; Song *et al.*, 2024; Vasconcellos *et al.*, 2026). While biochemical assays were historically the mainstay of laboratory identification, they have rapidly given way to faster, more precise alternatives, appearing in only three studies between 2017 and 2024. In their place, MALDI-TOF MS has emerged as the primary identification method, utilized in 70% of studies published since 2017, probably due to its accuracy and fast lead time (Nunes Ramos *et al.*, 2025).

Regarding molecular typing, a distinct technological shift has emerged: earlier studies (up to 2024) relied heavily on pulsed-field gel electrophoresis (PFGE), whereas the more actual studies (2024–2026) transitioned to WGS to achieve clonal resolution. Historically, PFGE was frequently employed in clinical settings to cluster genetically similar isolates; however, the technique lacks interlaboratory comparability and offers limited discriminatory power (Kubota *et al.*, 2019). While MLST provides better reproducibility for differentiating strains into sequence types (STs) (Li *et al.*, 2026; Simar; Hanson; Arias, 2021), no public database schema (PubMLST) is currently available for *R. pickettii*, a limitation likely driven by the high genomic similarity across the genus *Ralstonia*.

WGS has been applied equally for species identification (40%) and the precise differentiation of closely related strains (40%). This resolution is underscored by Li *et al.* (2026), who demonstrated that WGS yields substantially higher discriminatory power than traditional PFGE and MLST frameworks, making it highly effective for mapping transmission routes and pinpointing contamination sources, while simultaneously uncovering underlying resistance and virulence profiles (Simar; Hanson; Arias, 2021; Yuan *et al.*, 2024). In the most recent literature, this genomic approach has advanced to utilize cgMLST and single-nucleotide polymorphism (SNP) analysis (Li *et al.*, 2026; Yuan *et al.*, 2024). These high-resolution

workflows have proven efficient for delineating genetic clusters among *R. pickettii* isolates (Li *et al.*, 2026; Yuan *et al.*, 2024). Consequently, WGS stands out as tool for rapid outbreak detection, robust epidemiological surveillance, and can also contribute to CCS in pharmaceutical industries (Li *et al.*, 2026; Simar; Hanson; Arias, 2021; Yuan *et al.*, 2024).

### 3 FINAL CONSIDERATIONS

By mapping the dissemination dynamics of *R. pickettii*, this integrative review highlights how the pathogen's persistence in production utilities establishes a direct pathway to healthcare-associated outbreaks, with contaminated pharmaceutical inputs and fluid products serving as the primary vehicles of transmission. In addition, the findings demonstrate that overcoming current analytical bottlenecks relies heavily on transitioning to high-resolution diagnostic and molecular typing tools. These methods deliver the resolution necessary to reliably trace contamination sources across both clinical investigations and the factory floor, establishing definitive epidemiological links. Data from advanced identification methods must therefore be translated into actionable plant-floor measures. Rather than existing as isolated diagnostic metrics, these profiles should actively guide corrective and preventive actions (CAPA) systems, optimize sanitization programs via evidence-based disinfectant rotation, and strengthen the ongoing validation of critical production utilities. Therefore, integrating these precise typing workflows into comprehensive contamination control strategies is vital to prevent process deviations, manage outbreaks, and meet the strict standards of good manufacturing practices.

### ACKNOWLEDGMENTS

The authors are grateful to Pos-Graduate Program in Health Surveillance of the Instituto Nacional de Controle de Qualidade em Saúde (Fundação Oswaldo Cruz). Marcelo Luiz Lima Brandão is grateful for the support from CNPq in the form of a Productivity in Research Fellowship (PQ-C) (Process: 304877/2024-7) and FAPERJ in the form of a Bolsa de Jovem Cientista do Nosso Estado (Process: E-26/204.245/2025-BOLSA N.º de Matrícula: 2005.03225.2).

### REFERENCES

ANDERSON, R. L. *et al.* Factors associated with *Pseudomonas pickettii* intrinsic contamination of commercial respiratory therapy solutions marketed as sterile. **Appl. Environ. Microbiol.**, v. 50, n. 6, p. 1343-1348, 1985.

BAKER, M. A.; *et al.* *Ralstonia pickettii* and *Pseudomonas aeruginosa* bloodstream infections associated with contaminated extracorporeal membrane oxygenation water heater devices. **Clin. Infect. Dis.**, v. 75, n. 10, p. 1838-1840, 2022.

BASSO, M. *et al.* A case of persistent bacteremia by *Ralstonia mannitolilytica* and *Ralstonia pickettii* in an intensive care unit. **Infect. Drug Resist.**, v. 12, p. 2391-2395, 2019.

BATARILO, I. *et al.* Oxacillinases and antimicrobial susceptibility of *Ralstonia pickettii* from pharmaceutical water systems in Croatia. **Arh. Hig. Rada Toksikol.**, v. 73, n. 2, p. 103-109, 2022.

BERNAUS, C. G. Risk Assessment and Regulatory Response to Systematic Pharmaceutical Quality Failures: Lessons from Argentina's Contaminated Fentanyl and Dexamethasone Crisis. **J. Regul. Sci.**, v. 13, n. 1, 2025.

BRASIL. Agência Nacional de Vigilância Sanitária. **Farmacopeia brasileira: 7.ed.** Brasília: ANVISA, 2024. 1072 p. v.1

CHEN, Y. Y. *et al.* An outbreak of *Ralstonia pickettii* bloodstream infection associated with an intrinsically contaminated normal saline solution. **Infect. Control Hosp. Epidemiol.**, v. 38, n. 4, p. 444-448, 2017.

CHOUDHURY, H.; *et al.* An outbreak of *Ralstonia pickettii* endophthalmitis following intravitreal methotrexate injection. **Clin. Ophthalmol.**, v. 9, p. 1117-1120, 2015.

CLSI – Clinical and Laboratory Standards Institute. **Performance Standards for Antimicrobial Susceptibility Testing**, 34th Edition. CLSI Supplement M100. Wayne, PA: CLSI; 2024.

ÇELEN, M. K. *et al.* Central line-associated bloodstream infection outbreak related to *Ralstonia pickettii*-contaminated saline in a pediatric hematopoietic stem cell transplant center. **J. Hosp. Infect.**, v. 118, p. 45-52, 2024.

COSTA, A. S. *et al.* Standardization of an Effective Disinfection Methodology Against Microorganisms Isolated from a Pharmaceutical Industry Facility as a Contamination Control Strategy. **Standards**, v. 6, n. 2, p. 22, 2026.

de la TORRE B. G.; ALBERICIO, F. The Pharmaceutical Industry in 2024: An Analysis of the FDA Drug Approvals from the Perspective of Molecules. **Molecules**, v. 30, n. 3, p. 482, 2025.

DEMIRDAG, Y. *et al.* An outbreak of *Ralstonia pickettii* bloodstream infection among pediatric leukemia patients. **Pediatr. Infect. Dis. J.**, v. 41, n. 9, p. 370-373, 2022.

DESAI, M.; NJOKU, A.; NIMO-SEFAH, L. Comparing Environmental Policies to Reduce Pharmaceutical Pollution and Address Disparities. **Int. J. Environ. Res. Public Health**, v. 19, n. 14, p. 8292, 2022.

EUCAST. European Committee on Antimicrobial Susceptibility Testing. **Breakpoint tables for interpretation of MICs and zone diameters, version 14.3, 2024**. Disponível em: [https://www.eucast.org/clinical\\_breakpoints/](https://www.eucast.org/clinical_breakpoints/). Acesso em: 08 set. 2025.

EUROPEAN MEDICINES AGENCY (EMA). The Rules Governing Medicinal Products in the European Union. Volume 4: European Union Guidelines for Good Manufacturing Practice for Medicinal Products for Human and Veterinary Use. **Annex 1: Manufacture of Sterile Medicinal Products**. Bruxelles, 2022.

FLUIT, A. C. *et al.* Characterization of clinical *Ralstonia* strains and their taxonomic position. **Antonie Van Leeuwenhoek**, v. 114, n. 10, p. 1721-1733, 2021.

FOOD AND DRUG ADMINISTRATION (FDA). **Guidance for Industry Sterile Drug Products Produced by Aseptic Processing - Current Good Manufacturing Practice**. Bethesda, MD, USA, 2004.

FORERO, J. *et al.* Outbreak of *Ralstonia pickettii* bacteremia caused by contaminated hydromorphone in a university hospital in Bogota, Colombia. **Infectio**, v. 25, n. 3, p. 185-190, 2021.

GAOH, S. *et al.* A comparative metagenomic analysis of specified microorganisms in groundwater for non-sterilized pharmaceutical products. **Curr. Microbiol.**, v. 81, n. 9, p. 273, 2024.

GHIJS, S.; WYNENDAELE, E.; DE SPIEGELEER, B. The continuing challenge of drug recalls: insights from a ten-year FDA data analysis. **J. Pharm. Biomed. Anal.**, v. 249, p. 116349, 2024.

GÖKER, M. *et al.* List of Recommended Names for bacteria of medical importance: report of the Ad Hoc Committee on Mitigating Changes in Prokaryotic Nomenclature. **Int. J. Syst. Evol. Microbiol.**, v. 75, art. 6943, 2025.

GUPTA, S.; DESAI, S. Environmental impact and regulatory compliance: a comprehensive approach in managing institutional pharmaceutical waste. **Int. J. Pharm. Pharm. Sci.**, v. 16, n.6, p. 14–18, 2024.

HOLMES, B. *et al.* *Burkholderia pickettii* sp. nov., a new name for the species previously known as *Pseudomonas pickettii*. **Int. J. Syst. Bacteriol.**, v. 43, n. 2, p. 366–366, 1993.

JESUS, P. R. *et al.* Monitoring the quality of the water used in mobile dialysis services in intensive care units in the city of Rio de Janeiro. **J. Bras. Nefrol.**, v. 44, n. 1, p. 32-41, 2022.

JIMENEZ, L. *et al.* Analysis of FDA enforcement reports (2012–2019) to determine the microbial diversity in contaminated non-sterile and sterile drugs. **Am. Pharm. Rev.**, v. 21, p. 48–73, 2019.

JONES, K. E. *et al.* Global trends in emerging infectious diseases. **Nature**, v. 451, n. 7181, p. 990-993, 2008.

KRONE, M. *et al.* *Ralstonia pickettii* bloodstream infections with potential genomic link to internationally distributed contaminated saline solution, Germany, October 2023. **Euro Surveill.**, v. 29, n. 3, p. 2400010, 2024.

KUBOTA, K. A. *et al.* PulseNet and the Changing Paradigm of Laboratory-Based Surveillance for Foodborne Diseases. **Public Health Rep.**, v. 134, n. 2\_suppl, p. 22S-28S, 2019.

LI, Q. *et al.* Leveraging whole-genome sequencing for microbial contamination tracking and risk assessment in pharmaceutical manufacturing. **Front. Microbiol.**, v. 17, p. 1807989, 2026.

MATTOSO, J. M. V. *et al.* Quantitative and qualitative evaluation of microorganism profile identified in bioburden analysis in a biopharmaceutical facility in Brazil: criteria for classification and management of results. **PDA J. Pharm. Sci. Technol.**, v. 79, n. 2, p. 125-156, 2025.

MENGHWAR, H. S. *et al.* Application of cgMLST in outbreak analysis and surveillance. **Pathogens**, v. 11, n. 3, p. 351, 2022.

MOREIRA, B. M. *et al.* *Ralstonia pickettii* and *Burkholderia cepacia* complex bloodstream infections related to infusion of contaminated water for injection. **J. Hosp. Infect.**, v. 60, n. 1, p. 51-55, 2005.

NUNES RAMOS, J. *et al.* Challenges in the Identification of Environmental Bacterial Isolates from a Pharmaceutical Industry Facility by 16S rRNA Gene Sequences. **DNA**, v. 5, n. 3, p. 33, 2025.

ORME, J.; *et al.* Native valve endocarditis due to *Ralstonia pickettii*: a case report and literature review. **Case Rep. Infect. Dis.**, v. 2015, p. 324675, 2015.

PATEL, R. *et al.* A retrospective regulatory analysis of FDA recalls carried out by pharmaceutical companies from 2012 to 2023. **Drug Discov Today**. V. 29, n. 6, p. 103993, 2024.

PELLEGRINO, F. L. *et al.* *Ralstonia pickettii* bloodstream infections at a Brazilian cancer institution. **Curr. Microbiol.**, v. 56, n. 3, p. 219-223, 2008.

PEREZ-ALEMAN, P.; FERRETTI, T. Creating innovation capabilities for improving global health: inventing technology for neglected tropical diseases in Brazil. **J. Int. Bus. Policy**, v. 6, n. 1, p. 84-114, 2023.

RAJACHANDRAN, K. *et al.* Outbreak of nosocomial infection from an unusual source. **Indian J. Crit. Care Med.**, v. 26, n. 9, p. 1042-1044, 2022.

RALSTON, E.; PALLERONI, N. J.; DOUDOROFF, M. *Pseudomonas pickettii*, a new species of clinical origin related to *Pseudomonas solanacearum*. **Int. J. Syst. Bacteriol.**, v. 23, p. 15-19, 1973.

- ROSS, B. *et al.* Outbreak with *Ralstonia pickettii* caused by contaminated magnesium vials. **Dtsch. Med. Wochenschr.**, v. 139, n. 7, p. 323-326, 2014.
- RYAN, M. P.; ADLEY, C. C. *Ralstonia* spp.: emerging global opportunistic pathogens. **Eur. J. Clin. Microbiol. Infect. Dis.**, v. 33, n. 3, p. 291-304, 2014.
- RYAN, M. P.; PEMBROKE, J. T.; ADLEY, C. C. *Ralstonia pickettii*: a persistent gram-negative nosocomial infectious organism. **J. Hosp. Infect.**, v. 62, n. 3, p. 278-284, 2006.
- SANDFORT, M. *et al.* Nosocomial Outbreak of *Ralstonia pickettii* Infections Likely Linked to Saline Solutions in Germany from August 2023 to March 2024—Challenges in Medical Product-Related Outbreaks. **Microorganisms**, v. 13, n. 9, p. 2102, 2025.
- SAUNDERS, M. *et al.* Outbreak of *Ralstonia pickettii* associated with contamination of saline products distributed internationally, the United Kingdom, 2024. **Euro Surveill.**, v. 29, n. 27, p. 2400384, 2024.
- SCHNETZINGER, M. *et al.* Bacterial contamination of water used as thermal transfer fluid in fluid-warming devices. **J. Hosp. Infect.**, v. 141, p. 49-54, 2023.
- SHARMA, A.; GAMTA, V.; LUTHRA, G. The importance of good manufacturing practices (GMP) in the healthcare industry. **J. Pharm. Res. Int.**, v. 35, n. 18, p. 75–90, 2023.
- SHENG, Z. *et al.* Molecular epidemiological and clinical infection characteristics analysis of *Ralstonia*. **Eur. J. Clin. Microbiol. Infect. Dis.**, v. 43, n. 6, p. 1161-1170, 2024.
- SIMAR, S. R.; HANSON, B. M.; ARIAS, C. A. Techniques in bacterial strain typing: past, present, and future. **Curr. Opin. Infect. Dis.**, v. 34, n. 4, p. 339-345, 2021.
- SONG, M. *et al.* A comprehensive technology strategy for microbial identification and contamination investigation in the sterile drug manufacturing facility - a case study. **Front. Microbiol.**, v. 15, p. 1327175, 2024.
- SOUZA, P. A. *et al.* Evaluation of Antimicrobial Resistance Patterns of *Pseudomonas aeruginosa* Strains Isolated among COVID-19 Patients in Brazil Typed by Fourier-Transform Infrared Spectroscopy. **Life (Basel)**, v. 14, n. 9, p. 1079, 2024.
- SOUZA, P. A. *et al.* Evaluation of biofilm formation, antimicrobial pattern, and typing of *Stenotrophomonas maltophilia* isolated from clinical sources in Brazil. **Braz J Microbiol**, v. 56, n. 3, p. 1861-1871, 2025a.
- SOUZA, P. A. *et al.* Phenotypical and Molecular Characterization of *Acinetobacter baumannii* Isolated from Hospitalized Patients During the COVID-19 Pandemic in Brazil. **Life (Basel)**, v. 15, n. 4, p. 623, 2025b.
- STELZMUELLER, I. *et al.* *Ralstonia pickettii* - innocent bystander or a potential threat? **Clin. Microbiol. Infect.**, v. 12, n. 2, p. 99–101, 2006.

SUNDARAM, S. *et al.* Application of membrane filtration for removal of diminutive bioburden organisms in pharmaceutical products and processes. **PDA J. Pharm. Sci. Technol.**, v. 53, n. 4, p. 186–201, 1999.

UNITED STATES PHARMACOPEIA. **USP-NF Online**. Rockville: United States Pharmacopeial Convention, 2026. Disponível em: <https://online.uspnf.com>. Acesso em: 01 jul 2026.

VASCONCELLOS, L. *et al.* Phenotypical and molecular characterization of *Acinetobacter* spp. isolated from a pharmaceutical facility. **Lett. Appl. Microbiol.**, v. 76, p. ovad101, 2023.

VASCONCELLOS, Luiza *et al.* Phenotypical and molecular characterization of *Pseudomonas* spp. isolated from a pharmaceutical facility. **Processes**, v. 14, n. 2, p. 231, 2026.

VIANA-CÁRDENAS, E. *et al.* A large multicenter *Ralstonia pickettii* outbreak in critically ill patients during the COVID-19 pandemic: epidemiological and clinical characteristics of 66 cases. **J. Infect. Prev.**, v. 25, n. 3, p. 85–88, 2024.

YABUUCHI, E. *et al.* Transfer of two *Burkholderia* and an *Alcaligenes* species to *Ralstonia* gen. nov.: proposal of *Ralstonia pickettii* (Ralston, Palleroni and Doudoroff 1973) comb. nov., *Ralstonia solanacearum* (Smith 1896) comb. nov. and *Ralstonia eutropha* (Davis 1969) comb. nov. **Microbiol. Immunol.**, v. 39, n. 11, p. 897–904, 1995.

YUAN, C. *et al.* Genomic analysis of *Ralstonia pickettii* reveals the genetic features for potential pathogenicity and adaptive evolution in drinking water. **Front. Microbiol.**, v. 14, p. 1272636, 2024.