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The role of Ralstonia in the infection of patients with haematological malignancies

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Abstract

Ralstonia spp are oxidase-positive, non-fermentative rods that are aerobic Gram-negative bacteria commonly encountered in water and soil environments. The Ralstonia species, typically considered to be a low pathogenic environmental organism, has the potential to induce serious illnesses in specific vulnerable patient populations, such as individuals with haematological malignancies. Challenges in accurately identifying this pathogen within the clinical setting can lead to potential misidentification, resulting in its role as a disease-causing agent being underestimated. This review article examines *Ralstonia spp* infections in patients with hematologic malignancies. By examining various articles published since 1995 through search databases like Google Scholar, Pub Med, Web of Science, Scopus and Elsevier databases, we aim to gather the most effective information. In this study, we reported the number of infections caused by Ralstonia spp in patients with hematologic malignancies that have been reported in the world so far. In this review study, we showed that Ralstonia spp is increasing in these patients and requires more investigations and more accurate tests.

Keywords: *Ralstonia spp*, Haematological malignancies, *Pseudomonas spp*, *Burkholderia spp*, Antibiotic resistance

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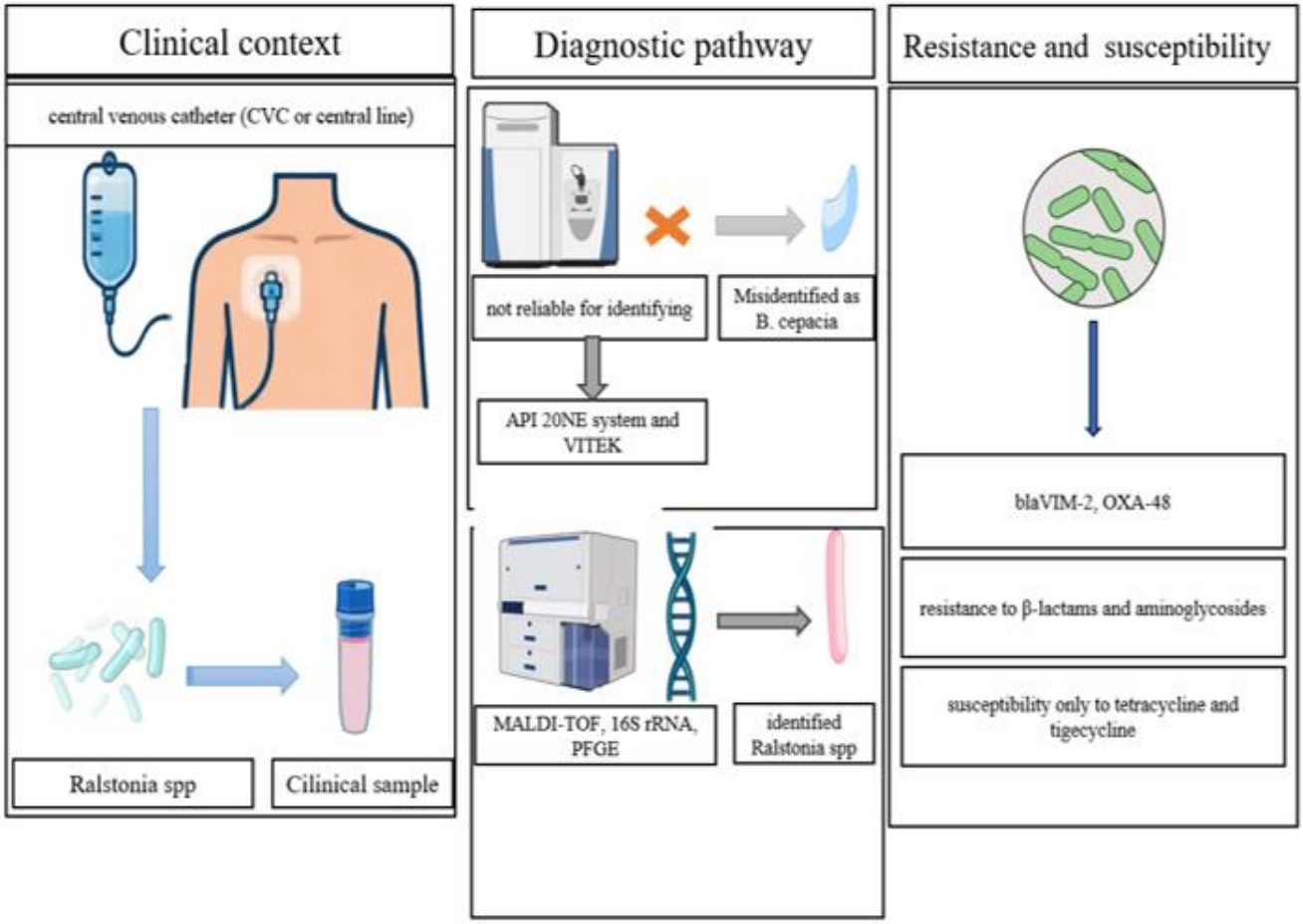
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Graphical abstract



Introduction

In 1973, *Ralstonia spp*. were initially discovered and placed within the category of *Pseudomonas spp.* It was later assigned to the genus Burkholderia as *Burkholderia picketti* and *Burkholderia solanacearum*. In 1995, Yabuuchi and colleagues identified the genus Ralstonia. There is still limited research on proteobacteria. Six distinct species have been identified as the official type strains that serve to distinctly categorize the genus Ralstonia. These species are *Ralstonia insidiosa*, *Ralstonia mannitolilytica*, *Ralstonia picketti*, *Ralstonia pseudosolanacearum*, *Ralstonia solanacearum*, and *Ralstonia syzygii*. The bacteria are usually isolated in nature from soil, water, and plants; they are non-fermentative, aerobic Gram-negative bacteria (NFGN), oxidase-positive and rod-shaped bacteria (1, 2). Ralstonia is able to proliferate in humid environments and survive for long periods of time in harsh conditions, especially in outdoor water treatment facilities such as municipal and medical water treatment systems (3). Certain species like *R. pseudosolanacearum*, *R. solanacearum* and *R. syzygii* are known to be plant pathogens. In recent times, *R. insidiosa*, *R. mannitolilytica*, *R. pickettii* have been found to cause infectious diseases, particularly in individuals with weakened immune systems, and are responsible for hospital-acquired infections (4). Earlier investigations indicate that the majority of infections were due to *R. picketti* and a small number to *R. mannitolilytica* (5). In 2003, the classification *R. insidiosa* was proposed, indicating that this species has the ability to cause various infections such as bloodstream infections, pneumonia, peritonitis, meningitis, endocarditis, spinal osteitis, osteomyelitis, septic arthritis, and prostatitis (6). Ralstonia nosocomial infections is thought to originate from contaminated solutions or water. The most frequent way of contracting an infection is likely through the contamination of medical supplies, as the bacteria can survive in antiseptic solutions, saline, and disinfectants

(7). Despite its relatively low virulence, previous studies have classified the bacterium as an opportunistic pathogen. Their small size allows them to pass through 0.2 µm filters commonly used for sterilizing solutions. There is limited knowledge of how *Ralstonia spp* is transmitted from person to person, but it appears that outbreaks are usually caused by contaminated solutions. *R. paucula* and *R. gilardii* have infrequently been detected in human clinical specimens including cerebrospinal fluid, bone marrow, wounds, and the respiratory system. Reports on the worldwide prevalence of *Ralstonia spp* are shown in Figure 1 (8).

Research method

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to ensure transparency and methodological rigor. A comprehensive literature search was performed across multiple electronic databases, including ScienceDirect, PubMed, Google Scholar, Medline, Springer, and Scopus (Web of Science), covering the period from 1995 to 2024. The search strategy employed a combination of keywords and Boolean operators (AND, OR) related to *Ralstonia spp*, *haematological malignancies*, *Pseudomonas spp*, *Burkholderia spp*, *antibiotic resistance* and *bacterial infections*. Additionally, reference lists of identified studies were manually screened to ensure the inclusion of all relevant literature. The study selection process followed the PICO (Population, Intervention, Comparison, and Outcomes) framework. To ensure the reliability of the synthesized data, the methodological quality of the included studies was rigorously assessed.

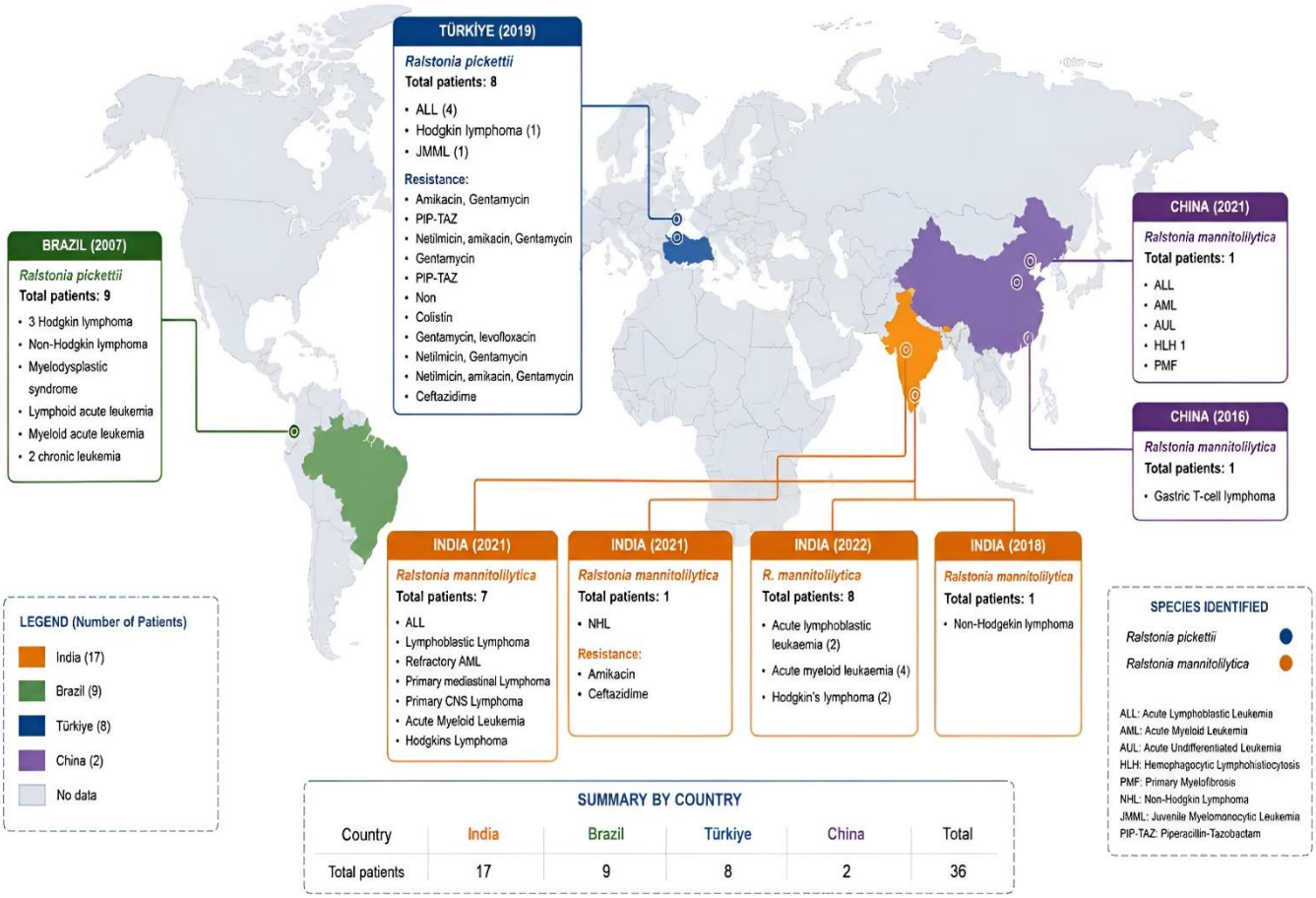


Figure 1. Geographic distribution of *Ralstonia spp* in patients with hematological malignancies in worldwide.

Ralstonia pickettii

R. pickettii was first isolated which was formerly known as *Pseudomonas pickettii* and *Burkholderia*

pickettii, is a waterborne microorganism capable of thriving in various types of water sources. It has the ability to contaminate intravenous drugs, blood culture bottles, distilled water, saline solutions, and other medical solutions employed in patient care within healthcare facilities. *Ralstonia pickettii* (*R. pickettii*) is recognized as a newly emerging bacteria that causes disease (9). *R. pickettii* has a tendency to biofilms formation in plastic industrial water conduits. Individuals with weakened immune systems may exhibit heightened susceptibility upon exposure to this microorganism. Bloodstream infections and catheter-related infections have been associated with contamination of products during the manufacturing process. (10). The identification and treatment of *Ralstonia picketti*i is complicated by its similarity to other similar bacteria (11). *R. pickettii* can be detected in various types of clinical specimens such as sputum, blood, wounds, urine, ear and nose swabs, and cerebrospinal fluid. It is commonly found in the respiratory secretions of individuals with cystic fibrosis. Most cases in scientific studies involve infections acquired in hospitals due to contaminated items such as blood products, sterile water, saline, chlorhexidine solutions, respiratory treatment fluids, and infected venous catheters (12). *Ralstonia pickettii* demonstrates the ability to thrive in environments with scarce nutrients by utilizing different compounds to fulfill its energy and carbon needs (13).

Ralstonia mannitolilytica

Ralstonia mannitolilytica are typically found in water and soil environments, but are increasingly being recognized as opportunistic pathogens that can cause infections in individuals with weakened immune systems. Previously, *R. mannitolilytica* was previously identified as "Pseudomonas thomasii" while *R. picketti*i biovar 3 was known as "thomasii". These organisms have the potential to trigger outbreaks of nosocomial infections by contaminating sterile parenteral fluids and medical devices (14). *R. mannitolilytica* has been identified as a cause of sepsis, meningitis, and central venous catheter-related bacteremia (15). Recently, numerous case studies have reported infections caused by *R. mannitolilytica* in India and around the world (16). Several case studies have emphasized that *R. mannitolilytica* is associated with bacteraemia and sepsis. Carreira et al, highlighted the importance of *R. mannitolilytica* in causing endocarditis (17). Currently, there are no specific treatment recommendations or cut-off points provided by the Clinical and Laboratory Standards Institute (CLSI) for *R. mannitolilytica*. Treating this species can be difficult because it often shows resistance to a variety of antibiotics (18). *R. mannitolilytica* is often resistant to various β-lactam antibiotics, such as carbapenems. Resistance is usually associated with the blaOXA-22 and blaOXA-60 genes, which are class D carbapenemases in Ralstonia species (19).The species name "mannitolilytica" refers to its ability to acidify mannitol in the past, *Ralstonia mannitolilytica* has been incorrectly identified as *Pseudomonas fluorescens* (12).

Ralstonia insidiosa

Ralstonia insidiosa has been detected in the respiratory secretion in individuals with cystic fibrosis, as well as in bodies of water such as rivers and ponds, soil, and activated sludges (20). The presence of *R. insidiosa* FC 1138 was detected in water distribution networks. When *E. coli* O157:H7 is cultured with *R. insidiosa*, the amount of *E. coli* in biofilms increases significantly in various test situations(21). Chryseobacterium gleum, formerly known as *Flavobacterium gleum* belongs to the phylum Bacteroidetes. It is frequently found in environments similar to *R. insidiosa* and may also be present in human infections (22, 23). *R. insidiosa* occasionally contributes to the formation of biofilms with various species such as *Listeria monocytogenes*, *Salmonella enterica* and *Escherichia coli.* In these cases, it is usually observed that *R. insidiosa* forms connections between cells of different species, whereby it is assumed that certain elements of the biofilm matrix contribute to an increased strength of the biofilms (21).

Characteristics of virulence and biochemistry linked to Ralstonia species

There was a difference between Ralstonia and Burkholderia. Analysis of cellular lipids and fatty acids, comparison of rRNA-DNA hybridisation results, and evaluation of 16s rDNA nucleotide sequences for phylogenetic analysis are some of the things that can be done. Ralstonia species can be identified by the

existence of one or multiple polar flagella in species capable of mobility. They can also produce acid from glucose and different types of carbohydrates(24). Moreover, Ralstonia species have shown resistance to colistin. This distinguishes them from *B. cepacia*, *B. andropogonis*, and *Alcaligenes faecalis*, the typical Alcaligenes species. Ralstonia and Xanthomonas each possess a pair of type II-SS secretory systems, which are used to move virulence factors like pect numerous Gram-negative bacterial pathogens, including those affecting humans, animals, plants and plant growth-promoting rhizobacteria, possess gene clusters responsible for encoding the T3SS (25). Type III gene clusters are present in plant pathogens like Ralstonia, Erwinia, Xanthomonas, and Pantoea. Sometimes, the genes that control the Type III secretion system can be found on plasmids, like in the case of *Ralstonia solanacearum*, a wilt pathogen that has a 2.09 Mb megaplasmid carrying these genes. Recent research has revealed the presence of *Ralstonia picketti*i, a bacteria that produces siderophores, in industrial wastewater (26). The research also indicated that these bacteria are capable of establishing a mutually advantageous partnership with the microalgae Chlorella sorokiniana, which breaks down dyes, in order to address challenges caused by the scarce presence of iron and carbon in bioremediation procedures(27). Ralstonia has the capacity to generate exopolysaccharides, with *Ralstonia pickettii* L4C6L displaying greater biofilm formation capability than other strains. *Ralstonia picketti* L4C6L is able to break down phosphate. The ability of this species to form biofilms is due to the secretion of signaling molecules like homoserine lactones(28).Zhang-Sun and colleagues undertook a study to examine the lipid A structures of three distinct species in this genus. The composition of the under-acylated lipid A from *Ralstonia picketti* was determined along with the attachment of AraN to the phosphate moieties. Both AraN substitution and deacylation lead to the release of Blebs from the bacterial membrane. *R. pickettii* showed a greater percentage of phosphate replacements in the findings(29). Due to this, *R. pickettii* was able to generate a higher amount of outer membrane vesicles. Various molecular types were detected at both the rough and semi-rough tiers, with LPSs having as many as twelve O-chain units and achieving a top size of 10kDa. At the intermediate level, the most notable LPS molecular species were recognized. *R. pickettii* exclusively makes LPS molecular species in rough and semi-rough types, all below 5kDa in size (30).

Methods for diagnosing ralstonia

The biochemical profiles of *R. mannitolilytica* and *R. insidiosa* are very similar to those of the *Burkholderia cepacia* complex. Commercial biochemical profiling methods such as the API 20NE system and VITEK are not reliable for identifying bacteria due to their similarities (31). *R. mannitolilytica* can readily be cultivated on standard culture mediums like trypticase soy agar supplemented with 5% sheep blood or Mac Conkey agar. *Ralstonia spp* Can be identified using biochemical tests as well as automated identification systems this particular species has the potential to be mistaken for Burkholderia spp Potential determination of Pseudomonas bacteria they are not part of the Aeruginosa group. Both the arginine dihydrolase test and pyrrolidonyl peptidase test are effective in distinguishing R. mannitolilytica from Pseudomonas and Burkholderia(30). The techniques used for recognition consist of the ViTek 2 system with 16sRNA gene sequencing (molecular techniques), PFGE, and MALDI-TOF,RAPD, and ribotyping have been created for *Ralstonia spp* (32). The bacteria's microbiological traits are not distinctive on blood agar or MacConkey Agar Biochemically, they have varied growth at 42 °C, produce gas from nitrate, oxidize lactose, and exhibit other specific properties due to its biochemical properties, *Ralstonia spp* can be misidentified as Burkholderia cepacia complex in lab environments. Specialized methods like MALDI-TOF, 16S rRNA, and other nucleic acid amplification tests are needed for precise species identification, but they are rarely carried out in regular microbiology laboratories(33). The Gram-negative bacteria exhibited the following outcomes in biochemical tests: positive results for catalase and oxidase, demonstrated motility, did not ferment, tested negative for methyl red and Voges–Proskauer, showed a negative reaction for indole, triple sugar iron agar indicated an alkaline/alkaline response, and tested negative for urease. Citrate was utilized for metabolism, while aesculin and gelatin were not broken down. Nitrate reduction was absent in R. mannitolilytica but present in *Ralstonia picketti*, and R. mannitolilytica did not

lower the pH of d-arabitol and mannitol unlike *R. picketti*i. Biochemical tests to distinguish Ralsutonia from Pseudomonas and Bulchordia species include: Reduction of nitrate, Reduction of nitrite, Colistin susceptibility, Desferrioxamine susceptibility ,Acidification of sucrose,Acidification of mannitol,Acidification of D-arabitol, Pyrrolidone peptidase, Trypsin, Arginine dihydrolase,Lysine decarboxylase,Alkalinization of tartrate, Pyoverdin production, Growth on salmonella-shigella agar. Ralstonia species are unable to thrive in Salmonella Shigella agar (Figure 2, 3) (34).

Ralstonia antibiotic resistance

Limited information is available regarding the surveillance and management of antibiotic resistance in these bacteria, with even less data on possible variations in resistance levels between clinical and environmental isolates. The antimicrobial susceptibility of these bacteria is determined using Etest and disc diffusion methods. Treating *Ralstonia spp* sepsis is complex and challenging due to its resistance to commonly used antibiotics like β-lactams and aminoglycosides, and susceptibility only to tetracycline and tigecycline. There is a lack of clear guidelines and limited trustworthy therapeutic information available due to the infrequent presence of *R. picketti* in human samples(35). Ryan and colleagues found that many strains of *R. picketti* and R. insidiosa exhibit significant resistance to the antibiotics aminoglycoside (gentamicin) and ß-lactam (aztreonam). as well as different levels of resistance to ticarcillin-clavulanic acid during their investigation. Studies have shown that the most effective antibiotics are sulfamethoxazole/trimethoprim and ciprofloxacin, a type of fluoroquinolone(36). *R. mannitolilytica* shows the ability to resist commonly used antibiotics like carbapenems. In Khajuria's study, the initial detection of the VIM-2 gene indicated the first instance. In the midst of the COVID-19 outbreak (37). Amani et al, were the first to discover the presence of the OXA-48 gene in human individuals with *Ralstonia spp* bloodstream infections attributed to drug-resistant Ralstonia strains (38). *Ralstonia spp* has developed resistance to a variety of antibiotics, including extended-generation cephalosporins, the majority of aminoglycosides, fluoroquinolones, and carbapenems. (5).

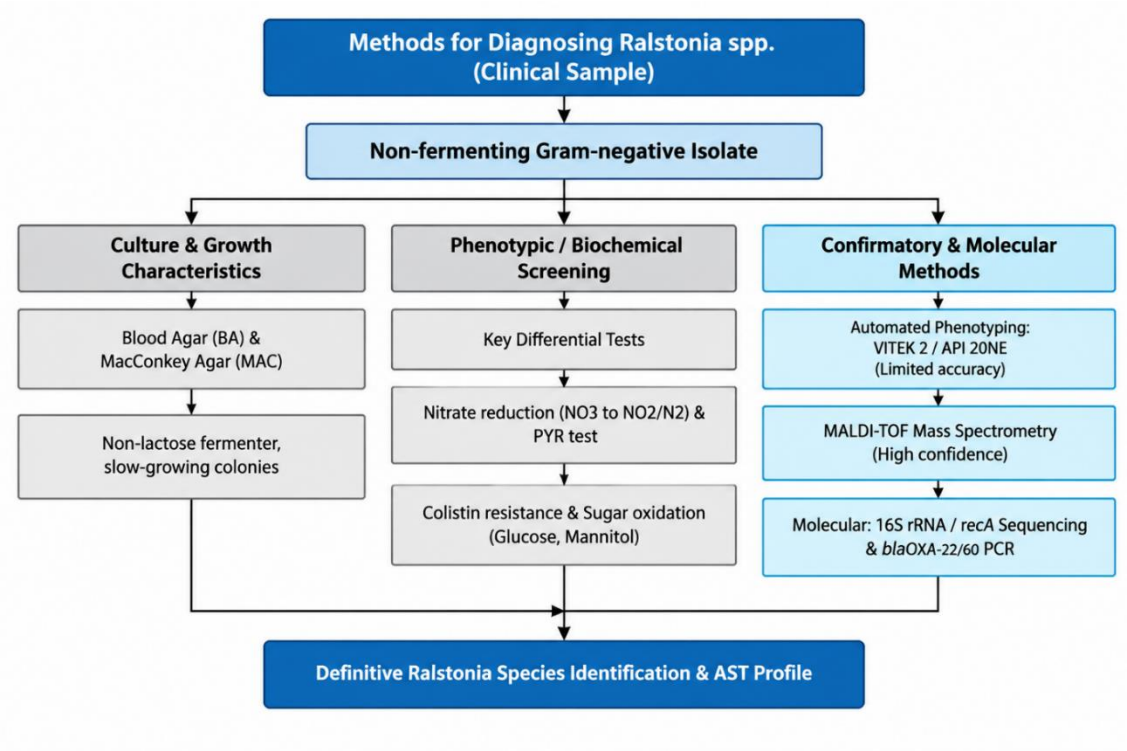


Figure 2. Methods for diagnosing *Ralstonia spp*.

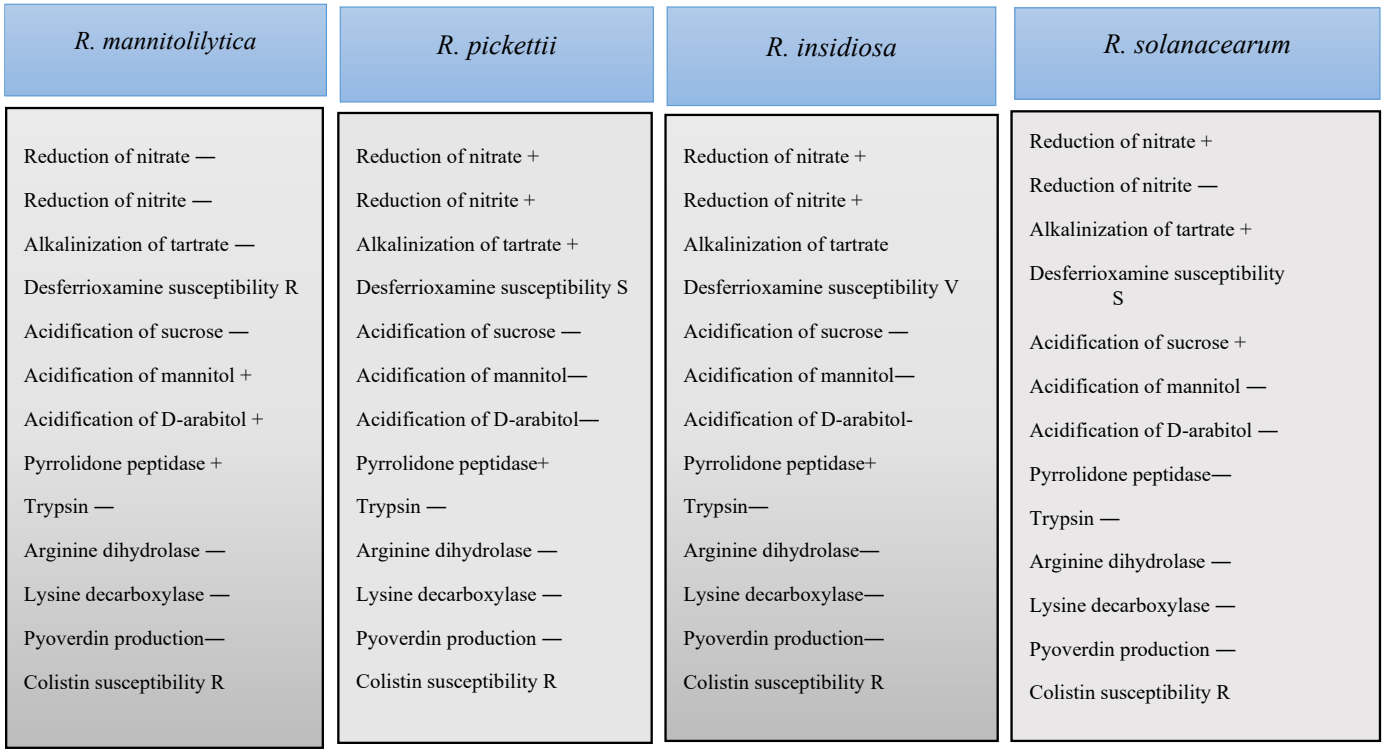


Figure 3. Biochemical characteristics of isolates of *Ralstonia spp* (34). R, resistant; S, susceptible, and V, variable, negative(-), Positive(+).

Ralstonia antibiotic resistance

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Table 1 . Antimicrobial susceptibility of *Ralstonia spp*

Antimicrobial agent or class	Reported pattern in the manuscript	Reference
Trimethoprim–sulfamethoxazole (TMP–SMX)	Reported among the most effective agents	(33)
Ciprofloxacin	Reported among the most effective agents	(33)
Tetracycline	Susceptibility reported	(33)
Tigecycline	Susceptibility reported	(33)
β-lactams (general)	Resistance reported	(33)
Gentamicin (aminoglycoside)	Resistance reported	(33)
Aztreonam	Resistance reported	(33)
Ticarcillin–clavulanic acid	Variable resistance reported	(33)
Carbapenems	Resistance reported in *R. mannitolilytica*; acquired carbapenemase genes are discussed	(5)(34)
Extended-generation cephalosporins	Resistance reported in the manuscript's broad summary	(5)
Other fluoroquinolones	Variable resistance reported in the manuscript's broad summary	(5)

Reported cases of the role of *Ralstonia spp* in clinical infections of patients with hematological malignancies

The clinical profile of *Ralstonia spp*. infections in hematology-oncology patients is characterized by a complex interplay between host immunocompromise and specific iatrogenic risk factors. Our synthesis of the identified cases reveals a consistent clinical hallmark across diverse patient demographics: the presentation of febrile neutropenia, which often mimics severe sepsis.(36). A pivotal observation across the reviewed studies is the central role of Central Venous Catheters (CVCs) as the primary portal of entry. The data suggests that these devices serve as the critical nexus for R. pickettii and R. mannitolilytica transmission, primarily facilitated by biofilm formation on catheter surfaces, including Hickman and Porta-Cath systems. The correlation between the duration of CVC maintenance and the onset of Ralstonia bacteremia appears statistically significant across multiple outbreaks (e.g., Brazil study; Siddiqui et al.). This finding underscores that the infection is rarely a primary host-driven event, but rather an iatrogenic complication exacerbated by the interplay of long-term vascular access and suppressed host defenses (37). The literature indicates a notable dichotomy in clinical presentation based on the underlying hematological pathology. In pediatric cohorts, particularly patients with acute lymphoblastic leukemia (ALL), Ralstonia infections often manifest as acute, high-grade bacteremia necessitating immediate intervention (Demirdag et al.; Wautrs et al.). In contrast, among adult patients specifically those with chronic lymphoproliferative disorders or stem cell transplant recipients the infection course frequently exhibits a more insidious or sub-acute pattern, often identified during routine surveillance rather than through overt clinical deterioration (Mikulska et al.; Brett D et al.). Despite these variations in age and underlying pathology, febrile neutropenia remains the unifying clinical finding across all subgroups (38-41) (42). In a study conducted in Korea from 2009 to 2023, out of 18 Ralstonia cases, three Ralstonia cases were found in the blood unit of children, and 10 of these 18 cases were patients who underwent hematopoietic stem cell transplantation and were hospitalized in the special unit. Reports on the worldwide prevalence of *Ralstonia spp* are shown in Table 2 (43). Siddiqui and colleagues conducted a study that identified a total of 14 cases of R. mannitolilytica at our hospital over a period of two years.All patients exhibited symptoms of fever and/or chills, and tested positive for R. mannitolilytica in their blood cultures. Eight out of the 14 infected individuals had blood cancers, while the others were diagnosed with different conditions such as breast cancer, , acute respiratory distress syndrome, necrotizing pancreatitis, dengue hemorrhagic shock syndrome, decompensated chronic liver disease and severe pneumonia caused by COVID-19 (44).

Table 2. Ralstonia isolates from patients with hematologic malignancies worldwide.

No. of patients	*Ralstonia* species	Primary diagnosis reported in the manuscript	Resistance / susceptibility reported in the manuscript	Country	/ Year	Reference
8	*Ralstonia pickettii*	Acute lymphoblastic leukemia (ALL); Hodgkin lymphoma; juvenile myelomonocytic leukemia (JMML)	Amikacin, gentamicin, piperacillin–tazobactam, netilmicin, colistin, levofloxacin and ceftazidime.	Türkiye	2024	Sreedhar Jayakrishnan Cherulil (45)
7	*Ralstonia mannitolilytica*	ALL; lymphoblastic lymphoma; refractory acute myeloid leukemia (AML); primary mediastinal lymphoma; primary central nervous system lymphoma; AML; Hodgkin lymphoma		India	2021	K,Ramani et al (46)
1	*Ralstonia Mannitolilytica*	Non-Hodgkin lymphoma (NHL)	amikacin, Ceftazidime	India	2022	Chauhan et al (41)
8	*R. mannitolilytica*	Acute lymphoblastic leukemia; AML; Hodgkin lymphoma		India	2022	Siddiqui T et al (44)
9	*Ralstonia pickettii*	Hodgkin lymphoma; non-Hodgkin lymphoma; myelodysplastic syndrome; acute lymphoid leukemia; acute myeloid leukemia; chronic leukemia		Brazil	2008	C. Pellegrino et al (47)
1	*Ralstonia mannitolilytica*	ALL; AML; acute undifferentiated leukemia (AUL) hemophagocytic lymphohistiocytosis (HLH); primary myelofibrosis (PMF)		China	2023	Zhang et al (48)
1	*Ralstonia mannitolilytica*	Non-Hodgkin lymphoma		India	2018	Chitre et al
1	*Ralstonia mannitolilytica*	Gastric T-cell lymphoma		China	2016	Xia Liu et al (36)

Conclusion

Ralstonia species, particularly *R. pickettii* and *R. mannitolilytica*, have emerged as formidable opportunistic pathogens capable of causing life-threatening healthcare-associated infections, particularly among immunocompromised patients, such as those with haematological malignancies. The high mortality rates associated with *Ralstonia*-related bloodstream infections underscore the gravity of these infections in hospital settings. A fundamental challenge in clinical practice remains the misidentification of

Ralstonia spp. by automated commercial systems, which often leads to inaccurate clinical management and delayed appropriate therapy.

Therefore, it is imperative to shift towards molecular-based diagnostic standards such as 16S rRNA gene sequencing or MALDI-TOF mass spectrometry—for definitive identification, especially in high-risk units. Effective control of *Ralstonia* requires a multifaceted strategy: rigorous environmental monitoring and maintenance of hospital water and plumbing systems to eliminate reservoirs, coupled with strict adherence to infection control protocols to prevent cross-contamination. Ultimately, improving patient outcomes demands a multidisciplinary approach, integrating rapid, accurate molecular diagnostics with prompt, targeted antimicrobial stewardship. Given the increasing incidence of multidrug-resistant strains, ongoing vigilance and collaborative efforts between microbiology laboratories and clinical teams are essential to mitigate the impact of this stealthy pathogen.

Author contribution

MR conceived and designed the study, coordinated the research, and wrote the main manuscript text. **EM**, **AG**, and **MK** performed the literature search, data extraction, and data analysis. **NA**, **MB**, and **MY** helped in drafting, editing, and revising the manuscript. All authors reviewed and approved the final manuscript.

Conflicts of interest

There are no conflicts of interest.

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