

Original Research Article

PHENOTYPIC CHARACTERISATION OF CLINICAL RALSTONIA STRAINS AND THEIR ANTIBIOTIC-RESISTANT PROFILE IN TERTIARY CARE HOSPITAL

S Aishwarya¹, Bala Murugan Govindhan², Bethsheba Fairy Rachel D³

¹Assistant professor, Department of Microbiology, Government Medical College and Hospital, Kallakurichi, Tamil Nadu, India.

²Senior Resident, Department of Microbiology, National Institute of Mental Health and Neurosciences, Bengaluru, India.

³Senior Resident, Department of Obstetrics and Gynaecology, Government Medical College, the Nilgiris, Tamil Nadu, India.

Received : 23/08/2026
Received in revised form : 20/09/2026
Accepted : 03/10/2026

Corresponding Author:

Dr. S Aishwarya,
Assistant professor, Department of
Microbiology, Government Medical
College and Hospital, Kallakurichi,
Tamil Nadu, India.
Email: aishusuri@gmail.com

DOI: 10.70034/ijmedph.2026.4.51

Source of Support: Nil,
Conflict of Interest: None declared

Int J Med Pub Health
2026; 16 (4); 339-348

ABSTRACT

Background: *Ralstonia* species are aerobic, non-fermenting, Gram-negative bacilli widely distributed in environmental sources, including water, soil and plants. Although traditionally regarded as organisms of low pathogenicity, *Ralstonia pickettii*, *Ralstonia mannitolilytica* and *Ralstonia insidiosa* are increasingly recognized as opportunistic pathogens associated with healthcare-associated infections, particularly among immunocompromised patients and those with indwelling medical devices. Their phenotypic similarity to other non-fermenting Gram-negative bacilli presents diagnostic challenges, while their antimicrobial susceptibility patterns remain incompletely characterized. **Aim:** To identify and characterize clinical *Ralstonia* species using conventional phenotypic methods and determine their antimicrobial susceptibility profiles in a tertiary care hospital.

Materials and Methods: A prospective observational study was conducted in the Department of Microbiology, Coimbatore Medical College and Hospital, Tamil Nadu, India, from March 2023 to February 2024. A total of 100 non-lactose-fermenting, oxidase-positive clinical isolates were included. The isolates were subjected to conventional culture, Gram staining, motility testing, catalase and oxidase testing, and a series of biochemical tests for identification and speciation. Antimicrobial susceptibility testing was performed using the modified Kirby–Bauer disk diffusion method on Mueller–Hinton agar. The results were interpreted using the criteria described in the dissertation, with the limitations of applying non-species-specific breakpoints to *Ralstonia* taken into consideration.

Results: Among the 100 isolates, *Pseudomonas* species accounted for 93 (93%), *R. mannitolilytica* for 4 (4%) and *R. pickettii* for 3 (3%). No *R. insidiosa* was identified. All seven *Ralstonia* isolates were obtained from male patients, and six were associated with the nephrology department. Blood was the predominant specimen type among the *Ralstonia* isolates, accounting for six of the seven isolates, while one *R. mannitolilytica* isolate was recovered from urine. All *Ralstonia* isolates demonstrated resistance to colistin and polymyxin B. They were reported as susceptible to gentamicin, amikacin, ciprofloxacin, ofloxacin, levofloxacin and aztreonam. Resistance to ceftazidime and cefepime was observed in all seven isolates. Variable resistance was observed to cefoperazone–sulbactam, piperacillin–tazobactam, imipenem and meropenem. Among *Pseudomonas* isolates, resistance was highest to cotrimoxazole (100%), followed by ceftazidime (68.8%) and ciprofloxacin (65.6%).

Conclusion: *Ralstonia* species constituted a small but clinically relevant proportion of the non-lactose-fermenting, oxidase-positive isolates investigated. Their recovery predominantly from blood specimens and their concentration in the nephrology department highlight the importance of accurate identification

and careful clinical correlation. The observed antimicrobial susceptibility patterns support continued laboratory surveillance, while the small number of *Ralstonia* isolates and the limitations of phenotypic identification and susceptibility interpretation warrant cautious application of these findings.

Keywords: *Ralstonia pickettii*, *Ralstonia mannitolilytica*, Non-Fermenting Gram-Negative Bacilli, Phenotypic Characterization, Antimicrobial Susceptibility.

INTRODUCTION

Non-fermenting Gram-negative bacilli are important organisms encountered in clinical microbiology laboratories. Their ability to survive in diverse environmental conditions, persist in healthcare-associated environments and exhibit variable antimicrobial susceptibility makes their identification and clinical interpretation challenging. Among these organisms, the genus *Ralstonia* has gained increasing attention because of its association with opportunistic infections and healthcare-associated transmission.^[1] *Ralstonia* species are aerobic, non-fermenting, Gram-negative bacilli commonly found in natural and artificial environments, including water, soil, plants and water distribution systems. Several species have been described within the genus, including *Ralstonia pickettii*, *Ralstonia mannitolilytica*, *Ralstonia insidiosa*, *Ralstonia solanacearum*, *Ralstonia pseudosolanacearum* and *Ralstonia syzygii*. Among these, *R. pickettii*, *R. mannitolilytica* and *R. insidiosa* have been associated with human infections, whereas several other members are primarily recognized as plant pathogens.^[2] Historically, *R. pickettii* was considered the principal clinically significant member of the genus. However, reports of infections caused by *R. mannitolilytica* and *R. insidiosa* have expanded the clinical importance of these organisms. They have been associated with bloodstream infections, pneumonia, meningitis, endocarditis, osteomyelitis and infections related to indwelling medical devices. These infections are frequently described in patients with underlying malignancies, immunosuppression, chronic kidney disease, respiratory disorders and prolonged hospital stays.^[3] One of the important epidemiological characteristics of *Ralstonia* species is their environmental persistence. They have been recovered from contaminated intravenous fluids, sterile pharmaceutical preparations, disinfectant solutions and medical equipment. Their ability to survive in aqueous environments and, in some circumstances, pass through sterilizing membrane filters has been implicated in healthcare-associated outbreaks. Contaminated water used in pharmaceutical manufacturing or healthcare procedures may also act as a potential source of transmission.^[4,5] In addition to environmental persistence, the ability of some *Ralstonia* strains to form biofilms may contribute to their survival on medical devices and other surfaces. Biofilm formation can promote persistence under unfavorable environmental conditions and may reduce the effectiveness of antimicrobial agents.

However, the virulence characteristics of these organisms remain incompletely understood, and their isolation from a clinical specimen does not necessarily establish an active infection.^[1,6] The laboratory identification of *Ralstonia* species presents considerable challenges because of their phenotypic resemblance to other non-fermenting Gram-negative bacilli, particularly *Pseudomonas* species and the *Burkholderia cepacia* complex.^[7] Conventional biochemical identification may produce overlapping or variable reactions, and commercial identification systems may not reliably differentiate all clinically relevant *Ralstonia* species. Tests involving nitrate reduction, urease activity, carbohydrate oxidation and growth characteristics can support differentiation, but results may vary among strains.^[8] Antimicrobial susceptibility testing of *Ralstonia* species is also difficult. Unlike several commonly encountered Gram-negative pathogens, species-specific clinical breakpoints are not well established for these organisms in standard susceptibility testing guidelines.^[9] Published reports have described variable susceptibility to fluoroquinolones, aminoglycosides, cephalosporins, carbapenems and trimethoprim-sulfamethoxazole. Intrinsic resistance to polymyxins is also an important characteristic of the genus.^[10] The increasing recognition of *Ralstonia* as an opportunistic pathogen emphasizes the need for improved laboratory identification, antimicrobial susceptibility surveillance and appropriate infection prevention practices. Data from individual healthcare facilities can help describe local isolation patterns and identify potential areas for further investigation.^[11] The present study was undertaken to characterize non-lactose-fermenting, oxidase-positive clinical isolates in a tertiary care hospital, identify *Ralstonia* species using conventional phenotypic methods and assess their antimicrobial susceptibility profiles. The study also examined the distribution of the identified organisms according to age, gender, clinical department, patient location and specimen type.

MATERIALS AND METHODS

Study Design and Study Setting

A prospective observational study was conducted in the Department of Microbiology, Coimbatore Medical College and Hospital, Coimbatore, Tamil Nadu, India, over a period of 12 months, from March 2023 to February 2024. The study was designed to identify and characterize clinical isolates of non-

lactose-fermenting, oxidase-positive Gram-negative bacilli, with particular emphasis on the phenotypic identification and antimicrobial susceptibility profiles of *Ralstonia* species.

Study Population and Sample Size

A total of 100 non-lactose-fermenting, oxidase-positive clinical isolates obtained from patients attending various clinical departments of the hospital were included in the study. Patients of all age groups were considered. The isolates were collected from different clinical specimens, including blood, pus and urine, as reported in the study results.

Inclusion and Exclusion Criteria

Inclusion Criteria

- Non-lactose-fermenting, oxidase-positive Gram-negative bacterial isolates obtained from clinical specimens.
- Clinical isolates from patients of all age groups.

Exclusion Criteria

- Lactose-fermenting bacterial isolates.
- Non-lactose-fermenting, oxidase-negative isolates.
- Patients already receiving antibiotic therapy, as specified in the study protocol.
- Repeated isolates from the same patient.

Specimen Collection and Processing

Clinical specimens were collected under aseptic precautions and transported to the Department of Microbiology for microbiological examination. Specimens were processed according to standard microbiological procedures. Except for blood specimens, the clinical samples were inoculated onto nutrient agar, blood agar and MacConkey agar and incubated aerobically at 37°C for 18–24 hours. Blood and catheter-tip specimens were inoculated into brain–heart infusion broth and incubated at 37°C for 48 hours. The broths were examined periodically for evidence of bacterial growth. Broths showing turbidity were subcultured onto nutrient agar, blood agar and MacConkey agar for further identification. Sputum specimens were evaluated using Bartlett's scoring system to assess specimen quality before processing. Endotracheal aspirates containing more than 10 epithelial cells per low-power field were rejected. For endotracheal aspirates, a bacterial count greater than 10⁵ CFU/mL was considered significant, whereas a count greater than 10⁴ CFU/mL was considered significant for bronchoalveolar lavage specimens, according to the study protocol. Urine specimens with colony counts greater than 10⁵ CFU/mL were considered significant for further processing. For catheterized patients, a colony count greater than 10³ CFU/mL was accepted, as specified in the study protocol.

Preliminary Identification of Bacterial Isolates

Bacterial isolates were initially identified based on colony morphology, cultural characteristics, Gram staining and biochemical reactions. Suspected non-fermenting Gram-negative bacilli were selected for further identification based on their growth characteristics and lactose fermentation on

MacConkey agar. Gram staining was performed using the Hucker modification to determine bacterial morphology and Gram reaction. The isolates were further subjected to motility testing by the hanging-drop method, catalase testing using 3% hydrogen peroxide and oxidase testing using tetramethyl-p-phenylenediamine dihydrochloride reagent. Oxidase-positive isolates showing characteristics consistent with non-fermenting Gram-negative bacilli were selected for additional biochemical characterization.

Phenotypic Characterization and Biochemical Identification

The selected isolates were subjected to a series of biochemical tests to differentiate *Ralstonia* species from other non-fermenting Gram-negative bacilli, particularly *Pseudomonas* species and the *Burkholderia cepacia* complex. The biochemical tests included indole production, Simmons citrate utilization, urease hydrolysis, triple sugar iron agar reaction, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, nitrate reduction, oxidation–fermentation testing, ONPG, leucine aminopeptidase, DNase, esculin hydrolysis and flagellar staining. The biochemical reactions were interpreted collectively to establish the presumptive identity of the isolates. Suspected *Ralstonia* species were further differentiated using their biochemical characteristics, including nitrate reduction, carbohydrate oxidation and growth at 42°C. *Ralstonia mannitolilytica* was differentiated primarily by its mannitol oxidation characteristics and growth pattern, while *Ralstonia pickettii* and *Ralstonia insidiosa* were assessed using their respective phenotypic and biochemical reaction profiles. Species identification was based on conventional phenotypic methods, without molecular confirmation.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed using the modified Kirby–Bauer disk diffusion method on Mueller–Hinton agar [12]. A few well-isolated colonies from a pure culture were inoculated into peptone water to prepare a bacterial suspension. The turbidity of the suspension was adjusted to match a 0.5 McFarland standard, corresponding approximately to 1.5 × 10⁸ CFU/mL. The standardized inoculum was uniformly swabbed onto the surface of Mueller–Hinton agar to obtain a confluent lawn culture. Antibiotic-impregnated disks were placed on the inoculated agar surface using sterile forceps, maintaining appropriate spacing between the disks. The plates were incubated aerobically at 37°C for 18–24 hours. Following incubation, the diameters of the zones of inhibition were measured in millimetres using a Himedia measuring scale. The antimicrobial susceptibility results were recorded according to the interpretative criteria adopted in the study. The antimicrobial agents tested included cotrimoxazole, gentamicin, amikacin, ciprofloxacin, ofloxacin, levofloxacin, ceftazidime, cefepime, aztreonam, cefoperazone–sulbactam, piperacillin–tazobactam, imipenem, meropenem, colistin and polymyxin B.

Interpretation of Antimicrobial Susceptibility Results

The antimicrobial susceptibility results for *Pseudomonas* isolates were interpreted using the Clinical and Laboratory Standards Institute (CLSI) M100 guidelines, 34th edition, as described in the study protocol, using the criteria applicable to *Pseudomonas aeruginosa*.^[12] As species-specific clinical breakpoints for *Ralstonia* species were not available in the interpretative criteria used in the study, the results were assessed using the alternative interpretative criteria adopted in the dissertation for other Gram-negative organisms. The susceptibility findings for *Ralstonia* were therefore considered descriptive laboratory observations and were not treated as validated clinical predictions of antimicrobial efficacy.

Data Collection and Statistical Analysis

Demographic and microbiological data were recorded, including patient age, gender, clinical department, hospital location, specimen type, bacterial identification and antimicrobial susceptibility patterns. The collected data were entered into a structured database and analyzed using descriptive statistics. Categorical variables were expressed as frequencies and percentages. The distribution of *Ralstonia* species and *Pseudomonas* isolates was summarized according to demographic characteristics, clinical departments, hospital locations, specimen types and antimicrobial susceptibility profiles.

Ethical Considerations

Institutional Human Ethics Committee approval was obtained before the commencement of the study, as documented in the dissertation. The study was conducted in accordance with the institution's ethical requirements. The ethics committee approval number and details of informed consent or waiver of consent should be included in the final manuscript according to the approved study protocol.

RESULTS

A total of 100 non-lactose-fermenting, oxidase-positive Gram-negative bacterial isolates obtained from clinical specimens were included in the study conducted at Coimbatore Medical College and Hospital, Tamil Nadu, India, from March 2023 to February 2024. The isolates were characterized using conventional phenotypic and biochemical methods and evaluated for their antimicrobial susceptibility profiles. The distribution of the isolates was analyzed according to bacterial species, age, gender, clinical department, hospital location and specimen type.

Distribution of Bacterial Isolates

Among the 100 clinical isolates investigated, *Pseudomonas* species were the predominant

organisms, accounting for 93 (93.0%) isolates. *Ralstonia mannitolilytica* was identified in four (4.0%) isolates, while *Ralstonia pickettii* was identified in three (3.0%) isolates. No isolates of *Ralstonia insidiosa* were detected during the study period. As shown in Figure 1, *Pseudomonas* species constituted the majority of the isolates, while *Ralstonia* species collectively accounted for seven (7.0%) isolates. Among the *Ralstonia* species identified, *R. mannitolilytica* was more frequently isolated than *R. pickettii*. The absence of *R. insidiosa* indicates that this species was not detected among the isolates investigated during the study period.

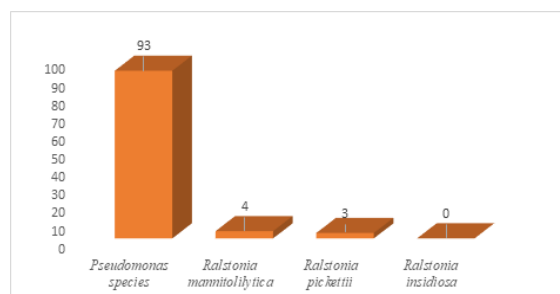


Figure 1: Distribution of bacterial isolates

Age-wise Distribution of Bacterial Isolates

The age-wise distribution of the 100 clinical isolates is presented in Table 1. The isolates were distributed across four age groups: below 20 years, 21–40 years, 41–60 years and above 60 years. The highest proportion of isolates was observed in the 41–60-year age group, which accounted for 33 (33.0%) isolates, followed by patients aged above 60 years, with 29 (29.0%) isolates. Patients aged 21–40 years contributed 25 (25.0%) isolates, while 13 (13.0%) isolates were obtained from patients below 20 years of age. Among the 93 *Pseudomonas* isolates, the highest number, 31 (33.3%), was observed in the 41–60-year age group, followed by 27 (29.0%) in patients above 60 years, 22 (23.7%) in the 21–40-year age group and 13 (14.0%) in patients below 20 years. Of the four *R. mannitolilytica* isolates, two (50.0%) were recovered from patients aged 21–40 years, while one (25.0%) each was identified in the 41–60-year and above-60-year age groups. All three *R. pickettii* isolates were distributed equally across the 21–40-year, 41–60-year and above-60-year age groups, with one (33.3%) isolate in each category. No *Ralstonia* species were identified among patients below 20 years of age. As detailed in Table 1, the largest proportion of the overall study population belonged to the 41–60-year age group. The *Ralstonia* isolates were identified exclusively among patients aged 21 years and above. Both *R. mannitolilytica* and *R. pickettii* were distributed across multiple adult age groups, without any single age group accounting for all isolates of either species.

Table 1: Age-wise distribution of bacterial isolates

Age group (years)	<i>Pseudomonas</i> species n (%)	<i>R. mannitolilytica</i> n (%)	<i>R. pickettii</i> n (%)	Total n (%)
Below 20	13 (14.0)	0 (0.0)	0 (0.0)	13 (13.0)
21–40	22 (23.7)	2 (50.0)	1 (33.3)	25 (25.0)
41–60	31 (33.3)	1 (25.0)	1 (33.3)	33 (33.0)
Above 60	27 (29.0)	1 (25.0)	1 (33.3)	29 (29.0)
Total	93 (100)	4 (100)	3 (100)	100 (100)

Gender-wise Distribution of Bacterial Isolates

The gender-wise distribution of the bacterial isolates is shown in Table 2. Of the 100 isolates included in the study, 63 (63.0%) were obtained from male patients and 37 (37.0%) from female patients. Among the 93 *Pseudomonas* isolates, 56 (60.2%) were recovered from male patients and 37 (39.8%) from female patients. All four *R. mannitolilytica* isolates (100%) and all three *R. pickettii* isolates (100%) were identified among male patients. No *Ralstonia* isolates

were recovered from female patients. As presented in Table 2, male patients accounted for a greater proportion of the total isolates. All seven *Ralstonia* isolates were recovered from male patients, whereas *Pseudomonas* species were identified in both male and female patients. This represents the observed gender distribution in the study population and does not establish a gender-associated risk of *Ralstonia* infection, given the small number of isolates.

Table 2: Gender-wise distribution of bacterial isolates

Gender	<i>Pseudomonas</i> species n (%)	<i>R. mannitolilytica</i> n (%)	<i>R. pickettii</i> n (%)	Total n (%)
Female	37 (39.8)	0 (0.0)	0 (0.0)	37 (37.0)
Male	56 (60.2)	4 (100)	3 (100)	63 (63.0)
Total	93 (100)	4 (100)	3 (100)	100 (100)

Department-wise Distribution of Bacterial Isolates

The distribution of bacterial isolates according to clinical department is presented in Table 3. The isolates were obtained from ten clinical departments, with Medicine contributing the highest number of isolates, 27 (27.0%), followed by Nephrology with 25 (25.0%) and Surgery with 14 (14.0%). Among the 93 *Pseudomonas* isolates, 26 (28.0%) were recovered from Medicine, 19 (20.4%) from Nephrology, 14 (15.1%) from Surgery and nine (9.7%) from Urology. The remaining isolates were distributed across Obstetrics and Gynaecology (OBG), Orthopaedics, Paediatrics, the Paediatric Intensive Care Unit (PICU), Pulmonology and ENT. All four *R. mannitolilytica* isolates were recovered from the

Nephrology department, accounting for 100% of the isolates of this species. Of the three *R. pickettii* isolates, two (66.7%) were obtained from Nephrology and one (33.3%) from Medicine. No *Ralstonia* isolates were detected in ENT, OBG, Orthopaedics, Paediatrics, PICU, Pulmonology, Surgery or Urology. As shown in Table 3, Nephrology accounted for all four *R. mannitolilytica* isolates and two of the three *R. pickettii* isolates. Thus, six of the seven *Ralstonia* isolates originated from this department, while the remaining isolate was identified in Medicine. This distribution highlights the observed departmental concentration of *Ralstonia* isolates; however, the study did not establish any specific clinical exposure or common source associated with these cases.

Table 3: Department-wise distribution of bacterial isolates

Clinical department	<i>Pseudomonas</i> species n (%)	<i>R. mannitolilytica</i> n (%)	<i>R. pickettii</i> n (%)	Total n (%)
ENT	3 (3.2)	0 (0.0)	0 (0.0)	3 (3.0)
Medicine	26 (28.0)	0 (0.0)	1 (33.3)	27 (27.0)
Nephrology	19 (20.4)	4 (100)	2 (66.7)	25 (25.0)
Obstetrics and Gynaecology	8 (8.6)	0 (0.0)	0 (0.0)	8 (8.0)
Orthopaedics	5 (5.4)	0 (0.0)	0 (0.0)	5 (5.0)
Paediatrics	4 (4.3)	0 (0.0)	0 (0.0)	4 (4.0)
PICU	3 (3.2)	0 (0.0)	0 (0.0)	3 (3.0)
Pulmonology	2 (2.2)	0 (0.0)	0 (0.0)	2 (2.0)
Surgery	14 (15.1)	0 (0.0)	0 (0.0)	14 (14.0)
Urology	9 (9.7)	0 (0.0)	0 (0.0)	9 (9.0)
Total	93 (100)	4 (100)	3 (100)	100 (100)

Distribution of Bacterial Isolates According to Hospital Location

The distribution of the isolates according to hospital location is shown in Table 4. Of the 100 isolates, 87 (87.0%) were obtained from inpatients, 12 (12.0%)

from patients in the intensive care unit (ICU) and one (1.0%) from an outpatient. Among the 93 *Pseudomonas* isolates, 81 (87.1%) were recovered from inpatients, 11 (11.8%) from ICU patients and one (1.1%) from an outpatient. All four *R.*

mannitolilytica isolates were obtained from inpatients, while two (66.7%) of the three *R. pickettii* isolates were obtained from inpatients and one (33.3%) from an ICU patient. As indicated in Table 4, the majority of the isolates were recovered from inpatients. All *R. mannitolilytica* isolates and most *R.*

pickettii isolates were obtained from this group. One *R. pickettii* isolate was recovered from an ICU patient. The predominance of inpatient isolates describes the hospital location distribution but does not, in itself, establish healthcare-associated acquisition or nosocomial transmission.

Table 4: Distribution of bacterial isolates according to hospital location

Hospital location	<i>Pseudomonas</i> species n (%)	<i>R. mannitolilytica</i> n (%)	<i>R. pickettii</i> n (%)	Total n (%)
ICU	11 (11.8)	0 (0.0)	1 (33.3)	12 (12.0)
Inpatient	81 (87.1)	4 (100)	2 (66.7)	87 (87.0)
Outpatient	1 (1.1)	0 (0.0)	0 (0.0)	1 (1.0)
Total	93 (100)	4 (100)	3 (100)	100 (100)

Distribution of Bacterial Isolates According to Specimen Type

The specimen-wise distribution of the bacterial isolates is summarized in Table 5. The clinical specimens represented in the final results were blood, pus and urine. Blood accounted for 40 (40.0%) isolates, followed by pus with 38 (38.0%) isolates and urine with 22 (22.0%) isolates. Among the 93 *Pseudomonas* isolates, 38 (40.9%) were recovered from pus, 34 (36.6%) from blood and 21 (22.6%) from urine. Among the four *R. mannitolilytica* isolates, three (75.0%) were obtained from blood and one (25.0%) from urine. All three *R. pickettii* isolates

(100%) were recovered from blood. No *Ralstonia* isolates were identified in pus specimens. As shown in Table 5, blood was the predominant specimen source among the seven *Ralstonia* isolates, accounting for six (85.7%) isolates. The remaining isolate, identified as *R. mannitolilytica*, was recovered from urine. All three *R. pickettii* isolates were recovered from blood specimens. These findings describe the specimen-wise distribution of the isolates and require clinical correlation to determine their significance as infections, colonization or possible contamination

Table 5: Distribution of bacterial isolates according to specimen type

Specimen type	<i>Pseudomonas</i> species n (%)	<i>R. mannitolilytica</i> n (%)	<i>R. pickettii</i> n (%)	Total n (%)
Blood	34 (36.6)	3 (75.0)	3 (100)	40 (40.0)
Pus	38 (40.9)	0 (0.0)	0 (0.0)	38 (38.0)
Urine	21 (22.6)	1 (25.0)	0 (0.0)	22 (22.0)
Total	93 (100)	4 (100)	3 (100)	100 (100)

Antimicrobial Susceptibility Profile of Bacterial Isolates

The antimicrobial susceptibility profiles of the 100 clinical isolates, comprising 93 *Pseudomonas* species, four *R. mannitolilytica* and three *R. pickettii*, were evaluated against 15 antimicrobial agents. The results are presented in Table 6, with the number and percentage of resistant and sensitive isolates reported for each organism. Among the 93 *Pseudomonas* isolates, the highest reported resistance was observed against cotrimoxazole (93/93; 100%), which was designated as intrinsic resistance in the dissertation. Among the remaining antimicrobial agents, resistance was highest to ceftazidime (64/93; 68.8%), followed by ciprofloxacin (61/93; 65.6%), cefepime (53/93; 57.0%) and gentamicin (47/93; 50.5%). Resistance to ofloxacin was observed in 40 (43.0%) isolates, while 38 (40.9%) isolates were resistant to amikacin. Levofloxacin resistance was identified in 27 (29.0%) isolates. Resistance to cefoperazone–sulbactam was observed in 26 (28.0%) *Pseudomonas* isolates, while 15 (16.1%) isolates were resistant to piperacillin–tazobactam. Both imipenem and

meropenem demonstrated resistance in 28 (30.1%) isolates. Aztreonam showed the lowest reported resistance, with one (1.1%) isolate resistant and 92 (98.9%) isolates sensitive. All *Pseudomonas* isolates were reported as sensitive to colistin and polymyxin B. Among the four *R. mannitolilytica* isolates, all four (100%) were reported as sensitive to cotrimoxazole, gentamicin, amikacin, ciprofloxacin, ofloxacin, levofloxacin and aztreonam. All four isolates were resistant to ceftazidime, cefepime, colistin and polymyxin B. Resistance to cefoperazone–sulbactam was observed in two (50.0%) isolates, while one (25.0%) isolate each was resistant to piperacillin–tazobactam, imipenem and meropenem. Similarly, all three *R. pickettii* isolates were reported as sensitive to cotrimoxazole, gentamicin, amikacin, ciprofloxacin, ofloxacin, levofloxacin and aztreonam. All three isolates were resistant to ceftazidime, cefepime, colistin and polymyxin B. Two (66.7%) isolates were resistant to cefoperazone–sulbactam, piperacillin–tazobactam, imipenem and meropenem, while one (33.3%) isolate was sensitive to each of these agents (Table 6).

Table 6: Antimicrobial susceptibility profile of *Pseudomonas* species and *Ralstonia* species

Antimicrobial agent	Susceptibility	<i>Pseudomonas</i> (n = 93) n (%)	<i>R. mannitolilytica</i> (n = 4) n (%)	<i>R. pickettii</i> (n = 3) n (%)	Total (N = 100) n (%)
---------------------	----------------	-----------------------------------	---	-----------------------------------	-----------------------

Cotrimoxazole	Intrinsic resistance	93 (100)	0 (0)	0 (0)	93 (93)
	Sensitive	0 (0)	4 (100)	3 (100)	7 (7)
Gentamicin	Resistant	47 (50.5)	0 (0)	0 (0)	47 (47)
	Sensitive	46 (49.5)	4 (100)	3 (100)	53 (53)
Amikacin	Resistant	38 (40.9)	0 (0)	0 (0)	38 (38)
	Sensitive	55 (59.1)	4 (100)	3 (100)	62 (62)
Ciprofloxacin	Resistant	61 (65.6)	0 (0)	0 (0)	61 (61)
	Sensitive	32 (34.4)	4 (100)	3 (100)	39 (39)
Ofloxacin	Resistant	40 (43.0)	0 (0)	0 (0)	40 (40)
	Sensitive	53 (57.0)	4 (100)	3 (100)	60 (60)
Levofloxacin	Resistant	27 (29.0)	0 (0)	0 (0)	27 (27)
	Sensitive	66 (71.0)	4 (100)	3 (100)	73 (73)
Ceftazidime	Resistant	64 (68.8)	4 (100)	3 (100)	71 (71)
	Sensitive	29 (31.2)	0 (0)	0 (0)	29 (29)
Cefepime	Resistant	53 (57.0)	4 (100)	3 (100)	60 (60)
	Sensitive	40 (43.0)	0 (0)	0 (0)	40 (40)
Aztreonam	Resistant	1 (1.1)	0 (0)	0 (0)	1 (1)
	Sensitive	92 (98.9)	4 (100)	3 (100)	99 (99)
Cefoperazone-sulbactam	Resistant	26 (28.0)	2 (50.0)	2 (66.7)	30 (30)
	Sensitive	67 (72.0)	2 (50.0)	1 (33.3)	70 (70)
Piperacillin-tazobactam	Resistant	15 (16.1)	1 (25.0)	2 (66.7)	18 (18)
	Sensitive	78 (83.9)	3 (75.0)	1 (33.3)	82 (82)
Imipenem	Resistant	28 (30.1)	1 (25.0)	2 (66.7)	31 (31)
	Sensitive	65 (69.9)	3 (75.0)	1 (33.3)	69 (69)
Meropenem	Resistant	28 (30.1)	1 (25.0)	2 (66.7)	31 (31)
	Sensitive	65 (69.9)	3 (75.0)	1 (33.3)	69 (69)
Colistin	Resistant	0 (0)	4 (100)	3 (100)	7 (7)
	Sensitive	93 (100)	0 (0)	0 (0)	93 (93)
Polymyxin B	Resistant	0 (0)	4 (100)	3 (100)	7 (7)
	Sensitive	93 (100)	0 (0)	0 (0)	93 (93)

DISCUSSION

The present study characterized 100 non-lactose-fermenting, oxidase-positive Gram-negative bacterial isolates obtained from clinical specimens at a tertiary care hospital in South India over a one-year period. Of these, 93 (93.0%) were identified as *Pseudomonas* species, while seven (7.0%) belonged to the genus *Ralstonia*, comprising four *Ralstonia mannitolilytica* and three *Ralstonia pickettii*. No *Ralstonia insidiosa* isolates were detected. The study highlights the importance of differentiating *Ralstonia* species from other non-fermenting Gram-negative bacilli in routine diagnostic microbiology, particularly because their phenotypic characteristics may overlap with those of other non-fermenters.

Distribution of *Ralstonia* Species

In the present study, *Pseudomonas* species were the predominant isolates, accounting for 93% of the total, whereas *Ralstonia* species constituted only 7%. Among the *Ralstonia* isolates, *R. mannitolilytica* was slightly more frequent than *R. pickettii*, representing 4% and 3% of all isolates, respectively. The absence of *R. insidiosa* may reflect its low frequency in the study population, although the limited sample size does not permit conclusions about its occurrence in the wider hospital setting. The predominance of *Pseudomonas* among non-fermenting Gram-negative bacilli is consistent with its established role as a frequently encountered organism in clinical microbiology laboratories. In contrast, *Ralstonia* species are less commonly recognized in routine clinical cultures and may be overlooked or misidentified when identification relies on conventional biochemical methods alone. Ryan and

Adley described the clinical relevance and identification challenges of *Ralstonia* species, emphasizing that these organisms can be associated with opportunistic infections and contaminated aqueous environments.^[1] The small proportion of *Ralstonia* observed in the present study is therefore relevant to the diagnostic laboratory, where even infrequently encountered non-fermenters may require further characterization.

The recovery of *R. mannitolilytica* and *R. pickettii* in this study is in keeping with previous reports identifying these species as potential opportunistic human pathogens, particularly in healthcare settings. However, because only seven *Ralstonia* isolates were identified and molecular confirmation was not performed, the species distribution should be interpreted as a phenotypic finding specific to the study population rather than a definitive estimate of local species prevalence.

Age-wise and Gender-wise Distribution

In the present study, the majority of isolates were recovered from patients aged 41–60 years (33.0%), followed by those aged above 60 years (29.0%). All seven *Ralstonia* isolates were recovered from patients aged 21 years and above. The four *R. mannitolilytica* isolates were distributed across the 21–40, 41–60 and above-60-year age groups, while the three *R. pickettii* isolates were equally distributed among these same age categories. A study by Siddiqui et al. (2022), conducted in a tertiary care hospital, described 14 cases of *R. mannitolilytica* bloodstream infection, of which seven (50%) occurred in patients aged 21–60 years. Their study also reported a male predominance, with a male-to-female ratio of 2.5:1.^[13] The present study similarly identified

Ralstonia isolates among adult patients and observed that all seven isolates were from males. However, the number of Ralstonia isolates in the present study was considerably smaller, and the age categories and patient characteristics were not identical between the studies. The male predominance observed in the present study is also comparable with the demographic distribution reported in a North Indian retrospective analysis of Ralstonia bacteremia conducted between April 2022 and April 2024. That study identified 26 cases, including 18 (69.2%) *R. mannitolilytica* and eight (30.8%) *R. pickettii* isolates, with a male-to-female ratio of 1.6:1.^[14] Although the present study and previous reports have documented a greater number of Ralstonia isolates among male patients, this observation should not be interpreted as evidence of an independent gender-related susceptibility. Differences in underlying illnesses, hospitalization patterns, invasive procedures and the distribution of patients across clinical departments may influence the observed demographic profile. Larger studies with detailed clinical information are required to examine these factors.

Department-wise and Hospital Location Distribution

In the present study, six of the seven Ralstonia isolates were recovered from the Nephrology department, including all four *R. mannitolilytica* isolates and two of the three *R. pickettii* isolates. The remaining *R. pickettii* isolate was obtained from Medicine. With respect to hospital location, six Ralstonia isolates were from inpatients and one was from an ICU patient. No Ralstonia isolates were reported from outpatients. The concentration of Ralstonia isolates in Nephrology is noteworthy because patients receiving renal care may undergo repeated hospital visits, vascular access procedures and other invasive interventions. Previous reports have documented Ralstonia bloodstream infections and outbreaks in patients receiving hemodialysis or other hospital-based care. Valente et al. (2024) described an outbreak involving Ralstonia and Burkholderia species associated with catheter-related bloodstream infections in a hemodialysis unit.^[5]

The North Indian retrospective study also documented Ralstonia bacteremia among hospitalized patients, including individuals with underlying comorbidities and healthcare exposure.^[14] In addition, Chen et al. (2017) reported an outbreak of *R. pickettii* bloodstream infection associated with an intrinsically contaminated normal saline solution, illustrating that contaminated medical fluids may act as a source of healthcare-associated transmission.^[12] Such reports demonstrate the importance of considering environmental and product-related sources when clusters of uncommon non-fermenting Gram-negative bacteria are detected. The departmental concentration observed in the present study, however, does not establish a common source, a transmission event or a healthcare-associated outbreak. The study did not include environmental

sampling, investigation of intravenous fluids, catheter-related assessments or molecular typing to establish epidemiological links. The findings should therefore be interpreted as a distribution of isolates by department and location, rather than proof of a specific route of acquisition.

Specimen-wise Distribution and Clinical Significance

Blood was the most frequent specimen source among the Ralstonia isolates in the present study, accounting for six of the seven isolates (85.7%). Three of the four *R. mannitolilytica* isolates and all three *R. pickettii* isolates were recovered from blood, while one *R. mannitolilytica* isolate was recovered from urine. No Ralstonia species were isolated from pus specimens. The predominance of blood isolates in the present study is consistent with the clinical importance of Ralstonia species as potential bloodstream pathogens. Siddiqui et al. (2022) reported a series of 14 *R. mannitolilytica* bloodstream infection cases in a tertiary care hospital.^[13] Basso et al. (2019) also described persistent bacteremia involving both *R. mannitolilytica* and *R. pickettii* in an intensive care unit.^[6] These reports indicate that Ralstonia species may be recovered from blood cultures in hospitalized patients and may be clinically significant in selected circumstances. A North Indian retrospective study identified 26 Ralstonia bacteremia cases from blood cultures over two years, further demonstrating the relevance of these organisms in bloodstream infection surveillance.^[14] In contrast, the present study included a broader set of clinical isolates and found Ralstonia in both blood and urine. This difference may reflect variations in the specimen types investigated, patient populations, referral patterns and study designs. The isolation of Ralstonia from blood should nevertheless be assessed in conjunction with clinical presentation, repeat blood culture findings, indwelling vascular devices, treatment history and other laboratory results. Ralstonia species are environmental organisms that may contaminate aqueous solutions and medical products, and their recovery from clinical specimens may sometimes represent contamination or pseudobacteremia. Because the present study did not include comprehensive clinical outcome data or a dedicated contamination assessment, it cannot confirm that every isolate represented an active infection.

Antimicrobial Susceptibility Pattern of Pseudomonas Species

In the present study, Pseudomonas species demonstrated variable antimicrobial resistance, with the highest reported resistance to cotrimoxazole (100%), followed by ceftazidime (68.8%), ciprofloxacin (65.6%), cefepime (57.0%) and gentamicin (50.5%). Resistance to amikacin, ofloxacin, levofloxacin, cefoperazone-sulbactam, piperacillin-tazobactam, imipenem and meropenem ranged from 16.1% to 43.0%, while resistance to aztreonam was reported in only one isolate (1.1%). All Pseudomonas isolates were reported as sensitive

to colistin and polymyxin B. The resistance to ceftazidime and ciprofloxacin observed in the present study is important from a local antimicrobial surveillance perspective, as these agents are commonly included in the assessment of non-fermenting Gram-negative bacilli. However, the study identified the organisms as *Pseudomonas* species rather than confirming all isolates as *Pseudomonas aeruginosa*. Consequently, direct comparison with studies specifically investigating *P. aeruginosa* should be made cautiously. The differences in resistance proportions across studies may be influenced by local antimicrobial prescribing practices, patient case mix, hospital-associated exposures, organism identification methods and the antimicrobial susceptibility testing criteria used. The present findings provide a descriptive account of isolates recovered at a single tertiary care centre and should not be generalized to other institutions without additional surveillance data.

Antimicrobial Susceptibility Pattern of *Ralstonia* Species

The antimicrobial susceptibility profile of *Ralstonia* species was an important finding of the present study. All seven isolates were reported as resistant to ceftazidime, cefepime, colistin and polymyxin B. In contrast, all isolates were reported as sensitive to cotrimoxazole, gentamicin, amikacin, ciprofloxacin, ofloxacin, levofloxacin and aztreonam. Variable resistance was observed to cefoperazone–sulbactam, piperacillin–tazobactam, imipenem and meropenem. These findings demonstrate that the two *Ralstonia* species identified in the present study had overlapping reported susceptibility patterns, although the proportion of resistance to some beta-lactam agents differed between them. All four *R. mannitolilytica* isolates were resistant to ceftazidime and cefepime, while one isolate each was resistant to piperacillin–tazobactam, imipenem and meropenem. Among the three *R. pickettii* isolates, two were resistant to piperacillin–tazobactam, imipenem and meropenem. The susceptibility results reported in the present study differ from those described in some previous investigations. Siddiqui et al. (2022), in a case series of 14 *R. mannitolilytica* bloodstream isolates, reported variable antimicrobial susceptibility and used CLSI criteria for *Pseudomonas* species to interpret the results. Their study described the use of agents such as cefoperazone–sulbactam and meropenem in clinical management.^[13] Basso et al. (2019) reported different susceptibility profiles between *R. mannitolilytica* and *R. pickettii*: the *R. mannitolilytica* isolate was resistant to several beta-lactams and aminoglycosides, while the *R. pickettii* isolate was susceptible to imipenem and aminoglycosides.^[6] A larger study from North India involving 26 *Ralstonia* bacteremia isolates also reported species-level variation in antimicrobial susceptibility. In that study, *R. pickettii* showed better reported susceptibility than *R. mannitolilytica* to some agents, including amikacin, imipenem, meropenem, aztreonam and

ceftazidime.^[14] In a separate molecular epidemiological study of *R. pickettii*, susceptibility varied by antimicrobial agent and testing method; the investigators also highlighted the difficulty of interpreting results in the absence of species-specific breakpoints.^[15] The resistance to colistin and polymyxin B in all seven *Ralstonia* isolates in the present study is consistent with published reports describing high rates of polymyxin resistance among clinical *Ralstonia* isolates. The literature review by Basso et al. (2019) identified colistin resistance in all four *R. pickettii* and all three *R. mannitolilytica* isolates for which data were available in the studies reviewed.^[6] Nevertheless, this similarity should be interpreted in the context of the small numbers of isolates and the susceptibility testing approaches used. The differences between the present findings and previous reports may be related to strain-level variability, differences in antimicrobial exposure, testing methodologies and interpretive criteria. The present study did not investigate the molecular mechanisms responsible for antimicrobial resistance, such as beta-lactamase genes, efflux systems or other resistance determinants. Therefore, no specific mechanism can be attributed to the resistance patterns observed. An important interpretive limitation is that species-specific clinical breakpoints for *Ralstonia* were not available for the study. The susceptibility categories were interpreted using criteria adapted from other Gram-negative organisms, as described in the methodology. These reported categories are therefore exploratory and should not be considered validated clinical predictions of treatment response. Further studies using reference susceptibility methods, such as broth microdilution where appropriate, alongside molecular identification and resistance gene analysis, are needed to clarify these patterns.

Strengths and Limitations

The present study provides a prospective, one-year description of non-lactose-fermenting, oxidase-positive Gram-negative isolates from a tertiary care hospital. It includes phenotypic characterization, species-level differentiation using conventional biochemical methods and antimicrobial susceptibility testing. The analysis of age, gender, department, hospital location and specimen type provides a descriptive overview of the clinical distribution of the isolates. Several limitations should be considered when interpreting the findings. First, the study was conducted at a single centre, and the results may not represent the distribution of *Ralstonia* species in other hospitals or geographical regions. Second, only seven *Ralstonia* isolates were identified, limiting the precision of species-specific proportions and antimicrobial susceptibility estimates. Third, molecular confirmation was not performed, restricting the certainty of species-level identification. Fourth, species-specific clinical breakpoints were unavailable for *Ralstonia*, and the reported susceptibility categories were based on adapted interpretive criteria. Finally, detailed clinical

information, patient outcomes, resistance mechanisms, environmental sampling and molecular epidemiological investigations were not included. Therefore, the study cannot establish clinical causality, treatment effectiveness, the source of isolates or the presence of healthcare-associated transmission.

CONCLUSION

This study provides local baseline data on the phenotypic characteristics, clinical distribution and antimicrobial susceptibility profiles of *Pseudomonas* and *Ralstonia* species in a tertiary care hospital. Continued microbiological surveillance, accurate species identification, appropriate antimicrobial susceptibility testing and correlation with clinical findings are essential to support antimicrobial stewardship and infection prevention practices. Further multicentre studies incorporating molecular identification, resistance mechanism analysis and epidemiological investigations are warranted to better understand the clinical significance and resistance patterns of *Ralstonia* species.

Availability of Data and Materials

The data supporting the findings of this study are available from the corresponding author upon reasonable request, subject to institutional policies and applicable confidentiality requirements.

Acknowledgements

The authors acknowledge the support of the Department of Microbiology, Coimbatore Medical College and Hospital, Tamil Nadu, India, and the laboratory staff who contributed to the conduct of this study.

REFERENCES

- Ryan MP, Adley CC. *Ralstonia* spp.: emerging global opportunistic pathogens. *European journal of clinical microbiology & infectious diseases*. 2014 Mar;33(3):291-304.
- Chen YY, Huang WT, Chen CP, Sun SM, Kuo FM, Chan YJ, Kuo SC, Wang FD. An outbreak of *Ralstonia pickettii* bloodstream infection associated with an intrinsically contaminated normal saline solution. *infection control & hospital epidemiology*. 2017 Apr;38(4):444-8.
- Liao L, Lin D, Liu Z, Gao Y, Hu K. A case of meningitis caused by *Ralstonia insidiosa*, a rare opportunistic pathogen. *BMC infectious diseases*. 2023 Aug 22;23(1):548.
- Papagrigorakis E, Vavourakis M, Vlachos C, Zachariou D, Galanis A, Marouglanis V, Polyzois V, Pneumatics S. Osteomyelitis caused by *Ralstonia mannitolilytica*, a rare opportunistic pathogen. *Cureus*. 2022 Apr 14;14(4).
- Valente M, Orecchioni F, Brigante F, Moretti MI, D'errico M, Moretti M, Tavio M, Cotorruello MS, Pasquali V, Marchi M, Ranghino A. Outbreak of *Ralstonia* spp and *Burkholderia* spp catheter-related bloodstream infection in a haemodialysis unit. *INNEPHROLOGY DIALYSIS TRANSPLANTATION* 2023 Jun 1 (Vol. 38, pp. I754-I755). GREAT CLARENDON ST, OXFORD OX2 6DP, ENGLAND: OXFORD UNIV PRESS.
- Basso M, Venditti C, Raponi G, Navazio AS, Alessandri F, Giombini E, Nisii C, Di Caro A, Venditti M. A case of persistent bacteraemia by *Ralstonia mannitolilytica* and *Ralstonia pickettii* in an intensive care unit. *Infection and Drug Resistance*. 2019 Aug 2:2391-5.
- Ryan MP, Pembroke JT, Adley CC. Differentiating the growing nosocomial infectious threats *Ralstonia pickettii* and *Ralstonia insidiosa*. *European journal of clinical microbiology & infectious diseases*. 2011 Oct;30(10):1245-7.
- Adley CC, Saieb FM. Comparison of bioMerieux API 20NE and Remel RapID NF Plus, identification systems of type strains of *Ralstonia pickettii*. *Letters in applied microbiology*. 2005 Aug 1;41(2):136-40.
- Ryan MP, Adley CC. The antibiotic susceptibility of water-based bacteria *Ralstonia pickettii* and *Ralstonia insidiosa*. *Journal of Medical Microbiology*. 2013 Jul;62(7):1025-31.
- Padmavathi BK, Ishwaryalakshmi R, Mathavi S, Rao AR, Rajan R. Phenotypic profiling of Carbapenem drug resistant *Pseudomonas* species in a tertiary care hospital in Tamilnadu. *Vascular and Endovascular Review*. 2024 Dec 27;7(2):224-8.
- Bajpai V, Govindaswamy A, Khurana S, Batra P, Aravinda A, Katoch O, Hasan F, Malhotra R, Mathur P. Phenotypic & genotypic profile of antimicrobial resistance in *Pseudomonas* species in hospitalized patients. *The Indian Journal of Medical Research*. 2019 Feb;149(2):216.
- Wayne PA. Clinical and laboratory standards institute. Performance standards for antimicrobial susceptibility testing. Testing. 34th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute; 2024.
- Siddiqui T, Patel SS, Sinha R, Ghoshal U, Sahu C. *Ralstonia mannitolilytica*: an emerging multidrug-resistant opportunistic pathogen in a tertiary care hospital setting. *Access Microbiology*. 2022 May 31;4(5):000367.
- Jamwal A, Varghese G, Tripathy S, Sarawat D, Patel SS, Kar M, Tejan N, Sahu C. Changing trends of *Ralstonia* bacteremia in patients at a tertiary care center in North India: a 2-year retrospective analysis. *The American Journal of Tropical Medicine and Hygiene*. 2024 Dec 10;112(3):539.
- Sheng Z, Li J, Han G, Fan R, Zhu P, Fang X. Molecular epidemiological and clinical infection characteristics analysis of *Ralstonia*. *European Journal of Clinical Microbiology & Infectious Diseases*. 2024 Jun;43(6):1161-70.