Title: *Ralstonia* spp: Emerging Global Opportunistic Pathogens

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Abstract

The bacterial genus *Ralstonia* (Gram negative non-fermenters) is becoming more prevalent in cases of infection with three bacterial species *Ralstonia pickettii*, *Ralstonia insidiosa* and *Ralstonia mannitolilytica* making up all cases reported (in the literature) to date. These organisms are prevalent in many different types of water supplies (including hospital water supplies) being well adapted to survive in low nutrient conditions. They have been shown to cause infections, sometimes serious such as osteomyelitis and meningitis, in hospital settings. Seventy cases of *R. pickettii*, 13 cases of *R. mannitolilytica* and 3 cases *of R. insidiosa* infection have been identified from the literature. Insight is given into the types of infections that are caused by these bacteria, the underlying conditions that are associated with these infections and potential treatments.

Introduction

Gram-negative non-fermenting bacteria are a growing concern in clinical environments, being one of the most common causes of nosocomial infections. The major opportunistic pathogens from this group comprise of many bacterial species including: *Acinetobacter baumannii*, *Pseudomonas aeruginosa, Burkholderia cepacia*, and *Stenotrophomonas maltophilia*. These bacteria take advantage of underlying medical conditions and diseases to cause infection. *Ralstonia* spp are amongst these non-fermenting gram-negative bacteria that are emerging as opportunistic pathogens.

Ralstonia is a genus which includes former members of *Burkholderia* spp. (*Burkholderia pickettii* and *Burkholderia solanacearum*). *Ralstonia* spp. are aerobic Gram-negative, non-fermentative rods that can be found in water and soil (1). *Ralstonia pickettii*, the type species of the genus, was until recently regarded as the only member of the genus of clinical importance (2) however two novel *Ralstonia* spp. have been described recently that are considered to be of minor clinical importance, *Ralstonia insidiosa* (3) and *Ralstonia* *mannitolilytica* (2). Investigation of instances of *Ralstonia* spp. infections in the literature showed many different types of infections (mostly with *R. pickettii* as the cause). This indicates that the genus may be a more extensive pathogen than was assumed previously with the types of infections being more invasive and severe.

Bacteriology

Ralstonia pickettii

As stated above *R. pickettii* is the type species of the *Ralstonia* genus. The bacterium has been recovered from a wide variety of clinical specimens including blood, urine and cerebrospinal fluid (4). The bacteria has been isolated from numerous water sources including municipal drinking water supplies (5), bottled water (6), dental water supplies (7), hospital water supplies (8), space shuttle water systems (9), standard purified water (10), laboratory based high-purity water systems (11) and industrial Ultra-pure/High Purity water (12, 13, 14). *R. pickettii* has been identified in biofilm formation in plastic industrial water piping (13, 15). *R. pickettii* is not thought of as a major pathogen and its virulence is thought to be low. It is consequently not routinely looked for in hospital analysis (2). All reported instances of infection attributed to *R. pickettii* infection are described in Table 1.

Ralstonia insidiosa

Ralstonia insidiosa is the closest related bacteria to *R. pickettii* (16) and has also been isolated from river and pond water, soil, activated sludge (16), laboratory purified water systems (3), and industrial Ultra-pure/High Purity water (16) and water distribution systems (18). The bacteria has also been found in the lung sputum of cystic fibrosis suffers (2). All reported instances of infection attributed to *R. insidiosa* infection are described in Table 2.

Ralstonia mannitolilytica

R. mannitolilytica is also closely related to *R. pickettii*. *R. mannitolilytica* had previously been called "*Pseudomonas thomasii*" and *R. pickettii* biovar 3/"thomasii" (19) and has been described in a number of hospital outbreaks (20, 21, 22, 23). Initial reports (24) described cases of bacteremia and bacteriuria in twenty patients. These infections were linked to *"P. thomasii*" contaminated parenteral fluids that had been prepared with deionized water contaminated with *"P. thomasii*". Pan *et al.*, reported an epidemic involving twenty-four patients that was caused by "*P. thomasii.*" contaminated saline solution that had been prepared by the hospital pharmacy (23). Pseudo-outbreaks linked to *R. mannitolilytica* have also been described (25). Reports have described it as causing meningitis and hemoperitoneum infection (26), renal transplant infection (27) and bacteremia (28). *R. mannitolilytica* is the most prevalent species of the *Ralstonia* genus to be found in Cystic Fibrosis suffers (29). All reported instances of infection attributed to *R. mannitolilytica* infection are described in Table 3.

Identification of *Ralstonia* spp.

The three *Ralstonia* spp. are difficult to identify and differentiate from one another using routine hospital analysis (3, 28). The three bacteria have very similar biochemical patterns to each other and to other bacterial species such as the *Burkholderia cepacia* complex and *Pseudomonas fluorescens* (2, 3, 28). The existing commercial biochemical identification systems on the market, e.g. API 20NE, RapID NE Vitek etc., do not give the best identification, especially for some genera or species including Gram-negative non-fermenting rods (30). Some identification methods may confound epidemiology as *Ralstonia* spp. can be easily misidentified as *Burkholderia cepacia* complex and *Pseudomonas fluorescens* using routine methods as stated above.

R. pickettii has been shown to give variable results using the standard biochemical test kits, e.g. API 20NE and *R. mannitolilytica* and *R. insidiosa* have not been added to test panels (13). The best way to tell the bacteria apart is though PCR with Species -Specific PCR primers available for all three bacteria (17, 31).

Epidemiology

The population at risk from these bacteria comprises almost exclusively immunocompromised patients with for example those with hematological malignancies (bacteremia, septicemia, etc.), patients in intensive care, patients with indwelling devices such as central venous catheters and neonates (32, 33, 34, 35, 36, 37, 38). Examples of underlying conditions and *Ralstonia* spp. infection includes: a 53-year-old man who had suffered a myocardial infarction and who contracted *R. pickettii*-related bacteremia while recuperating in hospital; (30) a 32-year-old man who was suffering from hepatitis-C-related liver cirrhosis who contracted *R. pickettii*-related peritonitis (3), a 41-year-old man with underlying diabetes mellitus who developed *R. pickettii* related pneumonia (39), a 7-year-old boy who received a cord blood transplant and then suffered an episode of *R. pickettii*-related bacteremia (40) and a 71-year-old man with underlying chronic renal failure, diabetes mellitus, hypertension and alcoholic cirrhosis who developed *R. pickettii*-related osteomyelitis (41).

R. pickettii and *R. mannitolilytica* have also been associated in infections with permanent indwelling intravenous devices such as catheters (33, 42).

Ralstonia spp. have been shown to be the causative agent of severe invasive infections including osteomyelitis, septic arthritis, meningitis, etc. (26, 41, 43). Another example of severe infection due to *R. pickettii* is the case of a 38-year-old female who contracted both bacteremia and meningitis (44). In many of the more serious infections the aetiology was unknown.

Reports that have indicated the occurrences of *Ralstonia* spp. infections (Tables 1, 2, 3) are increasing. This rise could be associated with the increase in the patient population at risk (older populations, people surviving with serious illness for longer, etc.), potentially as a result of the widespread use of antibiotics and the implementation of more invasive medical practices (2).

Factors responsible for infection

Contaminated solutions

Many of the cases of infection with *Ralstonia* spp. are due to contaminated solutions including water for injection, saline solutions made with purified water, respiratory solutions and sterile drug solutions (37, 45, 46, 47). Contamination of the products listed above generally occurs at the manufacturing stage. This contamination can occur through many different means but one of the most important is due to the ability of *Ralstonia* spp to pass through 0.2 µm filters that are used for the sterilization of many medicinal products, such as saline solution (48, 49). Another example of how solutions can become contaminated during the manufacturing stage was seen in 1983, when five infants became infected with *R. pickettii* that was associated with a contaminated respiratory therapy solution. *R. pickettii* contaminated the solution due to the bypassing of an 82°C holding tank during the manufacture of distilled water. This contaminated solution was then used for endotracheal suctioning and this allowed colonization of the patients (45).

Disinfectants

R. pickettii has been shown to survive in different hospital disinfectants including chlorhexidine. In 1983, six patients contracted *R. pickettii* related septicemia. The source of the contamination was traced back to the bidistilled water used to make up the 0.05% aqueous solution of chlorhexidine. This was then used for skin antisepsis before the insertion of a venous catheter and lead to infection (50). Contaminated chlorhexidine was also implicated in the recovery of *R. pickettii* from six pediatric patients in 1995 (51). As before the contaminated chlorhexidine was used for topical disinfection. *R. pickettii* has also been shown to survive ethacridine lactate (aromatic organic compound based on acridine) (52).

Pseudo-outbreaks

Ralstonia spp. have been linked with pseudo-outbreaks, with seven such outbreaks being described in the literature (Table 1 and 3). These pseudo-outbreaks have caused unnecessary treatments to be given to patients (e.g. wrong or unneeded antibiotics or the removal of indwelling devices) and are also a waste of valuable time and resources in hospital laboratories. Pseudo-outbreaks may be due to many different causes including contaminated distilled water used in the bacterial testing procedures, phlebotomist error or contamination at the manufacturing stage of materials used in laboratory testing. Verschraegen *et al*. reported that *R. pickettii* was the cause of pseudobacteraemia in a surgical ward. This was the first such case reported in the literature (53). The patients did not exhibit any signs of bacteremia, even though the organism was isolated from blood samples. The contamination was traced to distilled water (used in the testing procedures) and a 0.5% chlorhexidine solution (used to wipe the bench) prepared using the distilled water. Both of these were found in the hospital pharmacy. *R. mannitolilytica* has also been found to be the causative agent of pseudo-outbreaks in special-care baby wards (25).

Treatment

The treatment and management of *Ralstonia* spp. infections is often challenging as these pathogens are frequently resistant to numerous different types of antibiotics, including several β-lactams and most of the aminoglycosides. A major study into the antibiotic resistance of numerous strains of *R. pickettii* and *R. insidiosa* showed both bacteria were highly resistant to the aminoglycoside gentamicin and the ß-lactam antibiotic aztreonam and variably resistant to the ticarcillin-clavulanic acid mix (54). Two inducible β-lactamases in *R. pickettii*, *bla*$_{OXA-60}$ and *bla*$_{OXA-22}$ are considered to be the reason for the high level of resistance to β-lactams (55, 56). The presence of an aminoglycoside acetyl-transferase in *R. pickettii* genomes 12J and 12D also has an aminoglycoside acetyl-transferase accounts for the widespread resistance to aminoglycosides (see Table 1, and reference 54). The study carried out by Ryan and Adley showed that most strains were susceptible to the carbapenem meropenem and all isolates were susceptible to the quinolones (ciprofloxacin and ofloxacin), the tetracyclines (tetracycline and minocycline), the cephalosporins (cefotaxime and ceftazidime), the folate pathway inhibitors (trimethoprim/sulfamethoxazole) and the extended spectrum beta-lactam antibiotic of the ureidopenicillin class (piperacillin) (56). Various different antibiotics have been used to treat infections of *Ralstonia* spp. including trimethoprim/sulfamethoxazole, ciprofloxacin, piperacillin, cefotaxime, ceftriaxone, imipenem, meropenem, levofloxacin and cefepime (Table 1, 2 and 3). What can be seen from this data is the failure of meropenem and ceftriaxone to treat infection in several instances and the need to use an alternative which was mostly ciprofloxacin.

Conclusions

Ralstonia spp. are not recognized as major pathogens. However in this paper we have identified over a hundred examples of *Ralstonia* spp. infections. These species have certain characteristics such as resistance to disinfection practices and ability to survive in water supplies that allows them to cause many potentially harmful infections and death.

Methodology

All available publications discussing *R. pickettii, R. insidiosa* and *R. mannitolilytica* infections were searched for using the PubMed, Web of Knowledge, Science Direct and Google Scholar search databases from 1970 to 2013. The terms ‘*Ralstonia pickettii*, ‘*Pseudomonas pickettii’*, ‘*Burkholderia pickettii’*, ‘*Ralstonia*’ and ‘*pickettii’ ‘Ralstonia insidiosa*’ ‘*insidiosa*’ ‘*Ralstonia mannitolilytica’* ‘*mannitolilytica’* ‘*Pseudomonas thomasii’* were all searched for and any references that discussed infection were set aside. These papers/abstracts were then read and the required information extracted (year of outbreak, number/age/sex of patients, geographic location, underlying condition, condition caused by infection, susceptibility/resistance to antibiotics, antibiotics used to treat condition, clinical outcome). The reference sections of these publications were also checked for any infection related references that may have been missed during the electronic searches.

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Conflict of interest

The authors declare that they have no competing interests.

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