Incidence, Relevance and Response for *Ralstonia* Respiratory Infections

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BACKGROUND: Cases of *Ralstonia* colonization/infection occasionally reported by hospitals has generated increased interest in an organism previously little known to most clinicians. Our goal was to determine the incidence of respiratory colonizations and infections involving *Ralstonia* and the association of mechanical ventilation (limited to reports on respiratory-related occurrences in the USA) and propose a decision chart to assist response.

METHODS: We performed a secondary analysis of published clinical reports of *Ralstonia* to determine the potential risks for respiratory colonization and infection in the USA and if being on mechanical ventilation (MV) had an influence on colonization and conversion to infection (symptomatic).

RESULTS: The odds of developing colonization with *Ralstonia* were eight times higher and the likelihood of developing infection with *Ralstonia* was twelve times higher in those mechanically ventilated compared to those not mechanically ventilated.

CONCLUSIONS: Our results suggest that individuals who are currently on mechanical ventilation and are *Ralstonia* culture-positive have an increased risk for colonization and may have increased propensity to the development of infection (two decision trees for approaching diagnosis and therapy included).

KEY WORDS (MeSH): Ralstonia, Gram-Negative Bacterial Infections, Cross Infection, Ventilators, Mechanical, Equipment Contamination, Infection, Equipment, Disposable

ABBREVIATIONS: FDA – Food and Drug Administration, sp. – Specie (spp., plural), HIV – Human Immunodeficiency Virus, MV – Mechanical Ventilation, MMWR – Morbidity and Mortality Weekly Report, PCR – Polymerase Chain Reaction, VAP – Ventilator-Associated Pneumonia

Clin Lab Sci 2010;23(2):99

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INTRODUCTION

The impact of gram negative rods on clinical disease is well known. For example, it has been noted that gram negative rods (i.e., Burkholderia cepacia) have significant impact on patients (decline of lung function) with cystic fibrosis. *Ralstonia* is a lesser known member of the group. The Centers for Disease Control has published occasional reports over the past few decades of patients colonized or infected with *Ralstonia* bacteria.[1-3]

The *Ralstonia* genus is an aerobic, non-fermentative, oxidase-positive, Gram-negative bacillus commonly found in water and soil.[4] It may exist under harsh

conditions; for example, it is able to survive disinfectants such as chlorhexidine[5] and ethacridine lacatate[6], but it thrives best in moist environments. The *Ralstonia* genus includes *R. pickettii* and *R. solanacearum* (formerly *Burkholderia pickettii* and *B. solanacearum*), *R. insidiosa*[7], and *R. mannitolilytica*.[8] In CF patients, the prominent species detected via PCR is *R. mannitolilytica*.[9] However, its role in affecting disease/symptom severity is unknown. Recently in a review by Ryan et al, it was concluded that, "*R. pickettii* is still regarded as the main pathogenic species of this strain."[10]

Recently, the 2005 recall of a respiratory gas humidification device thought to be associated with cases of *Ralstonia* (75% *R. mannitolilytica*, 7% other *Ralstonia* spp.) colonization reported by hospitals in several states has generated increased interest in an organism previously little known to most clinicians.[1] The FDA Preliminary Public Health Notification regarding the Vapotherm respiratory device (Oct. 27, 2005) made the following recommendation, "Clinicians must continue to weigh the potential risks of *Ralstonia* spp. contamination of Vapotherm® devices against the benefits of using the device in patients requiring humidified oxygen therapy."[1] The device has been approved for return to market so clinicians need information on the potential risks of *Ralstonia* to patients, of which there is little, to follow this recommendation. Given this conclusion it is worth noting that this was not the strain identified in the Vapotherm recall. Our goal was to determine the incidence of respiratory colonizations and infections involving *Ralstonia* including (primary and secondary infections) and the association of mechanical ventilation (secondary infection defined as that which occurs because some initial primary infection has weakened the host). We limited our review to reports on occurrences in the USA.

The majority of results from an Internet search on "*Ralstonia*" would yield information on occurrences in plant nurseries rather than neonatal units. In that context *Ralstonia* is referred to by names such as "Bacterial Wilt" or "Geranium Wilt." Prior to 1995, this genus was typically identified as a species of *Burkholderia* or *Pseudomonas* and, depending on the assay used, there may still be misidentification .[4] Most of the reported human *Ralstonia* cases resulted from use of contaminated solutions such as distilled water[11] or injectable water[12], injectable saline[12-13] or purified respiratory ampules.[14]

The presence of *Ralstonia* spp. isolated from patients has led to confusion for many clinicians as to whether it is appropriate to treat the bacteria or to regard it as colonization. Another difficulty arises with properly identifying *Ralstonia* from other phylogenically similar organisms (i.e., *Burkholderia* sp. and *Pseudomonas* sp.). These factors have led to an aggressive treatment of the bacteria and this generalized "shotgun" approach to therapy has likely contributed to increasingly resistant strains,[15-17] which experts believe may be related to both constitutive and inducible mechanisms of the organism. This intensive therapy is quite unfortunate as most *Ralstonia* sp. lack the virulence factors seen in both *Pseudomonas* sp. and *Burkholderia* sp.[15,18-19]

It is often difficult to decide specific therapy for an organism without consideration of the host. If a host is immunocompromised (i.e. HIV infection or on immunosuppressive agents) or exhibits a long-term medical condition (i.e. cancer, cystic fibrosis), often a more aggressive therapy is enacted to treat a positive culture.[20-21] While there is some evidence pointing to this approach for the treatment in *Ralstonia*, a more complete examination of these host factors is certainly warranted.

To examine the impact of *Ralstonia* on patient clinical outcomes, we performed an analysis of published clinical reports of *Ralstonia* to determine the potential risks for respiratory colonization and infection in the USA. We specifically asked if being on mechanical ventilation (MV) had an influence on colonization and conversion to infection (symptomatic). These data would then lead to the generation of decision-making pathways for use in clinical respiratory care.

METHODS AND MATERIALS

Electronic databases searched included Medline/ PubMed, Web of Science®, Embase®, Scopus®, and BIOSIS Previews® from January 1995 through January 2008. Embase (over 4600 journals) and Medline/

PubMed (over 5000 journals) each cover about 1800 journals that the other database does not. Web of Science searches 8700 multidisciplinary research journals and Scopus covers over 15,000 research journals that include science, technology and medicine. BIOSIS Previews draws from 5,500 life science journals plus items from international meetings, review articles, books, and other unique references. Retrospective and prospective cited reference searches of key articles were also performed using Scopus and Web of Science.

Our literature review identified four reports that include data on respiratory colonization that allowed for calculating an average odds ratio.[2,22-23,3] The definition of infection versus colonization was based on the establishment of clinical parameters of disease (i.e., purulent sputum, fever greater than 38. *8* C, impaired oxygenation, or elevated white blood cell count).[24]

Four reports (two of which also provided colonization data) contained sufficient data to calculate an average odds ratio for infection.[2-3,25] Due to the variability of clinical environments, procedures, surveillance and therapies used internationally, we limited our search to occurrences in the USA. All studies represented new cases of Ralstonia. We focused on occurrences that involved the respiratory tract because of the relationship to hospital equipment/procedures raised by the humidification device recall and the greater potential for serious complications. Immunocompromised population examined included individuals with HIV, common variable immunodeficiency (CVID), extensive burn injury, and those individuals with greater than 20 mg of prednisone per day; patients with bronchiectatic conditions such as cystic fibrosis (CF) and idiopathic bronchiectasis were also examined as compromised populations.

Statistical Analysis

Our analysis sought to determine what the odds of becoming colonized or infected with *Ralstonia* between patients' receiving mechanical ventilation and those on other types of therapies. We performed a meta-analysis for these published multiple incidences of *Ralstonia* cultured in patients with respiratory infections in the USA. A meta-analysis seeks to systematically review all pertinent evidence, provide quantitative summaries, integrate results across studies, and provide an overall interpretation of these studies (National Library of Medicine Glossary). The results of each study included in the analysis were summarized as counts in a 2-by-2 table. The treatment group was patients on MV and the control group consisted of patients not on MV. We developed two different analyses; one for colonization with *Ralstonia* and the other for infection with *Ralstonia* based on reported constitutional and respiratory symptoms with isolation of bacteria. This analysis was carried out using the Fixed-effects model with NCSS Software (NCSS, Kaysville, UT, 2006). This analysis involved the calculation of a set of two-group, binary-event studies summarized from the published studies. In addition to limiting our search to reports of *Ralstonia* respiratory infections in the USA, our analysis required that case controls were reported (Jhung, 2007, used matched case controls).

RESULTS

Selection of Reports for Meta-Analysis

Four of the six published reports in this analysis contained colonization data and four of the reports contained infection data. A Morbidity and Mortality Weekly Report (MMWR) document from 1983 reported on an outbreak (multiple cases of culture-positive Ralstonia regardless of patient status, e.g., infection or colonization) in a special-care nursery traced to a contaminated respiratory therapy solution (no colonization detected in the prior 15 months).[2,14] The single dose vials of normal saline were under recall because of intrinsic bacterial contamination and were used in both infant and adult patients; no colonization was found in the adults under surveillance. Of the 12 reported infant patients, 11 were on mechanical ventilation and five of those were positive for *Ralstonia* colonization (one positive colonization without mechanical ventilation). Six of the seven control patients had endotracheal tubes. None of the 12 patients developed symptomatic *Ralstonia* infection.

An outbreak of multi-drug resistant Pseudomonas pickettii (now *Ralstonia* pickettii) in an 87-bed neonatal intensive care unit over a 26 month period was reported by Timm, et al, in 1995.[22] The ten premature infants with respiratory colonization had no extrapulmonary infections and all were intubated, had multiple

intravascular catheters, and patent ductus arterioses. Six of the ten patients developed P. pickettii pneumonia (one associated death). This particular P. pickettii was resistant to aminoglycosides, several extended spectrum beta-lactams and cephalosporins (some isolates were also resistant to ceftazidime and ticaricillin/clavulanate). The source of contamination was thought to be a vinegar cleaning solution used on the exterior of the infant isolettes.

Burns et al, reported on specimens from 595 patients with stable, non-exacerbated cystic fibrosis of which 559 were tested for gram negative pathogens as part of a phase III national collaborative study of aerosolized tobramycin from July 1995 through September 1996.[23] Only two (0.3%) of the specimens were positive for R. pickettii colonization and one was highly tobramycin-resistant.

A report of R. pickettii colonization associated with intrinsically contaminated normal saline solution (used for respiratory therapy) was published in MMWR (April 1998).[3] Seventeen patients (age range 4 days to 17 years) were culture positive for *Ralstonia* pickettii. The outbreak was associated with a contaminated saline solution used for endotracheal suctioning. Responding to the 1998 MMWR article request to report R. pickettii colonization or infection, Labraca and colleagues reported a total of 34 patients from four hospitals (three pediatric and one adult) colonized with R. pickettii over a three month period in 1999.[25] Twenty-six percent of these patients developed an infection and all had received mechanical ventilation. The source of contamination was identified as the intrinsically contaminated "sterile" normal saline solution identified in the prior MMWR report.

Jhung, et al,[26] reported both a case control cohort of 5 individuals at a single center and 38 patients nationally who were confirmed with culture positive R. mannitolilytica, many of whom had been previously on a respiratory device made by Vapotherm, Inc. These findings led to a recall of the product in 2005.

Results of meta-analyses

The summary results for colonization are shown in Table 1 and Figure 1 and the infection results are shown in Table 2 and Figure 2. The data indicate that the risk of infection or colonization is higher in the Treatment Group (mechanical ventilation group) compared to the control group (no mechanical ventilation). The wide 95% confidence intervals (CI) are due to the fact that the individual studies have small numbers. The overall average from combining all the studies is the statistic of interest. The first odds ratio was the ratio of the odds of *Ralstonia* colonization occurring in the pooled group of patients with MV to the odds of it occurring in the pooled group without MV. The second odds ratio was the odds of *Ralstonia infection* occurring in the pooled group of patients with MV to the odds of it occurring in the pooled group without MV. The odds of developing colonization with *Ralstonia* was eight times higher in those mechanically ventilated compared to those not mechanically ventilated. The patients in the pooled group who were on mechanical ventilation had 6 times the likelihood of developing *Ralstonia* infection compared to the rest of the cohort that didn't receive mechanical ventilation.

DISCUSSION

The current meta-analysis was conducted to examine one specific host factor, mechanical ventilation, and its associated risk for *Ralstonia* colonization and infection. These results suggest that patients receiving mechanical ventilation may be at increased risk for Ralstonia colonizations and infections. Our examination of these data offers the possibility that individuals who are being mechanically ventilated are more likely to develop Ralstonia-related infection. These findings have logical implications for treatment response.

It is well-accepted that, over time, the presence of endotracheal tubes for mechanical ventilation increases the risk for bacterial colonization and development of ventilator-associated pneumonias (VAP). Similarly, it is conceivable that *Ralstonia*, a water-borne bacteria cultured from respiratory devices, may have the capacity to colonize the airways; their capacity to generate biofilms, similar to Pseudomonas sp., have been recently reported.[27-28] While not specifically reviewed in this manuscript, it is worth speculating if intraspecies antibiotic resistance patterns may apply to the ongoing Ralstonia resistance, as is seen in other bacteria. Our data does hint at increased infection from *Ralstonia* in

Table 1. Colonization in the Respiratory Tract with *Ralstonia*.
The odds ratios shown are the odds of developing a *Ralstonia* colonization while on mechanical ventilation compared to non-mechanical ventilation. The 95% CIs are for the odds ratio and if it includes a value of 1.0 then the odds ratio is not significantly different from 1.0. The percent weight is the percent weight given by the meta-analysis when calculating the average values. The statistical procedures base this weight on sample sizes.

Study Name	Patients with Ralstonia	Odds Ratio	95% Lower CI	95% Upper CI	Percent Weight
MMWR 1983	12	34.48	2.03	586.04	22.47
MMWR 1998	17	10.32	4.23	25.22	30.96
Burns et al.	2	125.25	1.40	11219.63	15.37
Jhung et al.*	5	0.46	0.20	1.02	31.21
Weighted Average	---	**8.14**	**1.20**	**55.22**	**100.00 (Total)**

*Only patients that included mechanical ventilation data were used from this multi-site report.

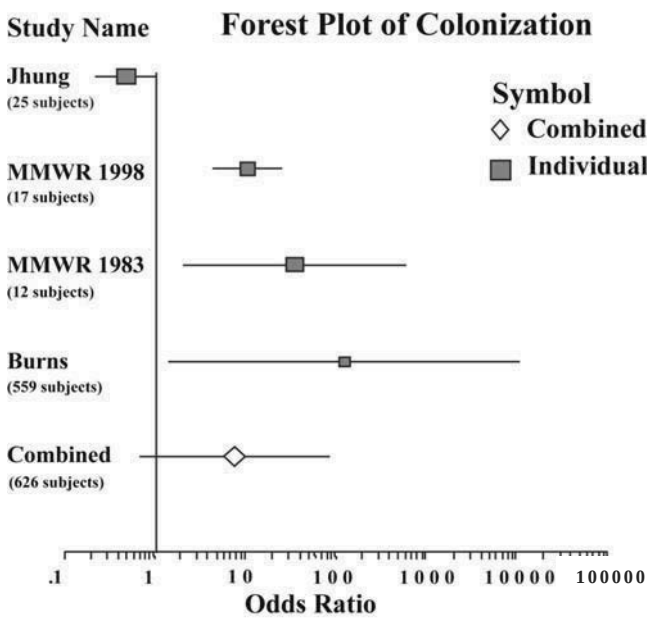

Figure 1. Odds ratios for colonization with *Ralstonia*.

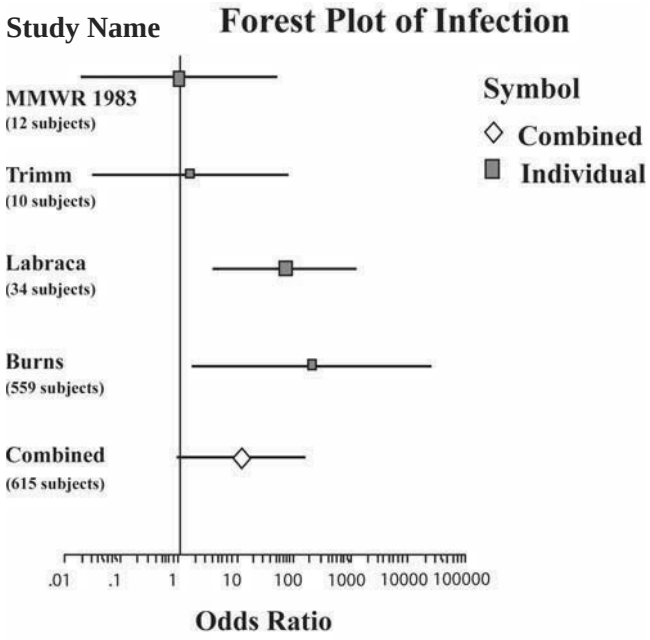

Figure 2. Odds ratios for infection with *Ralstonia*.

Table 2. Infection in the Respiratory Tract with *Ralstonia*.
The odds ratios shown are the odds of developing a Ralstonia infection while on mechanical ventilation compared to non-mechanical ventilation. The 95% CIs are for the odds ratio and if it includes a value of 1.0 then the odds ratio is not significantly different from 1.0. The percent weight is the percent weight given by the meta-analysis when calculating the weighted average values. The statistical procedures base this weight on sample sizes.

Study Name	Patients with Ralstonia	Odds Ratio	95% Lower CI	95% Upper CI	Percent Weight
MMWR 1983	12	1.00	0.020	50.89	29.82
Timm et al.	10	1.49	0.029	76.82	29.73
Labraca et al.	15	72.67	4.36	1211.39	40.45
Weighted Average	---	6.38	0.34	118.53	100.00 (Total)

those individuals who are mechanically ventilated, although this may be associated with mechanical ventilation as a marker of overall health status of these individuals.

Despite the results from our meta-analysis and other studies to help in clarifying *Ralstonia* status of patients, many clinicians continue to treat all of these bacterial isolates. Standard microbiological examination of biologic specimens (blood, urine, sputum) does not efficiently identify *Ralstonia* and may lead to misidentification of the organism. Therefore high clinical suspicion in susceptible populations may serve as a trigger for more in-depth examination for *Ralstonia*. In order to aid these caregivers, we have created a possible approach to the culture-positive *Ralstonia* patient (see Figures 3 and 4). Given an emerging concern for increased incidence of both *Ralstonia* and phylogenically similar (i.e., Pseudomonas sp., Burkholderia sp.) bacterial infection in immune-compromised hosts,[29-30] these decision trees start by dividing patients into either immunocompetent or immune-compromised. For immunocompetent patients, if there are clinical signs of infection, respiratory devices and IVs are replaced and antibiotics are started. In those individuals who demonstrate no signs of infection but are being mechanically ventilated, respiratory devices should be changed but no antibiotics should be started. In immunocompromised patients, it is crucial to correctly identify organisms by species-specific polymerase chain reaction (PCR) testing and to treat immediately upon the first signs of symptoms of infection (PCR specificity 99-100% for several *Ralstonia* species).[9-31] While this technique is not readily available throughout the USA, the importance of correct identification of the organism necessitates additional efforts for these results.

The small number of reports in the literature on respiratory colonizations and infections (those individuals who are culture-positive and exhibit signs of infection) associated with the *Ralstonia* organism is a limitation of this meta-analysis but it is a telling sign in itself. Respiratory infections due to *Ralstonia* do not appear to be common in the USA; at our university medical center there was no incidence of *Ralstonia* pickettii in microbiologic cultures over a ten year period

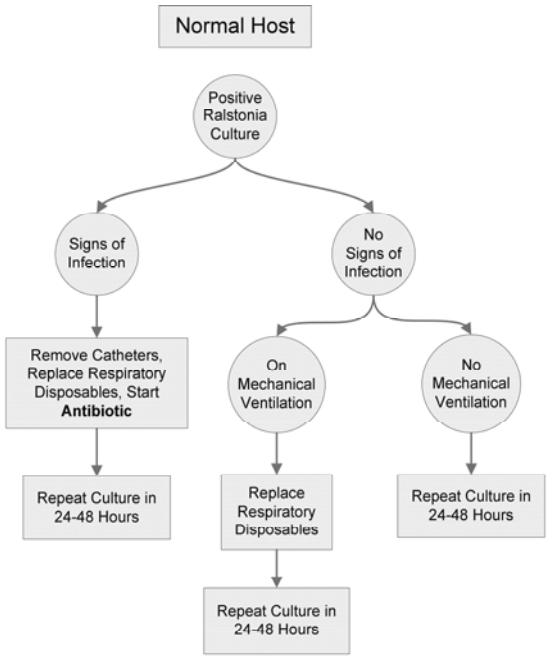

Figure 3. Decision chart for immunocompetent individuals.

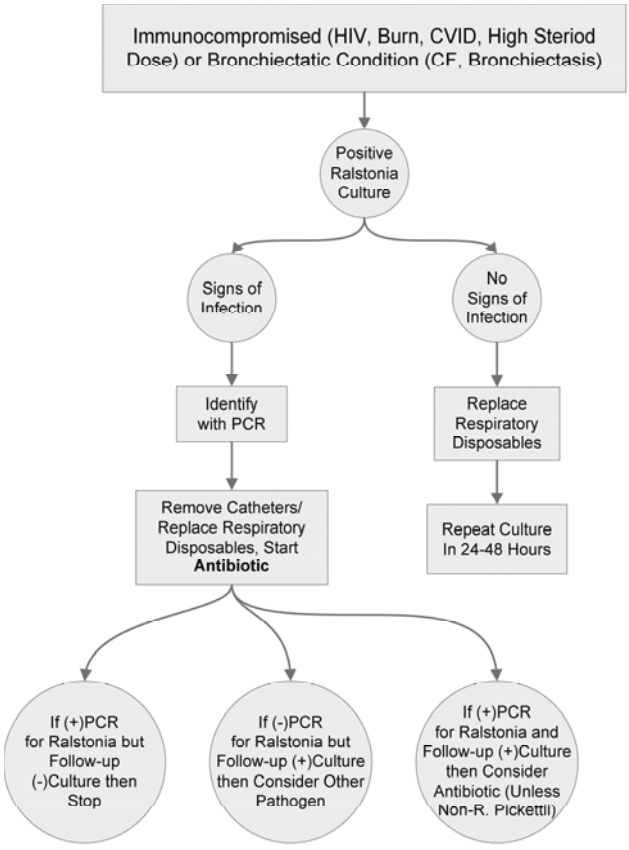

Figure 4. Decision chart for immunocompromised individuals.

(1997-2007). This may, in part, be due to the use of automated microbiological identification systems for identification of *Ralstonia* spp., which seem to have inadequate specificity. *Ralstonia* sp., are not common organisms in the clinical setting partly because surveillance in large scale should be performed to clarify the current position of the organism in various kinds of respiratory specimens. As previously mentioned, the patients suffering from *Ralstonia* infection in the available reports typically had weak/compromised immune responses, supporting the approach of utilizing this host factor as a means to stratify individuals in the appropriate identification and treatment of *Ralstonia* as an opportunistic organism.[10] To date there are only three reported cases of *Ralstonia* sepsis and they were in patients with immune insufficiency.[32]

Our results suggest that individuals who are currently on mechanical ventilation and are *Ralstonia* culture-positive may have increased propensity to the development of infection. Here, we provide two logical decision trees, taking into account host factors, clinical signs of infection, and mechanical ventilation status to aid the healthcare provider in approaching diagnosis and therapy for this organism. While interpatient transmission has not been well documented,[29] a common reservoir among multiple patients may serve to foster outbreaks (a reason to screen other patients in proximity). This data is suggested by various outbreaks reported with exposure to respiratory devices in the ICU. Our findings also support that simply culturing the organism from respiratory care devices in a mechanically ventilated patient is an inadequate stimulus to consider *Ralstonia*-specific-therapy. Rather, these devices should be replaced (i.e., ventilator tubing, etc.) and the patient should be recultured. In addition, a low-threshold for therapy should be applied to individuals exposed to possible contaminated reservoirs with immunocompromised states.

In conclusion, our data suggest that although rare, the presence of *Ralstonia* in clinical culture correlated with the use of mechanical ventilation. There was a significant correlation between mechanical ventilation and colonization. Given the potential for both a missed diagnosis and misdiagnosis, we presented a novel approach to surveillance and treatment of *Ralstonia pickettii* in susceptible populations. These findings will hopefully assist clinicians in the efficient identification and treatment of this organism.

ACKNOWLEDGEMENTS

The authors would like to thank Lee Vucovich, MS, MLS, AHIP; Assistant Director for Reference Services at the Lister Hill Library of the Health Sciences of the University of Alabama at Birmingham; for her assistance with searching electronic databases and George A. Fritsma, MS, MT(ASCP); for his careful review of the manuscript, and Linda Waugh for assistance with figure artwork.

REFERENCES

1. *Ralstonia* associated with Vapotherm oxygen delivery device--United States, 2005. MMWR Morb Mortal Wkly Rep 2005; 54(41):1052-3.
2. Pseudomonas pickettii colonization associated with a contaminated respiratory therapy solution--Illinois. MMWR Morb Mortal Wkly Rep 1983; 32(38):495-6, 501.
3. Nosocomial *Ralstonia* pickettii colonization associated with intrinsically contaminated saline solution--Los Angeles, California, 1998. MMWR Morb Mortal Wkly Rep 1998; 47(14):285-6.
4. Gilligan PH, Lum G, Vandamme PAR, Whittier S. *Burkholderia, Stenotrophomonas*, *Ralstonia*, *Brevundimonas, Comamonas, Delftia, Pandoraea* and *Acidovorax*. In: Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH, editors. Manual of clinical microbiology. Washington, DC: American Society for Microbiology 2003:729-48.
5. Verschraegen G, Claeys G, Meeus G, Delanghe M. Pseudomonas pickettii as a cause of pseudobacteremia. J Clin Microbiol 1985; 21(2):278-9.
6. Oie S, Kamiya A. Bacterial contamination of commercially available ethacridine lactate (acrinol) products. J Hosp Infect 1996; 34(1):51-8.
7. Coenye T, Goris J, De Vos P, Vandamme P, LiPuma JJ. Classification of *Ralstonia* pickettii-like isolates from the environment and clinical samples as *Ralstonia* insidiosa sp nov. International Journal of Systematic and Evolutionary Microbiology 2003; 53:1075-80.
8. De Baere T, Steyaert S, Wauters G, De Vos P, et al. Classification of *Ralstonia pickettii* biovar 3/'thomasii' strains (Pickett 1994) and of new isolates related to nosocomial recurrent meningitis as *Ralstonia* mannitolytica sp nov. International Journal of Systematic and Evolutionary Microbiology 2001; 51:547-58.
9. Coenye T, Vandamme P, LiPuma JJ. Infection by *Ralstonia* species in cystic fibrosis patients: identification of r. pickettii and r. mannitolilytica by polymerase chain reaction. Emerging Infectious Diseases 2002; 8(7): 692-6.

10. Ryan MP, Pembroke JT, Adley CC. *Ralstonia pickettii*: a persistent Gram-negative nosocomial infectious organism. Journal of Hospital Infection 2006;62(3):278-84.
11. Kahan A, Philippon A, Paul G, Weber S, et al. Nosocomial infections by chlorhexidine solution contaminated with Pseudomonas pickettii (Biovar VA-I). J Infect 1983; 7(3):256-63.
12. Roberts LA, Collignon PJ, Cramp VB, Alexander S, et al. An Australia-wide epidemic of Pseudomonas pickettii bacteraemia due to contaminated "sterile" water for injection. Med J Aust 1990; 152(12):652-5.
13. Chetoui H, Melin P, Struelens MJ, Delhalle E, et al. Comparison of biotyping, ribotyping, and pulsed-field gel electrophoresis for investigation of a common-source outbreak of Burkholderia pickettii bacteremia. J Clin Microbiol 1997; 35(6):1398-403.
14. McNeil MM, Solomon SL, Anderson RL, Davis BJ, et al. Nosocomial Pseudomonas pickettii colonization associated with a contaminated respiratory therapy solution in a special care nursery. J Clin Microbiol 1985; 22(6):903-7.
15. Stelzmueller, I., M. Biebl, Wiesmayr S, Eller M, et al. *Ralstonia pickettii* - innocent bystander or a potential threat? Clinical Microbiology and Infection 2006;12(2): 99-101.
16. Zellweger, C., T. Bodmer, Tauber MG, Muhlemann K. Failure of ceftriaxone in an intravenous drug user with invasive infection due to *Ralstonia pickettii*. Infection 2004;32(4):246-8.
17. Nordmann, P., L. Poirel, Kubina M, Casetta A, Naas T. Biochemical-genetic characterization and distribution of OXA-22, a chromosomal and inducible class D beta-lactamase from *Ralstonia (Pseudomonas) pickettii*." Antimicrob Agents Chemother 2000;44(8): 2201-4.
18. McKenney D, Brown KE, Allison DG. Influence of *Pseudomonas aeruginosa* exoproducts on virulence factor production in *Burkholderia cepacia*: evidence of interspecies communication." J Bacteriol 1995; 177(23): 6989-92.
19. Mahenthiralingam E, Urban TA, Goldberg JB. The multifarious, multireplicon *Burkholderia cepacia* complex. Nat Rev Microbiol 2005; 3(2): 144-56.
20. Hedrick TL, Smith PW, Gazoni LM, Sawyer, RG. The appropriate use of antibiotics in surgery: a review of surgical infections. 2007;Curr Probl Surg 44(10): 635-75.
21. Micozzi, A. and G. Bucaneve. Prophylaxis and treatment of bacterial infections: do we need new strategies? 2005; Rev Clin Exp Hematol 9(2): E4
22. Timm WD, Pfaff SJ, Land GL. An outbreak of multi-drug resistant Pseudomonas pickettii pneumonia in a neonatal intensive care unit. Proceedings of the 35th ICAAC 1995.
23. Burns JL, Emerson J, Stapp JR, Yim DL, et al. Microbiology of sputum from patients at cystic fibrosis centers in the United States. Clinical Infectious Diseases 1998; 27(1):158-63.
24. Talon D, Mulin B, Rouget C, Bailly P, et al. Risks and routes for ventilator-associated pneumonia with Pseudomonas aeruginosa. Am J Respir Crit Care Med 1998; 157(3 Pt 1): 978-84.
25. Labarca JA, Trick WE, Peterson CL, Carson LA, et al. A multistate nosocomial outbreak of *Ralstonia* pickettii colonization associated with an intrinsically contaminated respiratory care solution. Clinical Infectious Diseases 1999; 29(5):1281-6.
26. Jhung MA, Sunenshine RH, Noble-Wang J, Coffin SE, et al. A National outbreak of *Ralstonia mannitolilytica* associated with use of a contaminated oxygen-delivery device among pediatric patients. Pediatrics 2007; 119: 1061-8.
27. Ivnitsky, H., Katz I., Minz D, Volvovic G, et al. Bacterial community composition and structure of biofilms developing on nanofiltration membranes applied to wastewater treatment. Water Res 2007;41(17): 3924-35.
28. Kang, Y., H. Liu, Genin S, Schell MA, Denny TP. *Ralstonia solanacearum* requires type 4 pili to adhere to multiple surfaces and for natural transformation and virulence. Mol Microbiol 2002;46(2):427-37.
29. Lipuma JJ. *Burkholderia* and emerging pathogens in cystic fibrosis. Seminars in Respiratory and Critical Care Medicine 2003; 24(6): 681-92.
30. Woo PCY, Wong SSY, Yuen KY. *Ralstonia pickettii* bacteremia in a cord blood transplant recipient. New Microbiology 2002; 25(1): 97-102.
31. Kang MJ, Lee MH, Shim JK, Seo ST, et al. PCR-based specific detection of *Ralstonia solanacearum* by amplification of cytochrome c1 signal peptide sequences. J Microbiol Biotechnol 2007; 17(11): 1765-71.
32. Vitaliti SM, Maggio MC, Cipolla D, Corsello G. Neonatal sepsis caused by *Ralstonia pickettii*. Pediatr Infect Dis J 2008;27(3): 283.