Journal of Clinical and Nursing Research, 2026, Volume 10, Issue 1
http://ojs.bbwpublisher.com/index.php/JCNR
Online ISSN: 2208-3693
Print ISSN: 2208-3685

A Case of Ventilator-associated Pneumonia in Neonatology: Investigation and Analysis of the Source of *Ralstonia mannitolilytica* Infection

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Abstract: This study examined a case of ventilator-associated pneumonia caused by Ralstonia mannitolilytica in a neonatal department in order to determine the source of infection and mode of transmission. Symptoms of infection began to appear on day 16 of admission, and both sputum and catheter tip cultures revealed R. mannitolilytica. The infection control department of the hospital later sampled and identified R. mannitolilytica in the ventilator tube that was used by the patient with the exact gene sequence as the infecting strain. These findings indicate that ventilator tubing is a significant contamination source of this pathogen, and hospitals need to enhance their disinfection methods of ventilator-related devices.

Keywords: Neonatology; Ventilator-associated pneumonia; Ralstonia mannitolilytica; Infection source; Hospital infection control

Online publication: Feb 9, 2026

1. Introduction

1.1. Background of research

Ventilator-associated pneumonia (VAP) is pneumonia that develops following the placement of an artificial airway, either endotracheal intubation or tracheostomy, and the initiation of mechanical ventilation > 2 consecutive calendar days, including those where mechanical ventilation was initiated within 48 hours before the development of pneumonia [1]. VAP is a prevalent nosocomial infection in the neonatal intensive care unit (NICU) and is particularly prevalent in preterm or critically ill infants undergoing mechanical ventilation. Not only do this condition increase hospitalization and increase medical expenses, but can also lead to serious complications and even death. An evaluation of the global literature reveals that the occurrence rates of VAP among neonates have a broad spectrum of 2.7–37.2 cases per 1000 ventilator days [2]. Domestic surveys in China have also reported incidence rates of 12.5 to 58.4 and a mortality of 18.1 to 33.3% [3–5]. The broad variation in the incidence rates reported in the various hospitals

could be attributed to challenges in defining VAP, differences in the patient populations or unit-specific issues such as patient acuity, admission rates, and preventive measures. Since VAP may result in serious issues during ventilator weaning, the duration of hospital stays, medical expenses and even the survival of neonatal life in severe cases, it is important to prevent it as early as possible, diagnose it in time and treat it immediately. The main interventions towards VAP management are the rational use of antibiotics to minimize multidrug-resistant bacterial and fungal infections, the use of aseptic practices, and the minimization of the time spent on mechanical ventilation.

Ralstonia mannitolilytica is an opportunistic pathogen, which has been given more attention in hospital-acquired infections, but there are few reports in China. The available literature has indicated that this bacterium exhibits a high level of environmental flexibility and possible multidrug resistance (MDR), and hence a significant risk to immunocompromised patients and a significant contributor to the spread of the bacterium in healthcare institutions [6].

1.2. Research objective

This paper seeks to examine a case of VAP due to *R. mannitolilytica* in the neonatology department, the objectives of which are to determine the cause of the infection, and to learn how the infection spreads in the NICU. The results are supposed to be used as a guideline in preventing such instances of infections in the future.

2. Materials and methods

2.1. Case data

2.1.1. Patient data

The patient was a female who was very preterm with birth weight of 640 g. Our hospital was chosen to deliver the baby through cesarean section due to maternal conditions of "1. Acute fetal distress; 2. Pregnancy with identical twins (monochorionic diamniotic)." The patient was an extremely preterm female infant with a birth weight of 640 g. She was delivered by cesarean section in our hospital because of maternal complications, including

(1) Acute fetal distress

(2) Monochorionic diamniotic

Apgar scores were 6 minutes, 9 minutes, and 9 minutes. The amniotic fluid volume was normal and clear at birth. The placenta was delivered intact and the umbilical cord was not wrapped around the neck. The infant had no crying at birth and no meconium staining was seen. Admission diagnoses included the following:

(1) Extremely preterm infant;

(2) Extremely low birth weight infant;

(3) Neonatal respiratory distress syndrome; and

(4) Neonatal respiratory failure.

2.1.2. Condition changes

On the day of admission, after emergency cesarean section delivery, the infant was transferred to the neonatology department and assisted with oxygen via nasal catheter. On day 2 (August 8), the invasive mechanical ventilation was started. The invasive ventilator was taken off on day 8 and replaced with non-invasive ventilation. On day 10 post-admission, the infant developed a fever, with a temperature of 37.9 ℃. On day 14 the fever rose again, reaching a maximum of 38 ℃, with tachypnea and moist rales bilaterally. Chest x-ray examination showed coarse bilateral lung markings. Additional symptoms were worsening respiratory distress and falling oxygenation. Auxiliary examinations and test results after admission are as follows:

Table 1. Ancillary examinations and laboratory tests for the infant

Test / date	8-7	8-8	8-10	8-13	8-15	8-17	8-22	8-27	9-1
White blood cell ($\times 10^9$/L)	5.24	13.10	7.76	11.89	19.71	29.8	17.2	13.26	8.63
Neutrophils (%)	21.9	34.6	39.7	22.6	46.1	65.2	71.5	62.7	42.5
CRP	< 0.20	< 5	28.34	26.2	< 5	< 5	10.08	< 5	< 5
Hypersensitive C-reactive protein	< 0.20	2.24	> 5	-	-	-	> 5	< 0.50	< 0.50
Specimen culture		Blood culture (-), Urine mycoplasma culture (-)			Endotracheal tube tip culture: *R. mannitolilytica*	Sputum culture: *R. mannitolilytica*	Sputum culture: *R. mannitolilytica*	Sputum culture: *R. mannitolilytica*	Sputum culture (Normal flora)
Bedside chest X-ray	-	Decreased transradiancy in both lungs compared to previous; Pulmonary infiltrates worsened compared to previous	Pulmonary infiltrates showed absorption and improvement	Pulmonary infiltrates increased compared to previous	Pulmonary infiltrates showed absorption and improvement compared to previous	Pulmonary infiltrates showed absorption and improvement compared to previous	Pulmonary infiltrates slightly worsened compared to previous	Partial absorption of infiltrates in the left upper lobe compared to previous; No significant change in infiltrates in the remaining lung fields	No significant absorption of pulmonary infiltrates compared to previous

2.2. Sample collection and processing

2.2.1. Patient samples

Patient Specimens: The tip of the endotracheal tube was collected at the time of extubation on day 8 after admission. Sputum specimens were collected on the day of onset of fever (day 14 post-admission). Bacterial culture and identification of both samples showed the presence of *R. mannitolilytica* in the extubated tube tip and sputum cultures. Antimicrobial susceptibility test results on day 15 indicated that the isolate was sensitive to levofloxacin and cefepime but resistant to ampicillin and ceftriaxone. Following positive results from both the sputum and the tube tip cultures, more specimens including sputum, blood, urine and stool were taken for bacterial and fungal cultures.

2.2.2. Ventilator circuit samples

Following the second sputum culture that was positive for *R. mannitolilytica*, the Hospital Infection Control Department immediately restricted access to the intensive care unit (ICU) with the infant (who had already been isolated in a single room after the initial detection). Samples were collected from the ventilator's internal circuits, humidifier water tank and connection ports.

2.2.3. Environmental samples

Air samples were taken from the NICU ward and swabs from healthcare worker's hands and surfaces of frequently touched items.

2.3. Bacterial culture and identification

All samples were labeled with collection time, location and identity of the collector before being sent to the clinical laboratory. Isolation and culture were performed using standard bacteriological protocols. Bacterial identification was performed by matrix-assisted laser desorption ionization-time of flight mass spectrometry and by 16S rRNA gene sequencing.

2.4. Epidemiological investigation

The Hospital Infection Control Department, in association with the head of the Neonatology Department, the attending physician, and the primary nurse, made a retrospective analysis of all the diagnostic and treatment procedures during the hospitalization of the infant. The investigation was concentrated on specific time points, looking at personnel who had contact with the infant, the use of medical equipment and the management of the ward environment.

The sampling by the Hospital Infection Control Department showed that the *R. mannitolilytica* was isolated from the sputum of the infant and also from the endotracheal tube tip cultures. The isolated bacteria showed resistance to antibiotics such as ceftriaxone and amoxicillin/clavulanic acid. Meanwhile, blood, urine and stool cultures taken from the infant were negative.

3. Results

3.1. Sample test results of the ventilator circuit

Clinical laboratory results showed the presence of *R. mannitolilytica* in the humidifier water tank and internal

circuits of the ventilator.

3.2. Environmental sampling tests results

The clinical laboratory findings revealed that the *R. mannitolilytica* could not be found in the environmental samples of the NICU ward or on the hands of medical personnel or on the surfaces of the most touched objects.

4. Discussion

4.1. Analysis of the source of infection

In this study, which used environmental hygiene sampling and bacterial culture, *R. mannitolilytica* infection of the infant was identified to have been caused by the ventilator circuit. These results suggest that the ventilator circuits may be colonized and grow bacteria in the long-term of their use, especially in a humidifier water tank, which is most likely to be contaminated by bacteria.

4.2. Discussion of the route of transmission

The colonization of the ventilator circuit by *R. mannitolilytica* and the subsequent transmission of the infection to the respiratory tract of the infant through the ventilator was identified to be the greatest cause of infection. The current studies have classified risk factors that lead to development of VAP into two broad categories. The former is the first category, which includes patient-related variables like age, severity of underlying conditions and comorbidities. The second category is the iatrogenic ones, including medical interventions, treatment modalities, and drug interventions. Some of the factors that predisposed the infant to VAP in this instance were extremely low birth weight, gestational age < 32 weeks, a number of intubations, coexistent neonatal sepsis, and the use of acid-reducing agents. Together, long-term mechanical ventilation, and the immunocompromised condition of the infant were all important risk factors of infection. Also, the conditions in the NICU ward were most likely favourable to the growth of bacteria due to the high humidity of the environment.

4.3. Characteristics of R. mannitolilytica

R. mannitolilytica is an opportunistic pathogen and its biological properties enable them to survive and proliferate in a hospital over an extended period of time. Its incidence in healthcare facilities has been increasing annually particularly in high-risk units of the healthcare facility like ICUs, burn wards and respiratory units [7].

It has also been demonstrated that these bacteria may be spread using contaminated medical equipment, including ventilators and catheters, or by hand contact with healthcare providers, leading to nosocomial cross-infections. Patients with long-term broad-spectrum antibiotic or immunosuppressive therapy are more frequently reported to have infections. *R. mannitolilytica* is regarded as a mostly pathogenic bacterium due to its virulence factors that are secreted [8]. It has been found to release cytotoxins and invasive enzymes that have the potential to interfere with the host cell barriers. Over the last few years, there has been a significant rise in the number of isolates containing MDR and the expression of extended spectrum β-lactamases, complicating clinical management. Although antibiotic treatment is the primary mode of treating *R. mannitolilytica* infections, it is important to choose the appropriate agents due to the resistance trends. Research indicates that some strains are sensitive to carbapenems (e.g. imipenem) and combination therapy (cefoperazone/sulbactam). At the same time, the enhancement of the environmental cleaning of the hospital and the strict hand hygiene of the healthcare

workers is one of the key steps to prevent and control the spread of this pathogen.

4.4. Preventive interventions

In the light of this VAP case, the following bundled intervention plan is suggested:

(1) Strengthen disinfection management of ventilator circuits
Change ventilator circuits and humidifier water tanks regularly to prevent the use of one and the same equipment.

(2) Maximize humidifier control
Inject sterile distilled water or sterile water as humidification fluids, and do not store humidification water long.

(3) Standardize hand hygiene and aseptic techniques
Enhance hand hygiene awareness and compliance levels among medical personnel to minimize the chance of cross-infection.

(4) Strengthen environmental surveillance
Conduct regular microbiological surveillance of the NICU environment and equipment to aid in the rapid identification and correction of possible contamination sources.

5. Conclusion

This research could determine the origin of *R. mannitolilytica* infection in a neonatal VAP case as the ventilator circuit through the use of case investigation, laboratory bacterial culture and antimicrobial susceptibility analysis. This is a critical finding on which the improvement of hospital infection prevention and control strategies is based. The next step in preventing neonatal VAP should be put on enhancing disinfection and control of ventilator-related equipment to lower the rate of the disease.

Disclosure statement

The authors declare no conflict of interest.

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