Rhodococcus equi Infection in a Surgical Wound

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ABBREVIATIONS: ED= Emergency Department; WBC= White Blood Cell Count; BAP= Sheep's Blood Agar Plate

INDEX TERMS: *Rhodococcus equi,* surgical wound infections

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ABSTRACT: A 35-year-old male presented with abdominal pain one month after receiving a routine ventral hernia repair. Over the course of two months, repeated wound cultures were ordered and eventually produced growth of *Rhodococcus equi*. Appropriate antibacterial therapy was initiated to resolve the infection.

OBJECTIVES: Review the history, pathogenesis, diagnosis, and treatment of non-pulmonary *R. equi* infections; inform laboratory professionals of the possibility and severity of *R. equi* infections, and what can be done to facilitate prompt diagnosis and recovery.

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A 35-year-old diabetic male received routine day surgery to repair a ventral hernia. The surgical wound, upon follow-up visits, appeared to be healing properly. One month later, however, the patient presented to the ED with complaints of abdominal pain, after the patient had received an undisclosed minor trauma to the wound, while working outdoors for the local school department. The patient began having occasional feverish episodes along with erythema and the drainage of purulent material from the wound thereafter. There was no evidence of a pathogenic infection in the wound, as cultures yielded only 'normal skin flora', which included coagulase negative *Staphylococcus* and diphtheroids. Cephalexin was prescribed and the patient returned home.

One week later, the patient returned to the ED with what was described as a "non-healing surgical wound". The wound was cleansed and packed with gauze. With both wound and blood cultures appearing negative for significant bacterial growth, anti-fungal treatment was initiated due to the patient's continued failure to heal under the antibiotic treatment.

Due to the persistent infection, the wound was surgically reopened two weeks later, and the infected periumbilical patch was removed by an open approach. A gram stain from a sterile swab of the wound yielded no organisms. Two days later, the patient returned to the ED with severe abdominal pain. Additional microbiological workup was ordered on wound specimens collected using sterile swabs and anaerobic culturettes for both aerobic and anaerobic cultures, along with acid fast stains and cultures. Table 1 outlines the numerous tests ordered along with their corresponding findings.

LABORATORY RESULTS

The area surrounding the surgical wound was largely inflamed and erythemic with purulent discharge coming from the wound site. The patient's WBC count was slightly elevated at 13.7 x10^3/uL (3.9-10.0 x10^3/uL), though he was afebrile. Swabs taken from the wound, now over a month after the infection began, grew small, mucoid colonies on BAP, which at nearly 4 days after planting, developed a distinctive pink pigmentation. The colonies were gram-stained and showed long, gram-positive bacilli. An acid-fast stain was performed on the colonies, and the organisms were variably positive.

The organisms were catalase positive, oxidase negative, and urea positive. A CAMP test on BAP was performed using a streak of *Staphylococcus aureus* with a perpendicular streak of the organism in question. A striking zone of beta-hemolysis was noted near the intersection of the two streaks. A Remel™ Rapid CB Plus strip was inoculated yielding a 99.9% identification of *Rhodococcus equi*. The culture was sent to the State Laboratory for confirmation, and a preliminary identification of *R. equi* was reported. The State Laboratory later verified the identification.

BACKGROUND

The genus *Rhodococcus* is in the family *Nocardiaceae* in the suborder *Corynebacterineae* of the *Actinomycetales* order.[1] *R. equi* was originally termed *Corynebacterium equi* due to its morphological characteristics representing diphtheroids.[1] It wasn't until 1980, however, that the organism's cell wall composition and biochemical reactions were found to be more closely related to *Nocardia* and *Mycobacterium* than *Corynebacterium*, that the genus was changed to *Rhodococcus* ("red-pigmented coccus). [2,3]

Rhodococcus equi can be found in all continents of the world except Antarctica, and can flourish in both fresh and saltwater environments, and also within the intestines of bloodsucking arthropods.[4] *R. equi*, however, is more commonly associated with zoonotic infections mostly from horses, but also sporadically from cattle, sheep, pigs, goats, deer, dogs, wild birds and even cats.[3,4,5] *R. equi* colonizes the gastrointestinal tract of grazing mammals, and can be isolated from the manure and soil.[4] Exposure to *R. equi* can be via the oral route by ingesting products contaminated with soil or manure, inhalation of airborne organisms in dust, or direct inoculation due to trauma with soil or manure containing the organism.[5]

Table 1: Wound Cultures and Tests Ordered. There were no issues associated with the surgery, performed on 9/10, and for a month thereafter. However, after the patient received trauma to the wound, perhaps contaminating the wound with dirt, an infection began that persisted for nearly two months. This table outlines the course taken, involving repetitive wound cultures and gram stains, until the final, confirmed ID of *Rhodococcus equi* was reported, and the patient was properly treated allowing for full recovery.

Date	Test Ordered	Result
10/5	Wound Gram Stain	Few WBC No organisms seen
	Wound Culture	Light growth "Skin Flora" (Coagulase negative *Staphylococcus* & diphtheroids)
10/12	Wound Gram Stain	Few WBC No organisms seen
	Wound Culture	Light growth "Skin Flora"
	Blood Culture	No Growth @ 5 days
10/29	Wound Gram Stain	Few WBC No organisms seen
	Wound Culture	Light growth "Skin Flora"
10/30	Wound Gram Stain	Few WBC No organisms seen
	Anaerobic Wound Culture	No Anaerobes Isolated
	Blood Culture	No Growth @ 5 days
	Wound Culture	Positive growth with *Rhodococcus equi* (Preliminary ID reported 11/6)
11/6	Wound Culture (reference laboratory)	*Rhodococcus equi* (Confirmatory ID reported on 11/25

About 80-90% of patients with *R. equi* infections are immunocompromised.[6] Causes for immunosuppression can range from the most commonly seen AIDS/HIV, to malignancy, transplantation, chronic renal disease, alcoholism, immunosuppressive therapy (including prednisone, azothiopine and corticosteroid therapies) or, as in this case, diabetes mellitus.[5,6,7] On rare occasions, however, an infection with *R. equi* may be acquired in the immunocompetent. About 50% of the cases in the immunocompetent patient are due to trauma.[7]

Rhodococcus equi infections have ranged from necrotizing pneumonia, to "wound infections, subcutaneous abscess, thyroid abscess, retroperitoneal abscess, peritonitis, osteomyelitis, endophthalmitis, lymphadenitis, lymphangitis, septic arthritis, osteitis, bloody diarrhea, and fever of unknown origin among others."[7]

Rhodococcus equi is a facultative intracellular pathogen.[5] The pathogenicity of *R. equi* is based upon its ability to infect and live within macrophages, inhibiting their phagocytic capabilities, and eventually destroying them.[5,6] *R. equi* will cause inflammation, cell destruction and purulent granulomas. *R. equi* has the ability to disseminate from an initial infection site to many other sites in the host.[5] Due in part to its intracellular capability, *R. equi* is sometimes difficult to fully eradicate, and is commonly associated with relapse.[1]

Infections with *Rhodococcus equi* are associated with significant mortality due to the difficulty to eradicate the organism. The overall mortality rate of these infections is 25%, 50-55% in HIV patients, 20-25% in non-HIV, immunocompromised patients, and 11% in immunocompetent patients.[2] There are no racial differences in incidence of *R. equi* infections, however, there is a 3:1 male to female ratio, and the mean age of infection is 34-38 years old.[7] The strong prevalence of infection in males of this age group may be largely due to the fact that many occupations susceptible to soil and manure exposure, such as farming and landscaping, are mostly dominated by this population. A history of direct exposure to horses or pigs, however, is only present in one third of all patients with *R. equi* infections.[6]

LABORATORY IDENTIFICATION

Due to numerous shared characteristics, *Rhodococcus* is commonly misidentified as diphtheroid contaminant and normal flora, *Mycobacterium, Nocardia, Bacillus, Micrococcus* organisms or even fungi[6]. The organism's acid-fast nature can commonly result in a misidentification of other acid fast bacterium such as *M. tuberculosis*, which often causes more trouble for the patient's recovery. More often, however, *R. equi* is overlooked or ignored due to its diphtheroid-like morphology and the slow development of its characteristic pigmentation, often leading to the misdiagnosis as a contaminant.[6] *R. equi* can also be confused with *Nocardia* spp. because of its acid-fast nature, and fungal characteristics, such as the formation of aerial hyphae.[7] A misidentification of *R. equi* could lead to inappropriate antibiotic treatment, and if left untreated, death.

R. equi grows well on nonselective media when incubated aerobically at 37° C. On blood agar plates, large, smooth, irregular, mucoid colonies can appear within 48 hours, how-

Table 2: Reactions of *Rhodococcus equi*

Test	Reaction
Gram Stain	Gram positive bacilli
Ziehl-Neelsen acid-fast stain	Variably positive
Motility	Negative
Catalase	Positive
Urea	Positive
Alpha-Glucosidase	Positive
Alkaline Phosphatase	Positive
Nitrate Reduction	Positive
Oxidase	Negative
Citrate	Negative
Indole	Negative
Esculin	Negative
"Equi Factors" with CAMP method	Positive (zone of beta hemolysis)

ever less mucoid forms are possible.[2,5] At 24 hours, colonies can be 1 to 2mm in diameter, though are not distinctive.[5] *R. equi* will not grow on most types of MacConkey agar.[5] Generally, with *R. equi,* a salmon to coral pink pigmentation will develop after 4-7 days of incubation and is rarely seen in cultures <4 days old, although, the colonies may also be light yellow to colorless.[2,5,6] As seen in Figure 1, the gram stain of *R. equi* reveals pleomorphic, gram-positive bacilli varying from coccoid to long, curved, clubbed forms, often resembling diphtheroids.[2] Gram stains made from cultures on solid media, or purulent material from the patient will often show more coccoid forms, however long rods and branching filaments may be observed in stains made from liquid media.[5] The organisms may be acid-fast with the Ziehl-Neelsen stain, however, this too depends on its growth media and the age of the culture.[2] *R. equi* is non-motile, non-fermentative, and positive for catalase, urease, alpha-glucosidase, alkaline phosphatase and nitrate reduction, but is negative for oxidase, indole, citrate assimilation and esculin hydrolysis.[1] *R. equi* has been known to produce "equi factors" that interact with the beta-toxin of *Staphylococcus aureus* to produce a zone of beta-hemolysis that can be observed using the CAMP technique on BAP.[2] (Fig. 2). Table 2 summarizes *R. equi*'s different reactions to numerous laboratory tests.

TREATMENT

Rhodococcus equi is frequently difficult to treat due to its ability to thrive within the macrophage thus inhibiting its bactericidal functioning. *R. equi* is, however, susceptible to erythromycin, ciprofloxacin, vancomycin, aminoglycosides, rifampin, imipenem, and meropenem. Resistance has been observed to penicillins, ampicillin, carbenicillin and treatment is not suggested with such drugs, even if the organism appears susceptible, as rapid acquisition of resistance is possible.[2,6] *R. equi* has also shown moderate resistance to both first and second-generation cephalosporins.[2,5] It is often recommended to treat *R. equi* infections using a synergistic approach, with drugs such as erythromycin and rifampin together.[2] Antibiotics and lipophilic drugs with intracellular penetration capabilities are the most beneficial in treating these infections.[2] Failures in treatment have been associated with the poor penetration of macrophages by drugs such as gentamicin and penicillin.[2] Many patients suffering from an infection with *R. equi* should receive intravenous antibiotics for a minimum of 2 weeks, after which oral antibiotics can be substituted and continued, as long as positive cultures and symptoms have resolved.[6]

Figure 1. *Rhodococcus equi* gram stain at 1000x , showing gram positive bacilli which appear similar to diphtheroids in morphology.

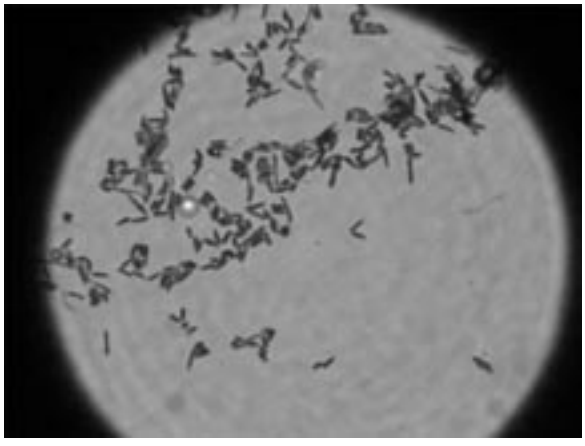
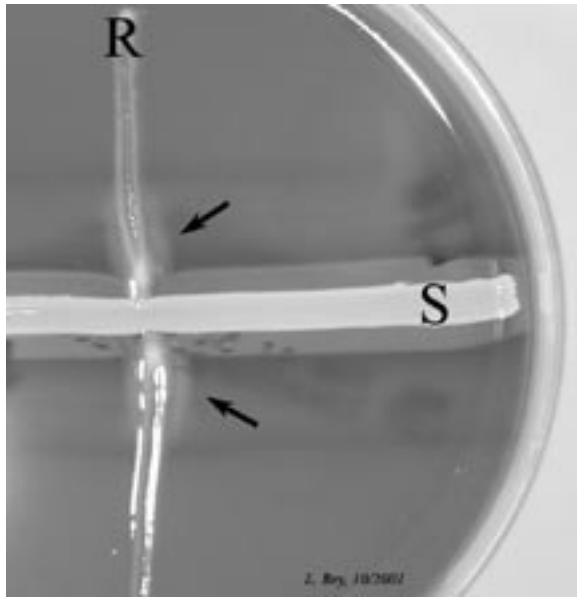


Figure 2. CAMP test for cholesterol oxidase produced by *R. equi*. R = Rhodococcus; S = lecithinase producing, hemolytic *Staphylococcus aureus*. Arrows point to additional zones of beta-hemolysis where secreted cholesterol oxidase works with lecithinase secreted by the Staphylococci to cause hemolysis of sheep RBCs in the media.



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DISCUSSION

This case classically defines the difficulties involved with the proper identification and treatment of *Rhodococcus equi* infections. Retrospective analysis of this case allows us to follow the diagnosis process; from a possible bacterial infection which failed to heal after treatment with cephalexin, to the question of a fungal invasion which failed to improve. We can now see why *R. equi* did not dissipate initially, as cephalexin is a first-generation cephalosporin, to which it was resistant. Finally the diagnosis of *Rhodococcus equi* was established, and was found to be susceptible to the prescribed doses of erythromycin. With the appropriate treatment, the patient was able to successfully eradicate the persistent infection that lasted over 2 months.

The difficulty in identification of a *Rhodococcus equi* infection lies in its ability to mimic other, more common, organisms. In this case, due to various reasons, there was a delay in finalizing the latest wound culture beyond 3 days, which was laboratory policy for wound cultures that were not producing any pathogenic growth or appeared to be contaminated with just skin flora. Since the characteristic pink pigmentation of this organism didn't develop until the fourth day of incubation, this delay, in turn, helped lead to the identification of *R. equi*. It is likely that *R. equi* was present in the previous cultures, but misidentified as normal skin flora, as they were all completed within 3 days, too soon for the pigmentation development needed to catch the eye of the laboratory scientist.

It is because of misidentification that *R. equi* can be so dangerous. According to the European Journal of Clinical Microbiology and Infectious Disease, "during the past decade an increase in the incidence of reported human *R. equi* infections has been noted, possibly because of greater attention being given to this pathogen, but certainly also because of the rising number of immunocompromised patients"[1]. The ability for laboratory professionals to properly recognize this organism, and produce, at minimum, a preliminary identification pending confirmation, will eliminate time needed to determine the proper treatment for the patient. Together, with appropriate laboratory work-up facilitating the physician's selection in proper treatment, a potentially dangerous organism can be easily eliminated and the patient cured.

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SUGGESTED READING

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