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Case Report

Rhodococcus equi AN UNUSUAL PATHOGEN IN PLEURAL EFFUSION IN A PATIENT WITH PRIMITIVE NEUROECTODERMAL TUMOR (PNET): A CASE REPORT

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ABSTRACT

Rhodococcus equi is anonsporing, nonmotile, aerobic Gram positive bacillus previously called as *Corynebacterium equi* is one of the important cause of zoonosis. The organism has ability to survive inside the macrophages. This property is considered important for its pathogenesis. Human infection with *R. equi* is a rare occurrence. Immunocompromised patients with HIV infection with CD4+ Helper T cell count <100 cells/mm^3 are predisposed. Cancer patients and patients undergoing organ transplantation receiving aggressive chemotherapeutic protocols are more susceptible. The degree and duration of neutropenia is an independent risk factor for these patients. *R equi* has a diverse clinical manifestations. Almost 80% patients have pulmonary system involvement with pleuritis, pleural effusion and cavitatorylung lesions. There is increased incidence of *R. equi* infections in humans reportedin recent times, may be due to the rising number of immunosuppressed patients as a result of increasing numbers of organ transplantations and use of immunosuppressive agents and aggressive antitumor chemotherapy.Here we describe an unusual case of *Rhodococcusequi from* pleural effusion in a patient with Primitive neuro-ectodermal tumor (PNET) of chest wallwith febrile neutropenia disease with unilateral lung opacities.

INTRODUCTION

Rhodococcus equi has been emerging as an opportunistic pathogen, especially in immunocompromised patients with HIV, cancer patients and patients with organ transplantations[1]. Most patients infected with *R.equi* present with a pulmonary syndrome. Pleuritis, pleural effusion and pneumonia are the common pulmonary manifestations. Other syndromesinclude gastrointestinal infections, pericarditis, meningitis, mastoiditis, and abscesses in the liver, kidney, psoas muscles, and cutaneous wounds[2]. Eighty-five percent of *R. equi* infections in humans occur in immunocompromised patients with HIV accounting for two-thirds of the cases[3]. Only a few cases of isolated bacteremia and pleural effusion have been reported in cancer patients with Hodgkin's lymphoma on chemotherapeutic drugs and patients undergoing bone marrow transplantation with immunosuppressive agents.Most patients present with sub acute onset of pulmonary disease showing abnormal chest radiographic findings and constitutional symptoms[4]. The diagnosis of *R. equi* infectionis often difficult due to microbiological and clinical similarities with other pathogens such as diphtheroids, mycobacterium species, or *Nocardia s*pecies.

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This case report is aimed to increase the awareness among the Physicians and Microbiologists about *R.equi* infection in immunocompromised especially cancer patients undergoing aggressive chemotherapy and radiotherapy protocols.

Case Report

A 7 year old male child from Bilaspur, Chhattisgarh, India presented with dry cough of 15 days duration and fever on and off since 15-20 days. For these complaints, child was worked up at Bilaspur. Blood counts showed neutropenia with absolute neutrophil count (ANC) 510 cells/mm^3. On examination general condition of child was good, KPS (Karnofsky Performance Scale Index) was 90%. There was no pallor, no icterus, no edema and no palpable lymphadenopathy and no hepatospleenomegaly. The child was symptomatic for chest pain & breathlessness. Air entry was decreased on the right side with tubular breath sounds. Blood pressure was 100/64 mmHg. Chest radiography showed mediastinal mass. Computed Tomographic scan showed well defined irregularly heterogeneously enhancing mass lesion in the Right mid/lower lung region eroding and destructing the ribs. The child was referred to Tata Memorial Hospital, Mumbai for further opinion and plan of management. Repeat CT scan of thorax and abdomen showed significant decrease in size and extent of right hemi thorax mass with resultant decrease in mass effect.

Residual lesion is seen eroding right seventh rib. Minimal soft tissue is seen in neural foramen at corresponding level. No obvious extension into spinal canal is seen. Axillary and supraclavicular lymphadenopathy noted. 9.4x9.3 cm mass lesion in right mid & lower lung zone with multiple necrotic centers & adherent to pleura, eroding & destruction of ribs with minimal pleural effusion suggestive of Neuroblastoma. There was no mediastinal lymphadenopathy.

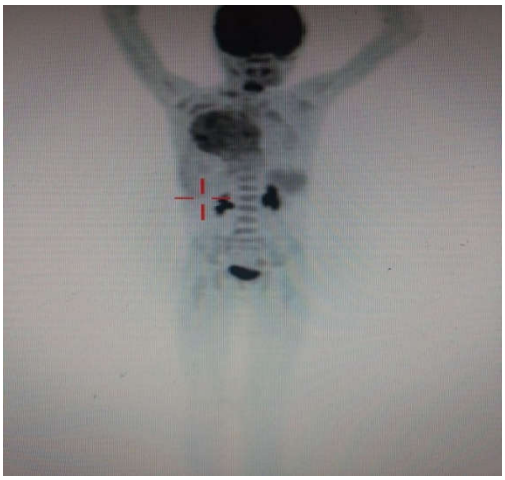

Fig 1a

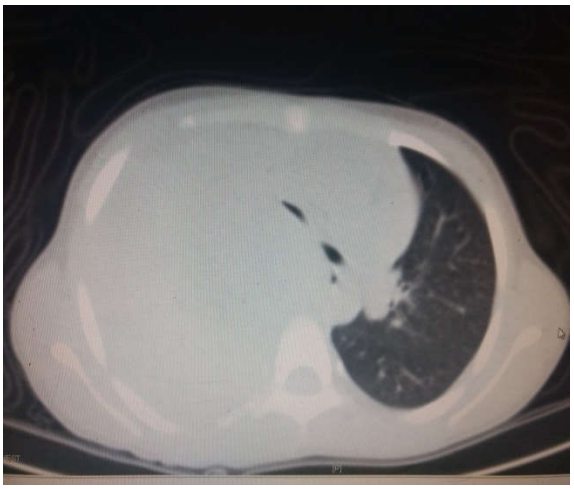

Fig1b

Fig.1a FDG Positron emission tomography scan showed right hemi thoracic mass and **Fig.1b.** Computed Tomographic scan showing well defined irregularly heterogeneously enhancing mass lesion in the Right mid/lower lung region eroding and destructing the ribs.

FDG Positron emission tomography scan showed right hemi thoracic mass with right 7th rib destruction pushing heart to left 10×10×11 cm with right pleural effusion and intraspinal extension @D5-9 level with metastatic right axillary and supraclavicular lymph nodes. Patientwas planned for induction chemotherapy and reassessed for definitive radiotherapy versus surgery. Patient received 12 cycles of VAC (vincristine 1.5 mg /m² IV, Doxorubicin 75 mg /m² IV and cyclophosphamide 1200 mg /m² IV) along with external beam radiotherapy (EBRT) with Cobalt 60 Gamma rays with two posterior oblique portals to a dose of 50.4 GY units over a period of 40 days. CT guided Biopsy was done which revealed malignant small round cell tumor in keeping with Ewing's sarcoma/Primitive Neuroectodermal Tumor (PNET). On immunohistochemistry tumor cells were positive for MIC-2, Fli-1, CD99 and NSE while they were negative for Desmin and LCA. The levels of tumor markers were as follows.Serum AFP (0.28 ng/ml), serum B-HCG (<0.13 mIU/ml), serum ferritin (604.81 ng/ml) and serum Vit D (12.6 ng/ml).Gram stain of ultrasound guided pleural tap showed polymorphonuclear leucocytes and Gram-positive bacilli which were pleomorphic with coccoidin appearance. ZiehlNeelson stain did not show any Acid fast bacilli and fungal stains were negative. Blood culture taken on the day of admission did not show growth after 48 hours of incubation. The patient did not improve after 48 days of intravenous Cefeperazone-sulbactam and Imipenemcombination. The pleural fluid was inoculated on Blood agar, MacConkey's agar, Chocolate agar and Sabouraud's Dextrose agar. On day3 of hospitalization, the smooth mucoidcolonies with salmon-pink appearance on sheep blood agar raised suspicion for *Rhodococcusequi*.

Fig 2 Culture of *R. equi*on Columbia blood agar with 5% sheep blood after 48 h at 37°C illustrating the typical mucoid teardrop appearance of the organism.

The organism was identified by using Vitek compact 2 automated identification system (Bio-Mérieux, France) including the manual biochemical reactions (Table.1) with CAMP (Cristie Atkins Munch Peterson) test.

Table 1 Microbiological characteristics of *Rhodococcusequi*.

Tests	Result
Catalase	Positive
Urea Hydrolysis	Hydrolysed
CAMP	Positive
Bile Esculin hydrolysis	Negative
Oxidase	Negative
Nitrate reduction	Positive

Susceptibility testing was performed by both disk diffusion method on Muller Hinton Blood agar using CLSI extrapolated cutoffs for *Staphylococcus aureus* as well as on Vitek Compact 2 automated system. The isolate was susceptible to vancomycin, teicoplanin, linezolid, clindamycin, rifampicin and levofloxacin. It showed intermediate susceptibility to gentamicin. Penicillinn, erythromycin, and trimethprim-sulfamethoxazole were resistant. The patient was started on intravenous Vancomycin at 40 mg/kg daily in two divided doses and oral levofloxacin at 16mg/kg per day based on susceptibility testing. Fever and respiratory symptoms gradually improved over 3 weeks of inpatient treatment.

Table 2 Antimicrobial susceptibility pattern of *Rhodococcusequi* from pleural effusion

Antibiotics	MIC Range (µg/ml)	Observed MIC (µg/ml)	Interpretation
Vancomycin	<=2 - >=16	0.5	Susceptible
Teicoplanin	<=8 - >=32	2.0	Susceptible
Linezolid	<=4 - >=8	2.0	Susceptible
Penicillin	<=0.12	>=0.5	Resistant
Erythromycin	<=0.5 - >=8	>=8	Resistant
Clindamycin	<=0.5 - >=4	0.25	Susceptible
Levofloxacin	<=1 - >=4	0.5	Susceptible
Rifampicin	<=1 - >=4	1.0	Susceptible

Gentamicin	<=4 - >=16	8.0	Intermediate
Cotrimoxazole	<=2/38 - >=4/76	>=8/76	Resistant

DISCUSSION

*R. equi*is an emerging pathogen which is usually misidentified as a contaminant Gram positive bacilli in clinical specimens. It is now recognized as an important opportunistic human pathogen. So far approximately 200 cases have been described in the literature from different anatomical sites. Almost Eighty-five percent of *R. equi* infections in humans occur in immunocompromised patients like HIV, cancer patients on chemotherapy and radiotherapy and patients undergoing organ transplantation especially bone marrow transplantation. Although mode of transmission of this pathogen is not yet clearly understood, most of the patients have some contact with herbivores or their manure[5]. Infection with these unusual pathogens tend to increase mortality and morbidity in addition to increased hospital stay and healthcare cost due to underlying disease with immune suppression. HIV and cancer patients are prone to develop infections with theserare organisms as there is prolonged and profound immune suppression either because of disease itself or due immunosuppressive agents used in the treatment of cancer and to prevent graft versus host disease in patients with organ transplantation. Prolonged and profound neutropenia is one of the most important independent risk factor to develop infections in cancer patients. Mortality rate in HIVinfected patients is 50% and is double the mortality in normal healthy individuals[6]. Respiratory system is commonly affected with *R. equi* infection, in the cases reported so far. Patients usually present with pulmonary disease of sub acute onsetwith fever, breathlessness and other systemic signs of infections with chest radiographic abnormalities. Almost 95% of the patient's shows abnormal chest radiographs and the most common radiographic findings are cavitatory lung lesions (75%) and pleural effusion (20%)[7, 8] and empyema. Diagnosis can be made via culture of biopsy specimens and blood and body fluids. All the specimens to be taken for culture before the administration of antimicrobial agents for better yield of the organisms. There have been cases reported, *R. equi*causing bacteremia and septicemia in cancer patients and patients undergoing allogenic bone marrow transplantation. Repeated isolation of the organism from blood and body fluids with presence of inflammatory cells is of more clinical relevance. Other less common manifestations of *R. equi* include gastrointestinal infections, pericarditis, meningitis, mastoiditis, and abscesses in the liver, kidney, psoas muscles, and contaminated cutaneous wounds. In a rare case report, *R. equi* was even isolated from malakoplakia lesions[9] which is an uncommon inflammatory disorder associated with immunosuppression. *R. equi* infection is said to be chronic and recurrent. It is difficult to eradicate the organism from the anatomical site isolated. Relapse may occur following a brief course of antimicrobial therapy but can also occur during the course of treatment. A combination of antimicrobial agents is given with good bactericidal activity and having good intracellular penetration. Mono therapy is avoided to prevent development of drug resistance and relapse. Therefore the antimicrobial treatment should be given for prolonged periodsat least 3-4 weeks duration. *R. equi* is said to be an intracellular pathogen that can survive inside macrophages and other inflammatory cells. The typical histological pattern shows a predominance of macrophages having granular cytoplasm that is filled with periodicacid-Schiff (PAS)-positive cocco-bacilli. Blood, bronchoalevolar lavage (BAL) and sputum cultures offer the best yield for the diagnosisof this infection. *R. equi*is non fastidious organism usually grows on sheep or Columbia blood agar. The optimum temperature for growth is 30°C. On solid ordinary non-selective media it forms large, irregular, mucoid colonies that turn to a salmon-pink color in 48-72 hours. Sometimes the growth may be delayed upto 7 days[10]. It is an obligate aerobe, encapsulated, non motile, non-spore forming, Gram-positive coccobacillus, which is partiallyacid-fast and bears a similarity to diphtheroids. The organisms such as *Staphylococcus aureus* and some *Listeria species*, which produces enzymes like sphingomyelinase, *R equi* causes synergistic hemolytic reaction with these organisms based on Christie Atkins Munch-Petersen (CAMP) test. Common biochemical properties used in routine clinical microbiology laboratories to identify *R. equi* (Table 1) are catalase, oxidase, carbohydrate fermentation, gelatin hydrolysis, indole production, urea and esculinhydrolysis, reduction of nitrates to nitrite. It is quite possible that *R. equi*may bemisidentified as diphtheroids. Isolation any of Gram positive bacilli from clinical specimen should be correlated with presence of inflammatory cells and repeated isolation from the same site is of more clinical importance. The repeated isolation of difficult- to-identify, Gram-positive bacilli with presence of pus cells from the patients with compromised immune status should raise the possibility of *R. equi*. As routine biochemical reactions do not provide the reliable identification of such rare pathogens, the other methods that can be employed for identification are automated systems like Vitek Compact (Bio-MeriuxFrance), MALDI TOF (Matrix assisted laser desorption ionization-Time of Flight mass spectrometry) and 16S rRNA sequencing. *R. equi* has generally shown in vitro susceptibility to vancomycin, rifampicin, fluoroquinolones like ciprofloxacin and levofloxacin, aminoglycosides, and carbamepenems like imipenem with cilastatin combination. Resistance to penicillin is commonly reported. The susceptibility pattern of an organism can vary in specific geographic areas and also depends on previous antimicrobial treatment[11]. There are no consensus guidelines on the optimal duration and regimen of antibiotic treatment, combination antimicrobial therapy using bactericidal and intracellularly active agents should be considered. A combination of carbapenem and one of theglycopeptides antibiotics, such as meropenem and vancomycin, are considered adequate for therapy[12].The combination of macrolide antibiotics like erythromycin and azithromycin and rifampicin can also be considered. The use of combination therapy may decrease the risk of developing resistance during therapy, which has been describedwith penicillin and other beta-lactam antibiotics. After an initial improvement, the patient can be treated with an oral regimen that could include combinations of floroquinolones, tetracycline, macrolides, and rifampicin. The optimal duration for treatment of initial disease and treatment of relapse is unknown. The duration of therapy depends upon the extent of infection, clinical and radiographical resolution, and immune status of the patient. Most experts recommend at least 6 months of therapy for immunocompromised patients[2].

CONCLUSION

Rhodococcus equi *should be considered as one of the potential pathogen in any immunocompromised patient and especially in cancer patients who are receiving radiotherapy and aggressive chemotherapeutic drugs. In routine clinical practice Isolation of any of rare pathogensfrom unusual site should be kept in mind for possible malignant diseases. There is a need to increase clinician'sawareness of this pathogen's morbidity and mortality in immunocompromised patients undergoing chemotherapy and organ transplantation and to improve the likelihood of its accurate and timely diagnosis. To better define the routes of transmission and the mechanisms of pathogenesis of* R. equi *infection, a thorough Clinical and laboratory research is needed.* Antimicrobial treatment should always be based on a combination of antibacterial drugs with bactericidal activity and drugs with good intracellular penetration and should be treated for prolonged period to prevent relapses and development of drug resistance.

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