



Case Report

A Rare Case of *M. marinum* Isolated from CSF of Two Months Old Baby with Non-Communicating Hydrocephalus

R. Lalngaihzuali, Ostwal.K*, Shaikh.N and Ingole.K

Dr. Vaishampayan Memorial Government Medical College, Solapur, India

ARTICLE INFO

Received 08 July 2015
Received in revised form 21 July 2015
Accepted 30 Aug. 2015

Keywords:

Atypical mycobacterium,
M. marinum,
Photochromogenic,
Septic shock.

Corresponding author: Ostwal.K Dr.
Vaishampayan Memorial Government
Medical College, Solapur, India.

E-mail address: karanost55@gmail.com

ABSTRACT

Background- This case reports a one month old male child presenting with abnormal body movements of Generalised Tonic Clonic Convulsion. CT scan revealed chronic sub-dural hematoma for which emergency craniotomy was done. Upon follow-up after 1 month of craniotomy, herniation from the bony gap was observed and CT scan and MRI revealed Non-communicating Hydrocephalus. So another operation was performed again placing VP shunt. The baby landed into septic shock like condition post operation.

Objective- To identify an atypical mycobacteria from CSF sample isolated from a one month old baby having hydrocephalus.

Method- Post VP Shunt CSF sample was sent for microbiological examination. ZN stain revealed acid fast bacilli. Culture on LJ media yielded smooth colonies after 7-10 days when incubated at 32°C. Colonies were cream in colour and turned yellow when exposed to light (photochromogenic). Sample was sent to National JALMA Institute for Leprosy & Other Mycobacterial Diseases in Agra, Uttar Pradesh for speciation. Biochemical tests were conducted by standard methods described in the CDC manual. The results were interpreted as per the method of Kent and Kubica.

Animal inoculation was done to check the pathogenicity of the strain.

Result- On the basis of cultural characteristics and biochemical tests, the strain was identified as *M.marinum*.

Conclusion- This case reports first instance of isolating *Mycobacterium marinum* in CSF of an infant with VP Shunt.

Introduction

Mycobacterium marinum is an intermediate-growing atypical mycobacterium that was first isolated from dead fish in a Philadelphia aquarium in

1926. Its pathogenicity in humans was confirmed in 1951 after its isolation from granulomatous skin lesions in swimmers from Sweden. It belongs to Runyon group



1(photochromogen) and colonies on LJ media produces pigment on exposure to light. Culture growth occurs over 7-14 days and is optimal at 32°C. It is the most common atypical Mycobacterium that causes opportunistic infection in humans.³ Infection is usually acquired by direct inoculation with the bacterium through broken skin in an aquatic environment.⁴ The infection is generally seen producing localized granulomas but they can also develop into an ascending lymphangitis that resembles sporotrichosis. These lesions are mostly localised but may sometimes extend to deep tissues.⁴ While *M. marinum* infections is due to aquatic trauma in healthy hosts, delayed diagnosis and immune suppression are also involved in the pathogenesis of invasive infections.⁵ Bone marrow invasion and bacteremia are rare and have been reported only in profoundly immunocompromised patients.⁶

Disseminated infection has been reported in immuno-competent patients and immune-compromised patients.⁷ This case highlights the isolation of an unusual pathogen i.e *M. marinum* from CSF in case of non-communicating hydrocephalus.

Case presentation

A 1 month old male child presented with abnormal body movements of Generalised Tonic Clonic Seizure for which he was admitted in the paediatrics ward. CT scan revealed chronic sub-dural hematoma for which emergency craniotomy was done. Upon follow-up after 1 month of craniotomy, herniation from the bony gap was observed and CT scan and MRI revealed Non-communicating Hydrocephalus. So another operation was performed again placing VP shunt. The baby went into septic shock-like condition post operation. CSF was sent for microbiological examinations which revealed acid fast bacilli on ZN staining and was further

identified to be *M. marinum*. The baby was started on injection Augmentin and Gentamycin and oral Cotrimoxazole suspension. After reporting of a photochromogen, treatment was shifted to rifampin and ethambutol for 2 months. With this treatment he recovered and got discharged.

Laboratory Investigations done

- i) CSF Sugars: 38mg/dL
- ii) CSF proteins: 124mg/dL
- iii) CSF WBC Count: 14.3/mm³
- iv) Blood culture: Sterile
- iv) HIV: Negative

Microbiological work up

Post VP Shunt CSF sample was sent for microbiological examination. Culture on Blood Agar, MacConkey Agar and Chocolate Agar were all negative for aerobic microorganisms. However, ZN stained smears of the CSF revealed acid-fast bacilli as shown in figure-1. A repeat sample of CSF was sent again for confirmation, it yielded the same result. Culture on LJ media yielded smooth colonies after 7-10 days when incubated at 32°C. Colonies were cream in colour and turned yellow when exposed to light (photochromogenic) as shown in Figure-2.

Atypical mycobacteria was suspected so subcultured colonies on LJ Media was sent to National JALMA Institute for Leprosy & Other Mycobacterial Diseases in Agra, Uttar Pradesh for speciation. It was then processed for acid fast staining using Ziehl-Neelsen technique. The bacterium was also tested for salt tolerance on Lowenstein-Jensen medium supplement with 5% NaCl, Iron Uptake Test, Aryl Sulfatase test, Tween 80 hydrolysis catalase activity and nitrate reduction. All these tests were conducted by standard methods described in CDC manual.

The results were interpreted as per the method of Kent and Kubica.⁸

Animal inoculation was done to check the pathogenicity of the strain.

Results

Results of biochemical tests performed are as follows

I. Salt tolerance on Lowenstein-Jensen medium supplemented with 5% NaCl

No growth on slant containing 5% NaCl while there was growth on slant of LJ Medium used as control after incubation at 28°C for 4 weeks.[Kent and Kubica,1985]⁸

II. Iron uptake test

No growth on iron citrate medium but growth on control LJ medium without iron citrate after incubation at 28°C for 2 weeks with loosened caps.[Kent and Kubica,1985]⁸

III. ArylSulfatase Test

Readings were taken on day 3 and day 7 of incubation at 35-37°C. A tube of uninoculated substrate medium used as negative control was negative on both days while a tube of substrate medium with standard strain of *M. fortuitum* used as positive control was positive on both days. The test strain was negative on 3rd day but positive by the 7th day producing red colour change immediately on addition of carbonate solution. [Kent and Kubica,1985]⁸

IV. Nitrate Reduction Test

Standard strain of *Mycobacterium tuberculosis* H37Rv showed positive i.e + 4 and standard strain of *Mycobacterium fortuitum* N2 showed +5. The test organism was negative as there is no development of pale pink to deep red colour change after addition of reagents #1,#2 and #3. [Kent and Kubica,1985]⁸

V. Catalase Activity Test

Readings were taken at room temperature and also after incubating at 68°C water bath for 20 minutes at pH 7 for testing the heat stable catalase after incubation with loosened caps for 2 weeks at 35-37°C. An uninoculated tube of medium used as negative control was negative on each occasions while the standard strain of *Mycobacterium tuberculosis* H37Rv was positive at room temperature and negative at 68°C and the standard strain of *Mycobacterium fortuitum* showed positive results at both temperatures. The test organism was slightly positive i.e produces few bubbles after addition of 1.0 ml of freshly prepared Tween-peroxide mixture at room temperature but was negative at 68C.[Kent and Kubica,1985]⁸

VI. Tween 80 Hydrolysis Test

Standard strain of *Mycobacterium tuberculosis* H37Rv showed positive result after 10 days of incubation at 35°C. The test organism too showed same result turning the substrate red on day 10 and not on day 5.[Kent and Kubica,1985]⁸

VII. Growth on MacConkey Agar Without Crystal Violet

No growth after incubation at 28°C examined on day 5 and day 11. [Kent and Kubica,1985]⁸ On the basis of the above mentioned tests performed by National JALMA Institute for Leprosy & Other Mycobacterial Diseases, Agra the pathogen was identified as *M.marinum*.

Animal Inoculation

0.1ml of the culture was inoculated in mice by intravenous route through the tail. The animal showed signs of sepsis and the acid-fast bacilli could be isolated from the spleen by the 3rd week.

Discussion

The diagnosis of *Mycobacterium marinum* infection is often delayed due to the indolent nature of early lesions and lack of clinical suspicion.⁹ In a 30 years retrospective study ninety-six percent of published cases with known exposures were accounted for injuries associated with swimming pools or other freshwater sources other than tap water.⁵ Another similar study revealed the main identifiable risk factors to be a history of trauma and water/fish-related hobbies or occupations.^{9,10} Hence for cutaneous infections, history of high-risk exposures that may have occurred up to 9 months before the onset of symptoms can be asked for.

However for non-cutaneous cases, diagnosis can often be missed especially in the absence of known exposure. Invasion into deeper structures such as synovia, bursae, and bone occurs in approximately one third of reported case-patients.⁶ Disseminated skin lesions can accompany deeper invasion but may be seen in isolation as well.¹¹ Bone marrow invasion and bacteremia are rare and have been reported so far mostly in profoundly immunocompromised patients,¹² though isolation of *M.gordonae* from CSF of a child with hydrocephalus has also been reported.¹³ Seventy-two percent of all published case reports did not include a description of exposure to a particular source.⁵ In this case too, *M marinum* infection was not suspected prior to ZN staining of the CSF as the source of exposure was clueless.

Clark & Shepard (1963) showed that intravenous challenge of mice, with an *M. marina* strain isolated from a human (ATCC 11564), caused infection of the lungs and liver, demonstrating that this strain was capable of replicating and surviving in deep tissues at temperatures close to 37°C.⁸ Work by Collins *et al.* (1975) reported that both regular and a high temperature-adapted

strain could cause systemic infections in both immunocompetent and immunocompromised mice.² The principle and drug regimen of management of a case of atypical mycobacteria is same as for mycobacteria tuberculosis.¹⁴ *M. Marinum* infection in otherwise healthy hosts can be self-limiting and disappear after several months, or it can be treated with a variety of antimicrobial drugs, including trimethoprim-sulfamethoxazole, rifampin and ethambutol, or doxycycline.¹⁴ *M. marinum* is resistant to many conventional antibiotics. In spite of this fact and very few cases reported in literature, optimal treatment has not yet been established.¹⁶

In this case the patient recovered completely after treatment with rifampicin and ethambutol for 2 months. Hence, the need for including *M. marinum* in the differential diagnosis of unknown pathogen in CSF.

Conclusion

This case highlights the importance of detecting infections caused by atypical mycobacteria which are generally not identified or rather often missed by clinicians and laboratory personnel. This is however the first instance of isolating *Mycobacterium marinum* in CSF of an infant with VP Shunt.

Acknowledgement

We wish to thank Dr. Srikant Prasad Tripathy, Director National JALMA Institute for Leprosy & Other Mycobacterial Diseases, Agra Uttar Pradesh for species level identification of our strain.

References

1. Felicia M. T. Lewis, Bryan J. Marsh, and C. Fordham von Reyn. Fish Tank Exposure and Cutaneous Infections Due to *Mycobacterium marinum*: Tuberculin Skin Testing,

- Treatment, and Prevention. *CID* 2003;37 (1 August).
2. Michael L. Kent, Virginia Watral, Martin Wu & Luiz E. Bermudez. In vivo and in vitro growth of *Mycobacterium marinum* at homoeothermic temperatures: *FEMS Microbiol Lett* 257 (2006) 69–75.
 3. Rallis E, Koumantaki-Mathioudaki E. Treatment of *Mycobacterium marinum* Cutaneous infections: *Expert Opin Pharmacother*. 2007 Dec; 8(17):2965-78.
 4. Holmes *et al*. Recurrent, Disseminated *Mycobacterium marinum* Infection Caused by the Same Genotypically Defined Strain in an Immunocompromised Patient: *Journal of Clinical Microbiology*, Sept. 1999, p. 3059–3061.
 5. John A. Jernigan and Barry M. Farr. Incubation Period and Sources of Exposure for Cutaneous *Mycobacterium marinum* Infection: Case Report and Review of the Literature. *Clinical Infectious Diseases* 2000;31:439–43.
 6. Centers for Disease Control and Prevention. Invasive *Mycobacterium marinum* Infections: Emerging Infectious Disease Journal ISSN: 1080-6059, Volume 9, No. 11—Nov 2003.
 7. Leslie J. Parent, Marguerite M. Salam, Peter C. Appelbaum, and John H. Dossett. Disseminated *Mycobacterium marinum* Infection and Bacteremia in a Child with Severe Combined Immunodeficiency: *Clin Infect Dis*. (1995) 21 (5): 1325-1327. doi:10.1093/clinids/21.5.1325.
 8. Kent, P.T and Kubica, G.P. Public health mycobacteriology: A guide for the level III laboratory. Atlanta, Ga.U.S. Dept of Health and Human Services, Centers for Disease Control, 1985
 9. Author: Shirin A Mazumder, MD; Chief Editor: Burke A Cunha, MD. *Mycobacterium Marinum*: Medscape Reference © 2011 WebMD, LLC
 10. Ang, P, Rattana-Apiromyakit, N. and Goh, C.-L. (2000), Retrospective study of *Mycobacterium marinum* skin infections: *International Journal of Dermatology*, 39: 343–347. doi:10.1046/j.1365-4362.2000.00916.
 11. Martin Bråbäck, Kristian Riesbeck, and Arne Forsgren. Susceptibilities of *Mycobacterium marinum* to Gatifloxacin, Gemifloxacin, Levofloxacin, Linezolid, Moxifloxacin, Telithromycin and Quinupristin-Dalfopristin (Synercid) Compared to Its Susceptibilities to Reference Macrolides and Quinolones: *Antimicrobial Agents and chemotherapy*, Apr. 2002, p. 1114–1116.
 12. Leslie J. Parent, Marguerite M. Salam, Peter C. Appelbaum, and John H. Dossett. Disseminated *Mycobacterium marinum* Infection and Bacteremia in a Child with Severe Combined Immunodeficiency: *Clin Infect Dis*. (1995) 21 (5): 1325-1327. doi:10.1093/clinids/21.5.1325
 13. Michael W. Scheld, W. Michael Scheld, M.D., Christina M. Marra, Richard J. Whitley Infections of the Central Nervous System: p.504-502.
 14. Koneman's Color Atlas and Textbook of Diagnostic Microbiology. Sixth Edition p1115-1117.
 15. Holmes *et al*. Recurrent Disseminated *Mycobacterium marinum* Infection caused by the same Genotypically defined strain in an Immunocompromised Patient. *Journal Of Clinical Microbiology* Sept. 1999, p. 3059–3061.
 16. Martin Braback, Kristian Reisbeck and Arne Forsfren. Susceptibilities of *M. marinum* to Gatifloxacin, emifloxacin, Levofloacin, Linezolid, Moxifloxacin, Telithromycin and Quinupristin-Dalfopristin (Synercid) compared to its susceptibilities to Reference Macrolides and quinolones: *Antimicrobial Agents and Chemotherapy*, April. 2002, p. 1114-1116..

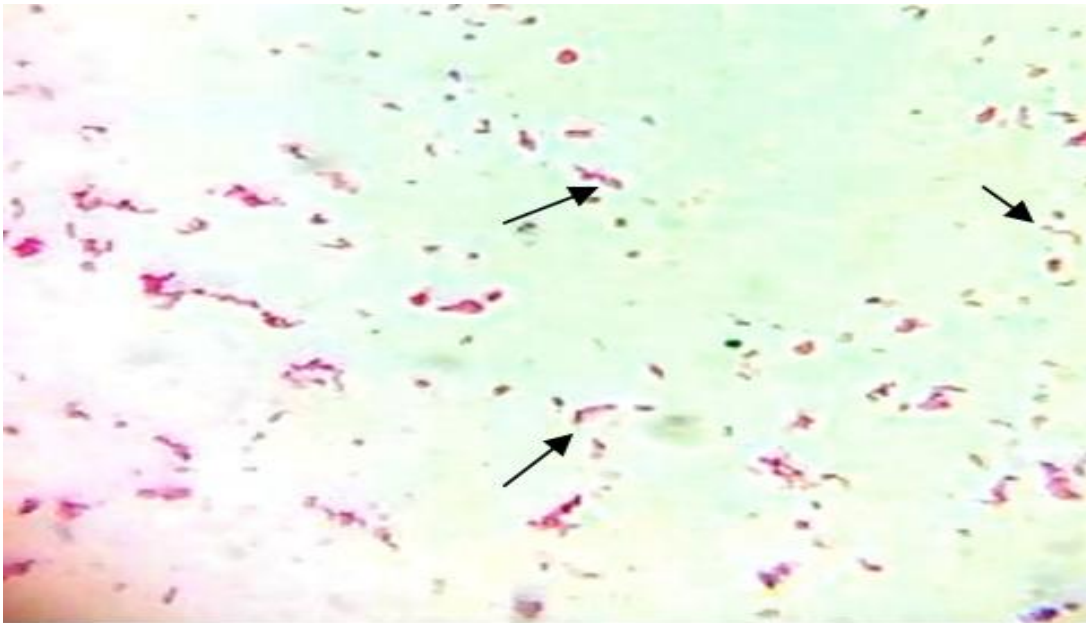


Figure 1: ZN stained smear showing acid-fast bacilli of *M marinum* on LJ media



Figure 2: Photochromogenic colonies