



ISSN Print: 2394-7500
 ISSN Online: 2394-5869
 IJAR 2015; 1(5): 215-217
 www.allresearchjournal.com
 Received: 13-03-2015
 Accepted: 23-04-2015

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Spingobacterium thalatothylum in H1N1 positive patient - first case report in India

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Abstract

Spingobacterium previously known as *Flavobacterium* is gram negative, oxidase positive, non motile bacilli. Very few case reports have been reported so far. This case reports a 35 year old male with fever, headache, nasal discharge, sore throat, cough and severe breathlessness since three days. He was suspected for H1N1 and was admitted in Infectious disease ward. His throat swab was collected and sent to National Institute of Virology (NIV), Pune for Real time Polymerase Chain Reaction (PCR). The sample was positive for H1N1. He was intubated due to continuous breathlessness and after two days tracheal aspirate was sent for microbiological examination. Yellow colonies on Blood agar, biochemical reactions and sugar fermentation test revealed *Spingobacterium thalatothylum*. Patient was treated with levofloxacin and meropenem. The patient recovered and was discharged later. To best of our knowledge, this is the first case report of *Spingobacterium thalatothylum* in India.

Keywords: *Flavobacterium*, H1N1, *Spingobacterium thalatothylum*, Tracheal aspirate, Yellow colonies.

1. Introduction

Spingobacterium species were previously described as unnamed bacteria (as a part of Centers for Disease Control and Prevention group- IIk). In 1981, Holmes *et al.*, proposed the genus name *Flavobacterium* for the bacteria ^[1], while in 1983, Yabuuchi *et al.* first proposed the name *Sphingobacterium* for the genus. ^[2] The genus *Sphingobacterium* was created to classify organisms that contain large amounts of sphingophospholipid compounds in their cell membranes, and have other taxonomic features that distinguish them from *Flavobacterium* species ^[3]. *Sphingobacterium* species have usually been isolated from soil, plants, foodstuffs, and water sources, but the isolation of the species from human clinical specimens has been rarely reported worldwide ^[4]. *Sphingobacterium* spp. are yellow-pigmented, aerobic, non-motile, oxidase-positive, indole-negative, saccharolytic, Gram-negative rods ^[5]. To date, 15 species, including *S. anhuiense*, *S. antarcticus*, *S. bambusae*, *S. canadense*, *S. composti*, *S. daejeonense*, *S. faecium*, *S. heparinum*, *S. kitahiroshimense*, *S. multivorum*, *S. piscium*, *S. shayense*, *S. siyangense*, *S. spiritivorum*, and *S. thalpothylum*, have been described in the genus *Sphingobacterium* ^[6,7]. The mean guanine-plus-cytosine content of the deoxyribonucleic acids of seven strains is 45.0 ± 0.8 mol%. The distinguishing features of the new species i.e *S. thalatothylum* include ability to reduce nitrate & thermotolerance (i.e growth at 42 °C) (thalat-warm and phylum-loving) ^[5], and an ability to produce acid from various carbohydrates and alcohols. In particular, the ability of *S. thalpothylum* strains to produce acid from adonitol distinguishes them from all other *Flavobacterium* species ^[5]. Previously reported, *Sphingobacterium* species isolated from human clinical specimens were *S. multivorum* and *S. spiritivorum*. All of the strains of *S. thalpothylum* were isolated from clinical specimens, and blood and wounds were the most common sources. Only one case report has been done till now in 1983 by Holmes *et al.* ^[5] to best of our knowledge, we are hereby reporting the first human case in India and third human instance in the world.

1.1 Case report- A 55 year old male presented with fever, headache, nasal discharge, sore throat, cough and breathlessness since three days. Fever was high grade and headache, myalgia followed. He was admitted in Infectious disease ward, and was started with amikacin and ceftazidime. Due to epidemic of H1N1 during that period, throat swab was collected and was sent to National Institute of Virology, Pune for real time PCR. The sample was positive for H1N1. So, patient was started with tamiflu. However, patient developed continuous,

progressive breathlessness. So he was on mechanical ventilation and then was intubated after two days. There was no significant past history. No history of travel, no history of any birds or animals in the house. No history of fever, cough, cold to any family members. His tracheal secretion was sent for microbiological examination.

2. Material and methods

Tracheal aspirate was sent for microbiological examination. Culture was done on blood agar and MacConkey agar. Colonies were subjected to biochemical reactions and Antibiotic susceptibility testing by Kirby Bauer disc diffusion method using standard disc of Hi-media laboratories, Mumbai. They were incubated at 37 °C overnight. Sugar fermentation test was done. Repeat sample was taken after two days to rule out contamination. (ref)

3. Results

3.1 Microbiological examination

Macroscopically, aspirate was turbid.

On gram staining there was evidence of pus cells and gram negative bacilli.

3.2 Culture

On blood agar after overnight incubation pure mono microbial, yellow, 1-2 mm, circular, smooth, regular margin, non haemolytic colonies were grown. On MacConkey agar, non lactose fermenting, oxidase and catalase positive, non motile colonies were grown. Subculture was done on Nutrient agar to check for pigment production. On nutrient agar, pale yellow, 1-2mm, circular, low convex, smooth colonies were grown at 30 °C. They were non diffusible and non fluorescent as shown in figure 1.



Fig 1: showing Pale yellow colonies of *Spingomonas thalatoaphylum* on Nutrient agar.

The biochemical reactions are as described in table 1-

Table 1: biochemical reactions [5, 8]

Biochemical test	Results
Indole	Not produced
Methyl red	Negative
Voges Proskauer	Negative
Citrate(Simmon's)	Not utilized
Urea	Was hydrolysed
Triple sugar iron	Alkali/no change
o-nitrophenyl- beta D galactopyranoside(ONPG)	Positive
Bile esuclin	Was hydrolysed
Nitrate	Was reduced to nitrite
DNase	Was hydrolysed
Gelatin	Was liquefied
Lysine decarboxylase	Negative
Arginine dihydrolase	Negative
Ornithine decarboxylase	Negative
Growth at 42 °C	Present
Growth at 5 °C	Absent

To rule out contamination, a repeat sample of patient was taken and it also revealed same colonies on blood agar and MacConkeys agar. Sugars were also put as the yellow pigment and biochemical reactions raised doubt of *Spingobacterium thalatoaphylum*. The results of Oxidative-fermentative sugars are as shown in table 2

Table 2: showing OF sugars. [5]

Sugars	Results-
OF glucose	Positive
OF mannitol	Negative
OF adonitol	Positive
OF lactose	Positive
OF maltose	Positive
OF salicin	Positive
OF arabinose	Positive
OF sucrose	Positive
OF xylose	Positive

The antibiotic susceptibility testing by Kirby Bauer disc diffusion method using standard disc of Hi-media laboratories, Mumbai. The results are as follows-[9].

Ampicillin (6mm), gentamycin (22mm), cefoxitin (19mm), tetracycline (22mm), levofloxacin (34mm), ceftazidime (21mm), ceftazidime+clavulanic acid (29mm), imipenem (26mm), imipenem+EDTA (32mm), ceftriaxone (22mm). The patient was resistant only to ampicillin and sensitive to all other antibiotics. Our isolate is ESBL producer as zone of ceftazidime + clavulanic acid is more than ceftazidime by 7mm. Treatment was shifted to levofloxacin and meropenem and he recovered completely after treatment in 10 days and was discharged later.

The biochemical reactions and OF sugars along with yellow pigment confirmed this microorganism as *Spingobacterium thalatoaphylum*. So, the isolate was not sent for identification by molecular methods.

3.3 Pathological examination

Haemoglobin-11.8g/dl, RBC's- 3.2million/mm, WBC's- 18,000, neutrophils-88%, lymphocytes-9%, monocytes- 1%, eosinophils-2%, basophils-0%, haematocrit-36%.

4. Discussion

In 1983, nomenclature of new isolate was done by Holmes *et al.* at California from seven different clinical samples as *Flavobacterium thalatoaphylum*. To best of our knowledge, after that only one case has been reported so far in wound swab in 1988. No case has been reported in India till date and hereby, we are reporting the first case report of *Spingobacterium thalatoaphylum* in India. *Spingobacterium* species have usually been isolated from soil, plants, foodstuffs, and water sources, but the isolation of the species from human clinical specimens has been rarely reported worldwide. Two species, *Spingobacterium multivorum* and *Spingobacterium spiritivorum* (previously classified as *Flavobacterium* spp.), have occasionally been associated with bacteremia, peritonitis and chronic respiratory infections in patients with severe underlying conditions. In our case the source may be mechanical ventilation. Our isolate is definitely not a contamination as repeat isolation was done and it also revealed same microorganisms.

S.thalatoaphylum is often confused with *S. spiritovorum* and *S. multivorum*. The distinguishing points between three are as follows as shown in table 3-

Table 3: showing differentiating features between *S. thalatothylum*, *S. spiritovorum* and *S. multivorum*.^[5]

Test	<i>S.thalatothylum</i>	<i>S.spiritovorum</i>	<i>S.multivorum</i>
Growth at 42 °C	+	-	-
OF Adonitol	+	-	-
Nitrate reduction	+	-	-

Our isolate was positive for growth at 42 °C, nitrate reduction and OF adonitol, so it was confirmed to be as *S. thalatothylum*.

As our patient was H1N1 positive, this represents immunocompromised state and later he developed super added infection of *Spingomonas thalatothylum*. This microorganism is usually of low virulence, but in immunocompromised patient it can cause severe degree of symptoms.

Resistance to antimicrobial agents is a characteristic that *S. thalatothylum* shares with the other *Flavobacterium* species. This contradicts with our study as our isolate is sensitive to all antibiotics except ampicillin. In study of Holmes *et al.*, 1983, the isolate was resistant to ampicillin, gentamycin and tetracycline. Our isolate is resistant only to ampicillin but sensitive to other antibiotics. Talking in general about *Spingobacterium*, according to some studies, they were sensitive to carbapenems, quinolones, and ceftazidime. This correlates with our study also. So, this can be concluded that *S. thalatothylum* is more resistant as compared to other *Spingobacterium* species. Prognosis also depends on immediate diagnosis. In our case, treatment was started immediately with levofloxacin and meropenem, and patient recovered completely in 10 days.

5. Conclusion

Cases may be confused with other yellow pigmented microorganisms like *Flavobacterium* and *Myroides* and hence complete biochemical tests including all the sugar fermentation tests should be done in all the cases.

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