Indian Journal of Medical Case Reports ISSN: 2319–3832(Online)
An Open Access, Online International Journal Available at http://www. cibtech.org/jcr.htm
2015 Vol. 4 (2) April-June, pp.56-59/Sharma et al.
Case Report

PAENIBACILLUS LAUTUS: A RARE CAUSE OF BACTEREMIA AND REVIEW OF LITERATURE

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ABSTRACT
Paenibacillus is a genus of aerobic, Gram positive bacilli, separated from genus bacillus based on phylogenetic studies using 16S rRNA gene. These organisms were not known to cause human disease, until recently; few species of this genus have been reported to cause infections in humans, commonly by *P alvei*. We report the case of *Paenibacillus lautus* bacteremia in a 68-year-old patient with type 2 diabetes which was identified using automated and mass spectrometry-based methods. To the best of our knowledge, this is the first report of human infection caused by this organism.

Keywords: *Paenibacillus Lautus, Bacteremia, 16S rRNA*

INTRODUCTION
Paenibacillus, a genus of aerobic, gram positive, endospore forming bacteria, belongs to the family *Paenibacillaceae*. The name *paeni* has been derived from the latin word *paene* which means almost and so the *Paenibacilli* are literally almost Bacilli (Sanghamitra *et al.,* 2013). Because of highly conserved genome encoding their 16S rRNA which differs from that of *Bacillus*, they were included in a novel genus called *Paenibacillus* which consists of more than 90 species (Sanghamitra *et al.,* 2013). Bacteria belonging to this genus have been isolated in a variety of environments such as soil, water; vegetable matter, and insect larvae, as well as clinical samples. Recently, several species of *Paenibacillus* have been reported to cause human infections like *Paenibacillus alvei* in prosthetic hip infections, cellulitis, and endophthalmitis, *P. macerans* in brain abscess, catheter associated infection, and wound infection, *P. polymyxa* in bacteremia. *P. alvei* is the commonest one (Sanghamitra *et al.,* 2013).
We describe a case of bacteremia in a diabetic, coronary artery disease patient caused by novel species of *Paenibacillus, P.lautus*

CASES
A 68 years old female patient presented with the complaints of shortness of breath, drowsiness and refusal of food since 2-3 days. Relatives noticed puffiness of face, cough and expectoration since one week. She was hypertensive, diabetic, dyslipidemic, non smoker and has positive family history of ischemic heart disease. She was a known case of coronary artery disease underwent CABG around 14 years back (1996). She had undergone bilateral knee replacement 8 years back. Intra cardiac device implantation was done around 6 months before admission. She also underwent debridement right diabetic foot 10 days prior to the admission.
On examination, afebrile, the pulse was 117/minute, BP 100/70 mm Hg. Bilateral wheeze and crept present on respiratory examination. Site of pacemaker implantation was normal.
On investigation, total leukocyte count was within normal range. Echo shows moderate TR, no vegetation. Sputum, blood samples were send for culture at the time of admission shows no growth. Leg wound was also send for the culture which grew *Klesiella pneumonia* sensitive only to colistin. The patient was on BIPAP. Inj ceftriaxone was started on admission and continued for 2 days. As there was no response to therapy, inj meropenem and inj colistin started. Blood cultures were repeated both from the central and peripheral line. At this time the blood culture grew *Paenibacillus lautus* from both the samples. The organism was sensitive to teicoplanin, gentamycin, vancomycin, rifampicin, amikacin, meropenem, ciprofloxacin, imipenem and resistant to ampicillin, oxacillin, erythromycin, trimethoprim-sulfamethaxole, penicillin, clindamycin. Repeat sample after 72 hours again grew the same organism

from both the sites. Line tip culture was negative. The blood culture after 10 days was no growth. The patient was discharged against medical advice.

Blood culture came positive around 40-60 hours of aerobic incubation. After 24 to 48 h of aerobic incubation, smooth, flat, and grayish colonies with few microcolonies were observed on blood agar plate (Figure 1). A Gram stain of the colonies revealed long and thin Gram-positive rods. Ellipsoidal and terminal spores swelling the sporangia became apparent after 48 to 72 h of incubation. Colonies from subcultures on Mueller-Hinton agar were catalase and oxidase positive. The isolate was identified by Vitek 2 compact using BCL card (BioMérieux). This was also identified by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) using a Microflex LT device (Bruker Daltonics, Wissembourg, France) and the Biotyper v3.1 database (Bruker).

Description of P. lautus (Heyndrickx et al., 1996)

Paenibucillus lautus (lau'tus. L. part. adj. *luutus,* washed, splendid). Motile, gram-positive rods with round ends that are 0.5 to1.0 µm wide by 4.0 to 7.0 µm long and occur singly and in pairs. Sporangia are regular and are swollen by sub terminal ellipsoidal spores. Facultatively anaerobic and catalase positive. The organism grows on routine media, such as nutrient agar, producing circular to irregularly shaped, low convex, grayish colonies (which become white as the cells sporulate) that are 1 to 2 mm in diameter after incubation for 2 to 3 days at 30°C; the colonies have a thin butyrous consistency and may have a tendency to spread across the agar surface with microcolonies. The optimum growth temperature is 28 to 30"C, the maximum temperature is 45 to 50"C, and the minimum temperature is 5 to 10°C. Voges-Proskauer reaction is positive, but citrate is not utilized, gelatin is not hydrolyzed, nitrate is not reduced, and argininedihydrolase, hydrogen sulfide, urease, and indole are not produced as determined with API 20E strips. Nakamura (27) has reported positive reactions for nitrate reduction and urease. In MI 50CH galleries, when API CHB suspension medium is used, esculin is hydrolyzed, and acid, but no gas, is produced from the following compounds: amygdalin, arbutin, D- and L-arabinose, D-cellobiose, D-fructose, galactose, gentiobiose, Dglucose, glycerol, glycogen, lactose, maltose, D-mannose, mannitol, D-melibiose, methyl-D-glumside, methylxyloside, N-acetylglucosamine, D-raffinose, ribose, salicin, starch, sucrose, D-trehalose, D-turanose, and D-xylose. Acid is not produced from adonitol, D- and L-arabitol, dulcitol, erythritol, D-fucose, 2-keto-~-gluconate,5 -keto-~-gluconate,D -lyxose, meso-inositol, methyl-D-mannoside, rhamnose, sorbitol, L-sorbose, D-taresults with L-fucose, gluconate, inulin, and D-melezitose. Nakamura reported a positive result for L-rhamnose fermentation. The following fatty acids are present and account for more than 1% of the total fatty acid content: 14:O iso, **14:0,** 15:O iso, 15:O anteiso, 16:O iso, 16:l o l l c, 16:0, 17:O iso, and 17:O anteiso. Casein is weakly hydrolyzed by some strains, growth is not inhibited by 5% NaC1, and ***5%*** horse blood agar is not hemolyzed. The average G+C content is 51 mol% as determined by the buoyant density method. Isolated from soil and human intestinal tracts. The type strain is strain LMG 11157 (= DSM 3035 = NRRL NRS-666).

In our case, the isolation of *P. lautus* from blood at 2 separate occasions associated with co morbid conditions suggests it as the causative agent and rule out possibility of contamination. Treatment with the IV antibiotics to which the organism was sensitive resulted in the disappearance of the organism from the blood cultures.

DISCUSSION

Discussion and Review of Literature

Based on the phylogenetic studies, bacilli were separated from genus Bacilli to form separate genus Paenibacillus. Several novel species of *Paenibacillus* have recently been described (Ve´ronique and Didier, 2004).

On review of literature it was found that blood was the common source of isolation from clinical samples and 8 cases of bacteremia were reported caused by *Paenibacillus sp*[3]. But none of the case was reported from India and none was caused by *P. lautus*. Different species isolated from blood were *P. alvei* (Annette *et al.*, 1989) *P. polymyxa* (Nasu *et al.*, 2003), *Bacillus licheniformis*, *B pumilus*, *P. popilliae* (Wu *et al.*, 1999), *P. sanguinis* (Ve´ronique and Didier, 2004), *and P. massiliensis* (Ve´ronique and Didier,

2004) *P. timonensis* (Ve´ronique and Didier, 2004) *P. thiaminolyticus* (Jie *et al.*, 2008). **Tab1** shows summary of cases caused by *Paenibacillus sp* (Jie *et al.*, 2008). *P alvei is* isolated in 26 years old female with sickle cell disease with hip prosthesis. *P. polymyxa* was isolated in case of 93 years old female patient with old cerebral infarction.

Table 1: Summary of infection caused by *Paenibacillus species*

Patient Age	Sex	Condition	Species	Source
n/a	n/a	Meningitis	P.alvei	Cerebrospinal fluid
20 da	F	Meningitis	P.alvei	Cerebrospinal fluid
26yrs	F	Sickle cell disease, hip prosthesis infection	P.alvei	Blood(hip prosthesis)
22 yrs	M	Traumatic injury,endophthalmitis	P.alvei	Foreign body from eye
62 yrs	M	Pneumonitis,pleuritis	P.alvei	Pleural fluid
62 yrs	M	Leg cellulitis	P.alvei	Culture from site
93 yr	F	Cerebral infarction	P.Polymyxa	Blood
18 yrs	F	Munchausen syndrome	P.Polymyxa, Bacillus licheniformis B pumilus	Blood from self inflicted compound
52 yrs	M	Periorbital trauma	P. marcerans	Brain abcess
9 yrs	M	Neutropenic fever	P. hongkongensis	Pseudobactermia
57 yrs	M	Endo carditis	P. polliae	Blood(heart valve)
49 yrs	M	Carcinoma of oropharynx	P. sanguinis	Blood
13 yrs	M	Lymphoblastic leukemia	P. massiliensis	Blood
75 yrs	F	Nephropathy,hemodialysis	P. timonensis	Blood
35 yrs	F	Drug abuse,night sweats, pulmonary nodules	P. provencensis	Cerebrospinal fluid
54 yrs	M	Cerebellar syndrome,Whipples disease	P. urinalis	Urine
80 yrs	M	Renal failure,hemodialysis,colon cancer	P. thiaminolyticus	Blood(possible permacath)

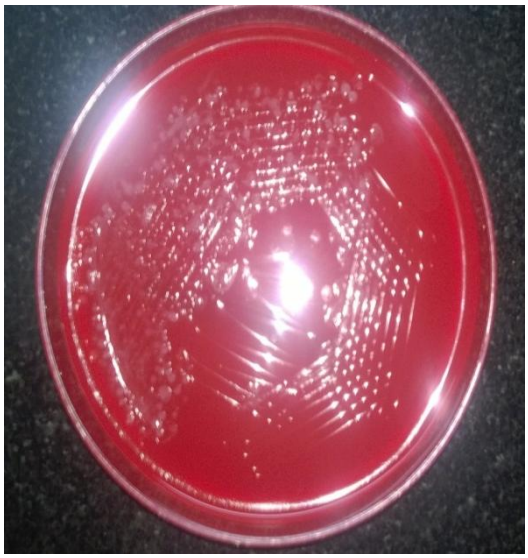

Figure 1: Growth and isolation on culture plates

Most common species isolated from different specimens was *P alvei. P. alvei* has also been isolated from life threatening conditions like meningitis and pneumonitis.[1] *P. alvei* originally isolated from honeycomb of bees and later on from soil, is found to produce thiol‑activated cytolysins which might be responsible for its virulence. *B. alvei* (Bacillus "of a beehive") was one of the earliest described species of the genus Bacillus [5].

Conclusion

Though in general, isolation of Gram‑positive spore bearing bacilli from culture are considered as contaminants, if isolated in pure form, from 2 blood culture sets and even in repeat culture, also in scenario of associated co morbid conditions should not be neglected and be processed for identification and sensitivity to reduce the morbidity and mortality of the patients.

No human disease has been found related to *P.lautus.*

The limitation of the report is that the genotype sequencing of the isolate could not be done. Also we cannot isolate the organism from the environment and could not identify the source. Since the patient was not showing signs and symptoms of bacteremia we cannot correlate the isolation and disappearance with the clinical condition of the patient.

This is, to the best of our knowledge, the first report showing that *P. lautus may* be a pathogen in humans causing bacteremia.

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