CASE REPORT

Journal of Clinical Microbiology and Infectious Diseases (*JCMID*) 2021, Volume 1, Number 2: 42-45
E-ISSN: 2808-9405

Published By : the Indonesian Society for Clinical Microbiology

Identification of *Paenibacillus amylolyticus* as the true causative agent for a nasal septum abscess: a case report

Rizalinda Sjahril[1,2*], Valentine Hursepuny[1], Prajayanti Palulun[1], Nadyah[1], Andi Rofian Sultan[1]

[1]Department of Microbiology, Faculty of Medicine, Hasanuddin University, Indonesia
[2]Microbiology Laboratory, Hasanuddin University Hospital, Makassar, Indonesia

*Corresponding to:
Rizalinda Sjahril; Department of Microbiology, Faculty of Medicine, Hasanuddin University, Indonesi:
rizalinda_sjahril@yahoo.com

Received: 2021-06-02
Accepted: 2021-11-30
Published: 2021-12-28

ABSTRACT

Introduction: A bacteria commonly found in the environment was identified in a nasal septum abscess in a diabetic patient. The isolation of a common environment bacteria *Paenibacillus amylolyticus* from nasal septum abscess, particularly in a diabetic type 2 patient, was initiated argumentation whether to consider it a true pathogen that needs to be treated with antibiotics or as a contaminant that does not require antibiotic treatment. Thus, in this case, report, an evaluation of the pre-analytical and analytical phase of the laboratory identification aided in determining whether the bacterium is a contaminant or causative agent.

Case description: A 58-year-old woman came to the Emergency Department with foul yellowish secretion from her left nostril that started three weeks before. She confessed to being diabetic but not under common control. Physical examination of the left nasal cavity showed signs of crust and fistula on the medial mucosal membrane, and an ulcer was seen at the upper sulcus ginggivo-buccal. Her blood pressure was 230/120 mmHg, her blood glucose was 270 mg/ dL, and her HbA1c was 13.5. Clinically, the diagnosis was nasal septum abscess with diabetes type 2 and hypertension grade 2. A swab sample was collected from the nasal lesion and transported to the laboratory at room temperature less than 2 hours. Direct Gram staining showed Gram-positive, rod-shaped bacteria1+, polymorphonuclear cells 3+ and epithelial 1+. Ziehl Neelsen staining was negative. While no growth was seen on chocolate agar, MacConkey agar and Saboraud dextrose agar, small greyish colonies grew on blood agar, which by Vitek2 System were identified as *Paenibacillus amylolyticus.* Conventional antibiotic sensitivity tests showed sensitivity to erythromycin and vancomycin. Accordingly, the patient was treated with erythromycin and metronidazole for ten days, and the nasal septum abscess was healed.

Conclusion: The presence of spore-forming rod-shaped bacteria on blood agar identified as *Paenibacillus amylolyticus.* It was confirmed as the true causative agent of the nasal septum abscess based on the acceptable pre-analytical and analytical phases.

Keywords: *Paenibacillus amylolyticus,* nasal septum, abscess, diabetic.
Cite This Article: Sjahril, R., Hursepuny, V., Palulun, P., Nadyah., Sultan, A.R. 2021. Identification of *Paenibacillus amylolyticus* as the true causative agent for a nasal septum abscess: a case report. *Journal of Clinical Microbiology and Infectious Diseases* 1(2): 42-45.

INTRODUCTION

The isolation of a common environment bacteria *Paenibacillus amylolyticus* from nasal septum abscess in a diabetic type 2 patient has initiated argumentation whether to consider it a true pathogen that needs to be treated with antibiotics or as a contaminant that does not require antibiotic treatment. The considerations from a conscientious clinical microbiologist may direct to the right decision.

It, formerly is known as *Bacillus amylolyticus,* was revealed to be phylogenetically different from genus *Bacillus.*[1] Molecular detection of 16S rRNA can detect genus Paenibacillus using specific PAEN515F primer and universal 1377R primer.[2] These rod-shaped bacteria are facultative anaerobe, which grows optimum at 37°C (temperature range 10 - 40°C) and pH 7.0. Growth is inhibited by 5% NaCl, 0.02% sodium azide and lysozyme. Cell size is 0.7 – 0.9 by 3.0 – 5.0 um and is motile due to peritrichous flagella. It can produce ellipsoidal spores in swollen sporangia at one end of the cell.[1] Colony is greyish, smooth, flat, circular and entire. No pigment is produced in nutrient agar. It is catalase-positive and oxidase negative does not produce acethymethylcarbinol (Voges Proskauer negative) and hydrogen sulfide, indole, dihydroxyacetone and lecithinase negative. It hydrolyzes casein, gelatin, starch, Tween 20 and Tween 60, but does not hydrolyze DNA, Tween 80, urea and hippurate. It does not decompose tyrosine

and does not deaminate phenylalanine. Citrate, propionate, acetate, fumarate, L-malate, lactate, mecinate, L-glutamate, L-Aspartate, alginate, gluconate, alpha-ketoglutarate, malonate. Produces acid but no gas from D-glucose, L-arabinose, D-fructose, D-galactose, maltose, sucrose, D-xylose, trehalose, glycerol, D-mannitol, D-cellobiose, D-ribose, Salicin, D-mannose, melibiose, inositol, inulin and starch. It does not produce acid nor gas from lactose, D-sorbitol, l-sorbose, L-rhamnose, raffinose and adonitol.[2] The ability to swim, swarm, produce biofilms and protease are other phenotypic characters that can be used to identify different bacilli and Paenibacilli strains. Automated phenotypic detection such as the Vitek2 System allows accurate and less laborious identification. The use of Matrix-assisted laser de-ionabsorption time of flight MALDITOF-MS showed the accuracy of identifying Paenibacillus strains.[3]

.

CASE DESCRIPTION

A 58-year-old woman came to the Emergency Department with foul-smelling discharge from her left nostril that started three weeks before. A painful reddish lump on the skin between her nostrils and upper lips preceded the yellowish pus-like and tainted with blood discharge. Blood-stained rhinorrhea, epistaxis, post-nasal drips, serial sneezing, nasal obstruction, smell (olfactory) disorder, facial pain, dysphagia, odynophagia, dysphonia, and odynophagia were denied. There was no coughing or shortness of breath. Otorrhoea, otalgia, tinnitus, cephalgia, and hearing disorders were denied. She confessed to being a diabetic but not under common control. She was not aware of having high blood pressure. Upon physical examination, blood pressure was 230/120 mmHg, pulse 104x/minute, respiration rate 20x/minute, and body temperature 36.5°C. The tympanic membranes were intact; the medial membrane of the left nasal cavity showed signs of crust and a fistula on the medial mucosal membrane of the left nasal cavity. Tonsils showed no enlargement, not hyperemic, T1-T1. An ulcer was present at the upper sulcus ginggivo-buccal. Chemical analysis of blood showed HbA1C 13.5. Complete blood count revealed white blood cells 9060/uL (normal range 4.000 - 11.000/uL), red blood cells 4036/uL (normal range 4500 – 5500/uL), haemoglobin 12.9 g/dL (normal range 13-16). Neutrophil 71.4% (normal range 50 - 70 %), lymphocytes 19 % (normal range 20 - 40 %), other measurements were within normal range. Kidney and liver function were within the normal range. Working diagnosis with nasal septum abscess, diabetes mellitus type 2 and hypertension grade 2 was finalized based on anamnesis, laboratory and clinical findings.

Antibiotic treatment

Antibiotic treatment was started directly by the Ear, Nose and Throat doctors: intravenous Ceftriaxone 1g/12 hours, Metronidazole 500mg/8 hours, Ketorolac 500 mg/8 hour, Ranitidine 50 mg/12 hours, and per-oral 10 mg Amlodipin/12 hours.

Microbiology Laboratory tests

Bacterial culture was ordered. Because the narrow space in the nares anterior and the abscess had ruptured, instead of abscess tissue collection, an ulcer swab was performed by pressing on the base of the ruptured abscess after cleaning excessive pus, crust and debris. The swab was transported to the laboratory at room temperature within 2 hours since collection.[4]

Culture on agar media

The ulcer swab that arrived within 2 hours at the Microbiology Laboratory of Hasanuddin University Hospital was streaked using the four-quadrant method on Blood agar (BA), chocolate agar (CA), MacConkey agar and Saboraud dextrose agar. According to the standard procedure, the agar plates were adjusted to room temperature beforehand. After streaking, the agar plates were stacked upside down and stored in the incubator in ambient air at 37°C for 24 hours.

Direct smear

The swab was rolled 360° several times on two objective glasses, then heat fixed. One was stained according to the procedure of Gram staining and the other for acid-fast staining.

Gram staining

On the heat-fixed slide, crystal violet was poured and left for 1 minute. Crystal violet was drained and washed using a wash bottle. Iodine was poured on the slide for 1 minute, drained and washed using a wash bottle. Alcohol 95% was dropped on the tilted slide for 5-10 seconds while observing that no more bluish color of crystal violet oozes from the smear and followed by washing with water to clear up alcohol remains. Safranin was poured on the smear for 30 seconds, then drained and washed using a wash bottle. The objective glass was either air-dried or blotting with paper. Observation under the microscope at low power was done to evaluate ulcer swab collection quality, followed by high power magnification using immersion oil.

Acid fast staining (Ziehl Neelson Method)

Carbol fuchsin was poured, and heating of the carbol fuchsin over a flame was done just until vapor began to rise. The process of immersing in heated carbol fuchsin was allowed for 5 minutes. De-coloration was done using 3 % acid alcohol (V/V). Counterstain with methylene blue for 1 minute was performed and washed using a wash bottle. The smear was allowed to air dry and avoid blotting with paper.

Identification using Vitek2 System

The isolated colony was suspended with sterile NaCl to a 1.8-2.2 MacFarland concentration, prepared to test using BCL card on Vitek2 System (Biomeriux). BCL cards were filled automatically in the VITEK vacuum chamber, incubated at 35°C and read automatically every 15 minutes for 14 hours.

Antibiotic Sensitivity Test (AST)

No Clinical and Laboratory Standards Institute breakpoints are available for Paenibacillus. Therefore we adopted the interpretative criteria described for Bacillus[5] on seven antibiotics on a lawn of *Paenibacillus amylolyticus* isolate at a concentration of 0.5 MacFarland on Mueller Hinton Agar (MHA) containing 5% goat's blood. The antibiotic discs were meropenem (10 µg), ciprofloxacin (5 µg), norfloxacin (10 µg), levofloxacin (5 µg), vancomycin (30 µg), erythromycin (15

µg), clindamycin (2 µg), and additionally tested was cefoxitin (30 µg). Bacterial discs were placed aseptically, allowing the equal distance between discs and slightly tapping the disc surface to ensure it touches the agar evenly. The bacterial plate was placed upside down and incubated at 37oC for 48 hours at ambient air. The inhibition area around the discs was measured by using a metal clipper.

RESULTS

Gram staining of direct smear revealed gram-positive bacilli bacteria one pos and polymorphonuclear cells three pos. No acid-fast bacilli were observed. Observation of bacterial growth on BA revealed two distinct bacteria on the first quadrant: many small colonies (alpha hemolysis, greyish colonies) and only one large colony (mucoid, white) at the first quadrant; the latter was considered as a contaminant (**Figure 1**). Gram staining of the small colony revealed Gram-positive, spore-forming rod-shaped (**Figure 2**). Meanwhile, the BCL card on Vitek2 System identified *Paenibacillus amylolyticus*. Antibiotic sensitivity testing revealed resistance to meropenem (10 µg), ciprofloxacin (5 µg), norfloxacin (10 µg), levofloxacin (5 µg), clindamycin(2 µg), and Erythromycin and Vancomycin sensitivity (**Figure 3**).

DISCUSSION

It is the first case of *Paenibacillus amylolyticus* isolated as the causative agent of abscess of the nasal septum of a diabetic patient. Other reported cases of *Paenibacillus amylolyticus* were an isolate reported from human blood[6] and another sepsis case in 2015 in which *Paenibacillus amylolyticus* and two other Lysinibacilli was detected as the cause of severe bacteremia in an immunocompromised narcotics addict.[7,8] Because the genus Paenibacillus have been reported in many environmental samples, eight and there is lacking evidence on the pathogenicity of the *Paenibacillus amylolyticus,* especially in the forming of abscess, and that normal flora residents are present at the location of the lesion, we provide our reasons to believe that this bacteria is a true pathogen. Another case report found anaerobic bacteria in the nasal septum (*Veillonella parvula* and *Peptostreptococcus sp*) due to upper dental trauma. After being treated with an antibiotic the patient had no nasal septum abscess.[9] We also found anaerobic bacteria in our nasal septum isolation but different species. Even though different bacteria were found, antibiotics effectively eradicate the nasal septum abscess.

Firstly, we evaluated the pre-analytic phase. The sample collection was performed by a competent medical doctor according to the standard abscess collection procedure, using sterile swab/container and aseptic technique, rapid time of transport (less than 2 hours) and correct condition of sample transport (room temperature).[4] The only miss was the type of specimen, which would have been better to collect tissue or aspirate samples. Secondly, the common normal flora of the anterior nares of the adult is dominated by Actinobacteria, Firmicutes and less dominantly by anaerobic Bacteroidetes. Among middle-aged adults (40-65 years), the bacterial

Figure1. Bacteria growth on BA at 24 hours

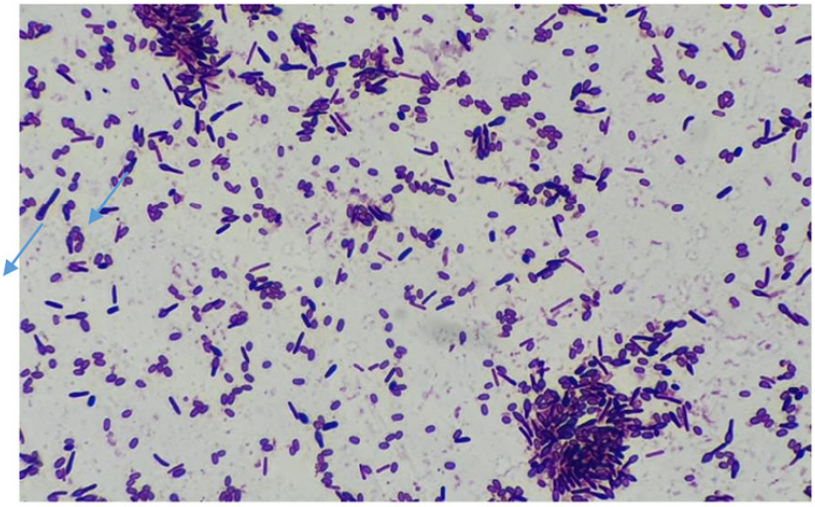

Figure2. Gram stain of a small greyish colony (positive Gram, rod-shape, spore-forming bacteria indicated by blue arrow)

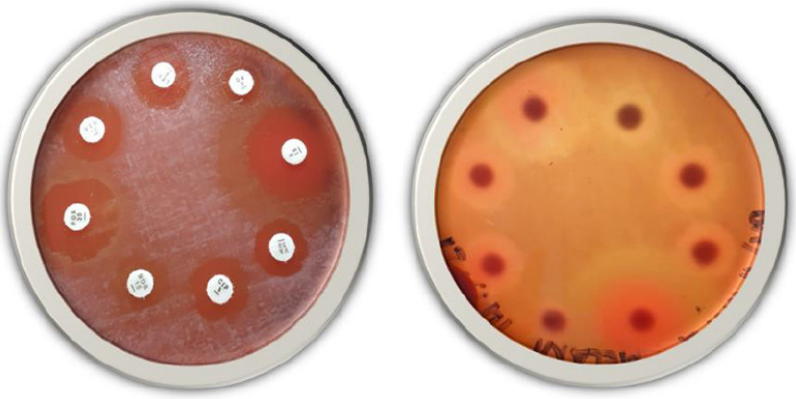

Figure3. AST result against eight antibiotics (cefoxitin, norfloxacin, ciprofloxacin, levofloxacin, meropenem, erythromycin, clindamycin, vancomycin)

community alters into a predominance of Cutibacterium, Corynebacterium, and Staphylococcus. The community changes towards a more oropharyngeal population among the elderly (> 65 years).[10] During the aerobic culture of this sample, none of the common bacteria grew on agar plates (BA, CA, Mac and SDA). Instead, Gram-positive, rod-shaped spore-forming bacteria were found abundant in the first quadrant of BA (**Figure.1**). Thirdly, the patient's immunocompromised status due to uncontrolled diabetes is a possible explanation for skin and soft tissue invasion of *Paenibacillus amylolyticus*. Pathogenicity of *Paenibacillus amylolyticus,* despite not yet frequently discussed, is reflected by possession of protease enzymes and the virulence genes nheA and nheC.[3]

In addition, *Paenibacillus amylolyticus* isolation was found in the blood.[6] Trauma in the upper dental might lead to an infection in the nasal septum, and an appropriate treatment caused nasal septum abscess.[9] Thus, we can predict that the *Paenibacillus amylolyticus* in blood might be one reason abscess forms in the nasal septum and infection in other body parts such as the upper teeth where bacteria migrate through the bone. However, different types of bacteria may be found. Further testing on more samples and using a more accurate detection system may reveal a better understanding of the pathogenic potential of *Paenibacillus amylolyticus.*

CONCLUSION

Direct and indirect gram stain showing Gram-positive, rod-shaped spore-forming bacteria, colony appearance on BA, catalase-positive and oxidase negative, and vitek2 system confirmed *Paenibacilli amylolyticus* as the etiology agent of a nasal septum abscess in a diabetic patient. The bacteria were sensitive to erythromycin and vancomycin.

DISCLOSURES

The authors have nothing to disclose.

FUNDING

No funding was involved.

CONFLICT OF INTEREST

The author(s) have no conflict of interest with any organizations or person that could influence the objectivity during the study, interpreting the result, and writing the manuscript.

CONSENT

Written informed consent was obtained from the patient to publish this case report.

AUTHOR CONTRIBUTION

Sjahril R contributed to the manuscript's conceptualization, designing, and writing; Hursepuny V, Palulun P, Haruna N, Sultan AR were involved in investigation and methodology. All authors agree for this final version of the manuscript to be submitted to this journal.

ACKNOWLEDGMENTS

The authors thank the Dean of the Faculty of Medicine Universitas Hasanudin and the Chief Director of Hasanuddin University Hospital for their support in developing and improving the Clinical Microbiology Specialist Study Program.

REFERENCES

1. Ash C, Priest FG, Collins MD. Molecular identification of rRNA group 3 bacilli (Ash, Farrow, Wallbanks and Collins) using a PCR probe test. Antonie Van Leeuwenhoek. 1994;64(3–4):253–60. Available from: http://dx.doi.org/10.1007/bf00873085
2. Shida O, Takagi H, Kadowaki K, Nakamura LK, Komogata K. Emended Description of Paenibacillus amylolyticus and Description of Paenibacillus illinoisensis sp. nov. and Paenibacillus chibensis sp. nov. Int J Syst Bacteriol. 1997;47(2):299–306. Available from: http://dx.doi.org/10.1099/00207713-47-2-299
3. Celandroni F, Salvetti S, Gueye SA, Mazzantini D, Lupetti A, Senesi S, et al. Identification and Pathogenic Potential of Clinical Bacillus and Paenibacillus Isolates. PLoS One. 2016;11(3):e0152831–e0152831. Available from: https://pubmed.ncbi.nlm.nih.gov/27031639
4. Baron EJ, Miller JM, Weinstein MP, Richter SS, Gilligan PH, Thomson Jr RB, et al. A guide to utilization of the microbiology laboratory for diagnosis of infectious diseases: 2013 recommendations by the Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM)(a). Clin Infect Dis. 2013/07/10. 2013;57(4):e22–121. Available from: https://pubmed.ncbi.nlm.nih.gov/23845951
5. Guinea J. Updated EUCAST Clinical Breakpoints against Aspergillus, Implications for the Clinical Microbiology Laboratory. J fungi (Basel, Switzerland). 2020;6(4):343. Available from: https://pubmed.ncbi.nlm.nih.gov/33291313
6. Sáez-Nieto JA, Medina-Pascual MJ, Carrasco G, Garrido N, Fernandez-Torres MA, Villalón P, et al. Paenibacillus spp. isolated from human and environmental samples in Spain: detection of 11 new species. New microbes new Infect. 2017;19:19–27. Available from: https://pubmed.ncbi.nlm.nih.gov/28702198
7. Wenzler E, Kamboj K, Balada-Llasat J-M. Severe Sepsis Secondary to Persistent Lysinibacillus sphaericus, Lysinibacillus fusiformis and Paenibacillus amylolyticus bacteremia. Int J Infect Dis. 2015;35:93–5. Available from: http://dx.doi.org/10.1016/j.ijid.2015.04.016
8. Grady EN, MacDonald J, Liu L, Richman A, Yuan Z-C. Current knowledge and perspectives of Paenibacillus: a review. Microb Cell Fact. 2016;15(1):203. Available from: https://pubmed.ncbi.nlm.nih.gov/27905924
9. Hyo Y, Fukushima H, Harada T, Hara H. Nasal septal abscess caused by anaerobic bacteria of oral flora. Auris Nasus Larynx. 2019;46(1):147–50.
10. Kumpitsch C, Koskinen K, Schöpf V, Moissl-Eichinger C. The microbiome of the upper respiratory tract in health and disease. BMC Biol. 2019;17(1):87. Available from: https://pubmed.ncbi.nlm.nih.gov/31699101