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First Report of Wound Infection with *Bacillus pumilus* in Iran

Hossein Azizi¹, Farnaz Kheirandish², Fatemeh Saleh^{1*}
and Mehdi Moazzami Gudarzi³

¹Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences,
Khorramabad, Iran.

²Department of Parasitology and Mycology, Razi Herbal Medicines Research Center,
Lorestan University of Medical Sciences, Khorramabad, Iran.

³Department of Microbiology, Faculty of Paramedical Science, Islamic Azad University,
Borujerd Branch, Borujerd, Iran.

Authors' contributions

This work was carried out in collaboration between all authors. Author FS wrote and edited. Authors HA and FS took over the microbiologic diagnosis. Author FK served as the molecular recognition process and also paper edited and author MMG served as the molecular recognition process. All authors read and approved the final manuscript.

Case Study

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ABSTRACT

Aims: *Bacillus pumilus* is a spore-forming bacillus that causes infections which has been rarely reported among humans. This research was the first report of isolating *B. pumilus* from bed sore in a patient who was a rancher in Iran.

Presentation of Case: In October 2012 a man with an injured livestock in the back area with fever, pain, bleeding, inflammation, accompanied by black necrosis tissue was hospitalized. According to the patient statements ulcer was due to contamination by animal. After the wound biopsy, the infectious specimen was cultured on the blood agar culture medium and then several biochemical tests were performed in order to identify. Polymerase chain reaction (PCR) was used for determining molecular identity.

Discussion and Conclusion: PCR was used for molecular identification. Result of sequencing and blast showed 99% homology with *B. pumilus*. According to physician orders, patient was treated with clindamycin and ceftriaxone. Results of the present research showed that *Bacillus B. pumilus* in addition to the gastrointestinal and skin infections in humans can be a factor in bed sore disease.

*Corresponding author: Email: f.saleh11@yahoo.com;

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1. INTRODUCTION

Genus of *Bacillus* includes rod-shaped, gram-positive, spore-forming and aerobic bacilli which is usually arranged like a chain. Most genera of *Bacillus* are found as saprophytes in soil, water and also some species are reported in insects and animals. Because the spores have a high resistance to different environmental conditions [1,2,3]. For example, spore of *Bacillus pumilus* has been even found in the interior volcanic rocks [1,2]. Due to production of peptide-antibiotic, lipopeptides and bacteriocins derivatives, *B. pumilus* has anti-bacterial and anti-fungal properties.

One of the remarkable points about *B. pumilus* is its resistance to H₂O₂¹, which can be used in bioreduction reactions. Compared with other species indicates a higher resistance to UV radiation and also has high resistance to disinfectants [4,5,6]. Rare cases of infection with *B. pumilus* have been reported in human [7]. Various species of *Bacillus* because of spore production can cause different diseases [8,9]. *Bacillus anthracis*, the causative agent of anthrax is more than 90% of its virulent form is cutaneous. Diseases spread by spores in the environment. Pollution of the environment, including animals, followed by contact with animals can be a major factor in causing disease. In 2007, 3 cases of food poisoning by *B. pumilus*, were reported, which were isolated species producing a kind of lipopeptide called pomilacidin with toxic effects on epithelial cells [5,8].

Some reports have been presented that *B. pumilus* can lead to cutaneous ulcer which is very similar to skin infection created by *B. anthracis*. Similar to present study, Bacteria was isolated from rancher that cause of ulcer was contact with animals [4,7].

2. PRESENTATION OF CASE

In October 2012, in Shohadaye Ashayer Hospital of Khorramabad city, Lorestan province, a man 41 years old was hospitalized. He was a rancher and had symptoms of fever, chills and dizziness. The patient referred to the hospital complaining about a wound in the back area which had inflammation, swelling, pain and bleeding. Blisters were observed in some areas of the back skin. There were also necrosis and black tissues around the wound (Fig. 1). The first diagnosis of physicians was bedsores. According to the patient statements appearance of infection signs have been created after contact with infected animals. By Additional reviews and tests indicated the patient had a primary lesion that caused by contact with infected animal get ulcers form. Biopsy of the lesion was done by sterile swab which was then inoculated to Stuart transport medium. Then, the wound biopsy was cultured on Blood Agar culture medium [7]. After incubation of the culture medium at 37°C both in aerobic and 5% CO₂ conditions, diagnostic biochemical tests were performed for the identification. Catalase positive, oxidase negative and colonies were observed in large, rough and beta-hemolytic forms. Spores were seen central and paracentral, motility, citrate utilization, VP reaction, growth in NaCl 7%, 45°C, gelatin hydrolysis, ONPG activity, lecithinase are positive, Acid production from Mannitol, Glucose, Maltose, Sucrose, Arabinose. In addition, Anaerobic growth, Urase, Starch hydrolysis, Indol, are Negative, did not produce acid from Lactose [7] (Table1). Antibiogram test was done by disk diffusion method according to CLSLI for various disks, in which *B. pumilus* showed sensitivity to antibiotics of gentamicin, amikacin, imipenem, ampicillin, tetracycline, ciprofloxacin, sulfamethoxazole, ceftriaxone and

¹ Hydrogen proxide

vancomycin and resistance to cefotaxime [7]. Finally, according to physician orders, patient was treated with clindamycin and ceftriaxone.



Fig. 1. Bedsore infection with black tissues

2.1 Gene Amplification of 16S rRNA

PCR was used for molecular identification. After DNA extraction using protocol of DNG Plus kit (Cinnagen; Tehran, Iran), a part of the 16S rRNA gene was amplified by universal primer according to the previous studies (10): Forward, RW01 (5-AACTGGAGGAAGGTGGGGAT-3) and Reverse, DG74 (5-AGGAGGTGATCCAACCGCA-3). Electrophoresis of PCR product was loaded on 1% agarose (Fig. 2)

2.2 Determining the sequence and performing BLAST on the sequences

PCR product was prepared for sequencing. The obtained sequence was compared with the sequences reported in Gene Bank and the results showed 99% homology in the considered gene based on gene sequence with strain of *B. pumilus*. The considered gene was submitted in Gene Bank with access number AB894358.

Table 1. Evaluation of biochemical tests in different species of Bacillus, (+) 85-100%, (-) 0-14%, (v) Variable, (w) Weak

	<i>B. pumilus</i>	<i>B. anthracis</i>	<i>B. cereus</i>	<i>B. megaterium</i>	<i>B. subtilis</i>	<i>B. coagulans</i>	<i>B. brevis</i>	<i>B. sthearothermophilus</i>	<i>B. thuringiensis</i>	<i>B. lateroporus</i>
Motility	+	-	+	+	+	+	+	+	+	+
Spore:										
Terminal/sub terminal	-	-	-	-	-	-	v	+	-	-
Central/paracentral	+	+	+	+	+	v	+	-	+	+
Anaerobic growth	-	+	+	-	-	+	-	-	+	+
Citrate utilization	+	w	+	w	+	w	w	-	-	+
VP Reaction	+	+	+	-	+	v	-	-	+	-
Indol	-	v	+	-	-	-	-	+	+	-
Lecithinase	+	+	+	-	-	-	-	-	-	+
Growth in:										
Nacl 7%	+	+	+	-	+	+	-	+	-	-
45 ^o c	+	-	+	+	+	+	+	+	w	+
Urase	-	-	-	+	-	-	-	-	+	+
Starch hydrolysis	-	+	+	+	+	+	-	+	-	-
Gelatin hydrolysis	+	+	+	+	+	-	-	+	-	-
Acid From:										
Manitol	+	-	-	+	+	+	-	w	+	-
Lactose	-	-	-	+	-	-	-	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	-	+	+	-
Arabinose	+	-	-	+	+	+	-	-	w	+
Xylose	+	-	-	+	+	+	w	+	+	+
Sucrose	+	+	v	+	+	+	+	+	v	-
ONPG activity	+	-	-	+	+	v	w	+	+	+

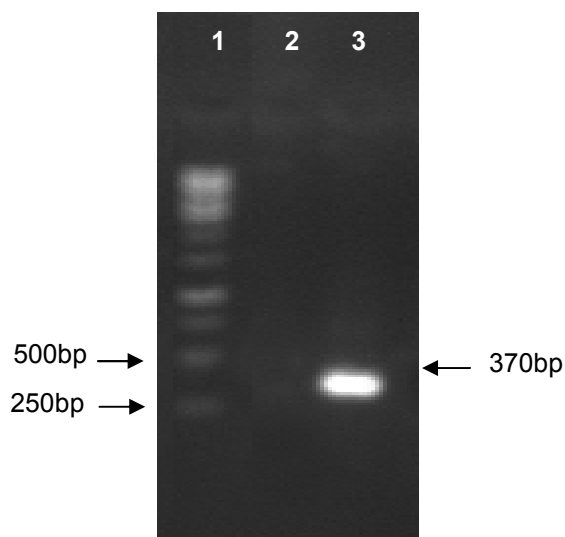


Fig. 2. Electrophoresis of PCR product on 1% agarose gel

Lane-1: 1000bp DNA ladder marker

Lane-2: Negative control

Lane-3: Specific band of B. Pumilus (370bp)

3. DISCUSSION

B. pumilus has received less attention as human pathogens because this bacterium often attacks plants, fruits and animals and is factor of disease for them [11]. It has several enzymes such as amylase, lipase [9]. Also it has cytopathic effects in Vero cells, haemolytic activity, lecithinase production proteolytic action on casein [7]. *B. pumilus* produces a toxin that has been detected in guinea pigs with experimentally induced enterocolitis associated with clindamycin [7]. Due to the enzyme activities can be explained toxicity of the *B. pumilus*.

It is possible that skin lesions can be related to toxic properties of *B. pumilus* [7]. There are rare reports on its pathogenicity in humans [1,2]. According to the previous studies, *B. pumilus* is considered one of the causes of gastroenteritis and food poisoning like other bacilli. However, this Bacillus has been often introduced as the cause of secondary infections such as septicemia and meningitis [7,8,9,12].

A number of reports were announced the bacteria cause skin infections and reason of infection diagnosed animal contact and like present study the ulcers were similar to anthrax [7].

This study is the first report of infection by *B. pumilus* in Iran. In our case, infection was clinically similar to cutaneous anthrax. Furthermore, using biochemical diagnostic tests, PCR and sequencing were used to confirm the diagnosis. included Catalase positive and oxidase negative, Spores were seen Central and Paracentral, Motility, Citrate utilization, VP Reaction, Growth in NaCl 7%, 45°C, Gelatin hydrolysis, ONPG activity, are positive, Acid production from Manitol, Glucose, Maltose, Sucrose, Arabinose. In addition, Anaerobic growth, Urase, Starch hydrolysis, Indol, Lecithinase are Negative, did not produce acid from Lactose. However, the same study only biochemical tests were carried out for diagnostic of infection [7]. Based on our findings, the patient in present research was contacted with

animals or their products such as hides or wool. Patient was exposed to the occupational factors. According to the patient statements, he had a wound in lumbar area that contact with animals caused infection *B. pumilus*. Due to the similarity of clinical signs of *B. pumilus* ulcer to anthrax and releasing spores into the environment by *B. pumilus*, as a result, it could has led to problems in hospital. The results of this study showed that other *Bacillus* species also have the ability to create diseases like anthrax. It is recommended that further studies should be done in this regard. Rapid diagnosis of infection and prescribe appropriate antibiotics can be effective in preventing disease progression. Due to the growing resistance of bacteria to antibiotics commonly and geographical differences, there are also suggestions that the experimental treatment appears to be necessary to identify the resistance pattern of the bacteria. Therefore, in this case, skin infections similar to that of *B. anthracis* are being progression as a nosocomial infection is important [13].

4. CONCLUSION

According to fast process of pathogenic bacteria and hospital infections is recommended that further studies on the relationship between *B. pumilus* toxin and other microbial toxins such as *B. anthracis* toxin be done. Also in patients with suspected lesions of cutaneous anthrax are more precise studies should be performed and this ulcer should not be construed as culture contamination.

CONSENT

All authors declare that written informed consent was obtained from the patient for publication of this case report and accompanying images

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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