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Case Report: *Bacillus pumilus*–Caused Bacteremia in a Patient with Food Poisoning

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Abstract. *Bacillus pumilus* has rarely been reported as a cause of human infections. We report a case of a *B. pumilus* causing food poisoning in an adult male. A 51-year-old Japanese man complained of severe abdominal cramps, fever with chills, diarrhea, dizziness, and loss of appetite after eating reheated rice with stewed minced meat purchased from a Kenyan restaurant. *Bacillus pumilus* was isolated from blood culture and was identified using a biochemical test and 16S rRNA gene sequencing analysis. The patient was treated with probiotics and ciprofloxacin and recovered after 3 days. To our knowledge, this is the first report describing the potential role of *B. pumilus* as a foodborne pathogen in Kenya and highlights the importance of good hygiene and food preparation practices.

INTRODUCTION

Food poisoning is a common, yet distressing, and sometimes a life-threatening problem. According to the World Health Organization's estimation, as many as 600 million people, or almost 1 in 10 people in the world, fall ill after consuming contaminated food each year. Of these, 420,000 people die, including 125,000 children younger than 5 years, with particularly high incidence rates reported in Africa and South-East Asia.[1] Toxic or infective agents such as bacteria, viruses, parasites, or prions can cause food poisoning by contaminating food at different points in the food production and preparation process.[1] It has also been shown that foodborne diseases can spread faster, once they emerge. A recent estimate suggests that approximately 30% of all newly emerging pathogens are commonly transmitted through food.[2] Most of the food poisoning cases, including diarrhea, vomiting, and stomach cramps usually start 4–36 hours after eating contaminated or spoiled food. Recovery can be achieved through rehydration treatment without a clinical diagnosis, although occasional severe outbreaks create a newsworthy public health hazard. In this study, we describe a food poisoning case caused by *Bacillus pumilus* in Kenya, and to the best of our knowledge, this is the first report.

CASE REPORT

A 51-year-old Japanese male patient visited an outpatient facility at a Nairobi hospital on November 26, 2013, because of severe abdominal pain/cramps, fever (38.1°C) with slight chills, diarrhea, dizziness, and loss of appetite. Initially, it was suspected to be an acute food poisoning because according to the information provided on November 25, 2013, the patient started to experience strong chills several hours after eating reheated rice and stewed minced meat for dinner, purchased from a Kenyan restaurant. Initial diagnosis at the hospital showed elevated levels of neutrophils (9.6×10^9/L). Both malaria and influenza A and B test results were negative. On the same day, the patient also reported to the Bacteriology Department of the Center for Microbiology Research, Nagasaki University Institute of Tropical Medicine-Kenya Medical Research Institute (NUITM-KEMRI) where stool and blood culture tests were recommended. Probiotics and oral ciprofloxacin tablets (500 mg/8 hours) were prescribed at the hospital, and he completely recovered after 2 days.

The stool sample was cultured on xylose lysine deoxycholate agar (Oxoid Ltd., Basingstoke, UK), deoxycholate hydrogen sulfide lactose agar, bromothymol blue agar, and Salmonella–Shigella agar (Eiken Chemical Company, Ltd., Tochigi, Japan), and incubated for 24–48 hours at 37°C. For *Campylobacter* spp. detection, the stool sample was cultured on Butzler selective medium (Oxoid Ltd.) and incubated under a micro-aerophilic condition with an Anaeropack® system (Mitsubishi Gas Chemical Company, Inc., Tokyo, Japan) at 42°C for 48 hours. Only lactose fermenting colony was observed, and nonpathogenic *Escherichia coli* was confirmed after the PCR test.[3] A blood sample was also drawn both for aerobic and anaerobic culture and incubated in BD BACTEC 9050 blood culture system (Becton Dickinson, Franklin Lakes, NJ). Following a positive alarm from the automated BACTEC system, the isolate was subcultured on 5% sheep blood agar at 37°C overnight. The colonies observed were large, rough, and beta-hemolytic forms. Microscopic examination showed sporulated Gram-positive rods. The organism was identified as *B. pumilus* by a battery of conventional biochemical tests using API 20E and API 50CHB (bioMerieux, Marcy-l'Étoile, France) kit according to manufacturer's instruction. The sample was found to be positive for o-nitrophenyl-b-D-galactopyranoside, gelatin, esculin, and the Voges-Proskauer reaction, but negative for citrate, urea, and indole (Table 1). Acid production was observed from glucose, mannitol, sucrose, arabinose, fructose, and mannose. Also, the isolate was catalase and oxidase positive, motile, can grow at 5–50°C temperature, 10% NaCl, and under UV light. A serum sample was additionally drawn and examined after 20 weeks of infection to determine the immune response against isolated *B. pumilus*, and the formation of agglutination was detected visually. Also, the organism was further confirmed by 16S rDNA sequence analysis using universal primers,[4] and the sequence was compared by National Center for Biotechnology Information, Basic Local Alignment Search Tool (NCBI, BLAST) program. The GenBank DNA database revealed 99.7% homology (1,044/1,047 base pairs) to *B. pumilus* (GenBank accession number MK192094).

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TABLE 1
Biochemical characteristics identifying the *Bacillus pumilus* isolate

Characteristic	*Bacillus pumilus*
API 20E tests for utilization of	
β-Galactosidase, acetoin production, gelatinase, glucose, sucrose, mannitol, amygdalin, arabinose.	Positive
Arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citrate utilization, H_2S production, urea hydrolysis, tryptophan deaminase, indole production, inositol, sorbitol, rhamnose, melibiose.	Negative
API 50CHB tests for utilization of	
Glycerol, L-arabinose, D-ribose, D-xylose, D-galactose, D-fructose, D-mannose, D-mannitol, α-methyl-D-mannoside, α-methyl-D-glucoside, N-acetylglucosamine, arbutin, esculin, salicin, cellobiose, D-maltose, D-trehalose, D-turanose, D-tagatose.	Positive
Erythritol, D-arabinose, L-xylose, D-adonitol, methyl-β-D-xylopyranoside, L-sorbose, L-rhamnose, dulcitol, inositol, D-sorbitol, D-lactose, inulin, melezitose, raffinose, starch, glycogen, xylitol, gentiobiose, D-lyxose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium gluconate, potassium 2-ketogluconate, potassium 5-ketogluconate.	Negative
Additional tests	
Motility, catalase, oxidase, casein hydrolysis, growth in 10% NaCl, growth under UV light, growth maximum at 50°C.	Positive

Antimicrobial susceptibility testing was performed by broth microdilution method as per the Clinical and Laboratory Standard Institute guidelines.[5] The isolate was found to be sensitive against amikacin, amoxicillin, ampicillin, amoxicillin/clavulanate, azithromycin, cefazolin, ciprofloxacin, doxycycline, erythromycin, gentamicin, levofloxacin, meropenem, ofloxacin, streptomycin, tazobactam/piperacillin, tetracycline, and vancomycin, but resistant to cefepime (128 μg/mL), and cefotaxime (64 μg/mL).

DISCUSSION

Unlike *Bacillus anthracis* and *Bacillus cereus*, *B. pumilus* has rarely been reported as a human pathogen. Therefore, few clinical reports on *B. pumilus* causing infections are documented; including food poisoning and bloodstream, wound, and cutaneous infections.[6–8] The lower rate of isolation could be because of the fact that many clinical laboratories may not attempt to identify *Bacillus* organisms at the species level.

Previous studies reported that meat dishes, eggs, baked products, and canned tomato juice had been involved in a presumptive food poisoning by *B. pumilus*.[9] A heat-stable toxin-producing *B. pumilus* was further isolated from dairy milk in Finland.[10,11] Recently, pre-cooked rice contaminated with pumilacidin-producing *B. pumilus* strain was implicated in a case of food poisoning in Norway.[12] Ingestion of cooked and reheated rice was identified among the main risk factors for *B. cereus* emetic food poisoning in earlier studies.[13,14] Similarly, the patient in our case had eaten reheated rice and stewed meat before getting sick. Therefore, we suspected that either reheated rice or stewed meat could be the source of infection, although consumed food samples were not available for laboratory analysis and there was no direct evidence provided for *B. pumilus* food contamination. We, however, speculate that the organism was responsible for the symptoms because except *B. pumilus*, no other pathogen was isolated from the blood.

Toxin-producing *B. pumilus* has been detected in guinea pigs with experimentally induced enterocolitis, and components produced by *B. pumilus* have shown toxicity against mice and eukaryotic cells.[15–18] In addition, cytopathic effects on Vero cells, hemolytic activity, lecithinase production, and proteolytic action on casein was documented which was also observed in our study.[17,19] However, in the present study, we could not confirm the production of toxin by *B. pumilus*; thus, additional studies are needed to determine the involvement of the toxin in food poisoning.

In this study, *B. pumilus* was found highly sensitive against commonly used antibiotics except for cefepime and cefotaxime. The reason behind this resistance pattern was not known, although Azizi et al.,[8] have described a similar result and further investigation is required.

Finally, this case report provides evidence that *B. pumilus* is a potential bloodstream pathogen that can cause acute food poisoning and highlights the importance of careful identification of *B. pumilus* in the clinical microbiology laboratory. It also expands the clinical spectrum of this microorganism as a causative agent of human infections. We recommend the promotion of good hygiene and food preparation practices, particularly in restaurants.

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REFERENCES

1. World Health Organization, 2015. *WHO Estimates of the Global Burden of Foodborne Diseases: Foodborne Disease Burden Epidemiology Reference Group 2007–2015.* Available at: http://www.who.int/iris/handle/10665/199350.
2. Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, Daszak P, 2008. Global trends in emerging infectious diseases. *Nature 451:* 990–993.
3. Toma C, Lu Y, Higa N, Nakasone N, Chinen I, Baschkier A, Rivas M, Iwanaga M, 2003. Multiplex PCR assay for identification of human diarrheagenic *Escherichia coli*. *J Clin Microbiol 41:* 2669–2671.
4. Shah MM, Iihara H, Noda M, Song SX, Nhung PH, Ohkusu K, Kawamura Y, Ezaki T, 2007. dnaJ gene sequence-based assay for species identification and phylogenetic grouping in the genus *Staphylococcus*. *Int J Syst Evol Microbiol 57:* 25–30.

5. Clinical and Laboratory Standard Institute (CLSI), 2018. *Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Eighth Informational Supplement*. Wayne, PA: Clinical and Laboratory Standard Institute. (CLSI document, M100).
6. Kimouli M, Vrioni G, Papadopoulou M, Koumaki V, Petropoulou D, Gounaris A, Friedrich AW, Tsakris A, 2012. Two cases of severe sepsis caused by *Bacillus pumilus* in neonatal infants. *J Med Microbiol 61:* 596–599.
7. Tena D, Martinez-Torres JA, Perez-Pomata MT, Sáez-Nieto JA, Rubio V, Bisquert J, 2007. Cutaneous infection due to *Bacillus pumilus*: report of 3 cases. *Clin Infect Dis 44:* e40–e42.
8. Azizi H, Kheirandish F, Saleh F, Gudarzi MM, 2014. First report of wound infection with *Bacillus pumilus* in Iran. *Annu Res Rev Biol 4:* 3220–3226.
9. Kramer JM, Gilbert RJ, 1989. *Bacillus cereus* and other *Bacillus* species. Doyle MP, ed. *Foodborne Bacterial Pathogens*. New York, NY: Marcel Dekker.
10. De Jonghe V, Coorevits A, De Block J, Van Coillie E, Grijspeerdt K, Herman L, De Vos P, Heyndrickx M, 2010. Toxinogenic and spoilage potential of aerobic spore-formers isolated from raw milk. *Int J Food Microbiol 136:* 318–325.
11. Nieminen T, Rintaluoma N, Andersson M, Taimisto AM, Ali-Vehmas T, Seppälä A, Priha O, Salkinoja-Salonen M, 2007. Toxinogenic *Bacillus pumilus* and *Bacillus licheniformis* from mastitic milk. *Vet Microbiol 124:* 329–339.
12. From C, Hormazabal V, Granum PE, 2007. Food poisoning associated with pumilacidin-producing *Bacillus pumilus* in rice. *Int J Food Microbiol 115:* 319–324.
13. Shinagawa K, 1990. Analytical methods for *Bacillus cereus* and other *Bacillus* species. *Int J Food Microbiol 10:* 125–141.
14. Ehling-Schulz M, Fricker M, Scherer S, 2004. *Bacillus cereus*, the causative agent of an emetic type of food-borne illness. *Mol Nutr Food Res 48:* 479–487.
15. Brophy PF, Knoop FC, 1982. *Bacillus pumilus* in the induction of clindamycin-associated enterocolitis in Guinea pigs. *Infect Immun 35:* 289–295.
16. Obi SK, 1980. Lecithinase and toxin production in *Bacillus* species. *Zentralbl Bakteriol A 246:* 415–422.
17. Hoult B, Tuxford AF, 1991. Toxin production by *Bacillus pumilus*. *J Clin Pathol 44:* 455–458.
18. Suominen I, Andersson MA, Andersson MC, Hallaksela AM, Kämpfer P, Rainey FA, Salkinoja-Salonen M, 2001. Toxic *Bacillus pumilus* from indoor air, recycled paper pulp, Norway spruce, food poisoning outbreaks and clinical samples. *Syst Appl Microbiol 24:* 267–276.
19. From C, Pukall R, Schumann P, Hormazabal V, Granum PE, 2005. Toxin-producing ability among *Bacillus* spp. outside the *Bacillus cereus* group. *Appl Environ Microbiol 71:* 1178–1183.