Phylogenetic Tree Analysis of First *Psychrobacter* Sp. Strain From Blood of Iraqi Patient; A Case Report

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Abstract

Psychrobacter spp. are a Gram negative bacteria, aerobic, non-motile, small, with coccobacilli shape. Originally isolated from seaweed samples and marine environments. Recently considered as rare opportunistic human pathogens. Sixty –five years old women admitted to hospital with diabetic mellitus and stage 4 pressure ulcers (PU) with seizure and mild fever 37.9 °C. A gram staining of blood culture revealed gram negative bacteria have a cocobacilli shape. The VITEK2 system (bioMérieux) misidentify the isolate as *Acinetobacter bumannii* complex with low discrimination. The submission of the bacterial isolate to the GenBank BLAST search tool revealed that the Iraqi isolate show 100% homology with *Psychrobacter* sp. From china with accession number ID: MK205167.1, the next matches with Uncultured *Psychrobacter* sp. (ID: KF859544.1 China) *Psychrobacter pulmonis*(ID: KU364058.1, India), *Psychrobacter pulmonis* (ID: MH550129.1, China)with 99% similarity for each one. This *Psychrobacter* sp. was the first isolate from bacteremia patients in Iraq. The identification based on 16S rRNA gene sequence for precisely identify this bacteria that misidentified by VITEK2 system.

Key Words: *Psychrobacter sp., 16S ribosomal RNA gene, Bacteremia*

Introduction

PPsychrobacter species are gram-negative coccobacilli, aerobic, nonpigmented, nonmotile [1]. Latest taxonomic classification system of involve three bacterial genera in the family of Moraxellaceae: Moraxella, Psychrobacter and Acinetobacter [2].The Psychrobacter genus includes 33 species of validly submitted names [3]. P. phenylpyruvicus (formally known as Moraxella phenylpyruvica) was correlated with bacteremia, septic arthritis, endocarditis, foot abscess and wound infection [4]. A novel species of P. sanguinis, has also been isolated from samples of human blood [5]. Recent taxonomic classification of Psychrobacter species was revised and most bacteria isolates from human sources other than P. phenylpyruvicus belong to the newly identify P. pulmonis species and P. faecalis [6]. Therefore, the spectrum of infectious diseases in human associated with thevarious species of the Psychrobacter genus could rapidly change [4]. Psychrobacter spp. Isolated from marine species (fish, crustaceans); marine environments (seaweed and seabed); food products (cheese, seafood and meat); lamb lungs and digestive tracts of pigs. Psychrobacter spp. It may also be a part of human microbiota; studies have shown the existence of P. faecalis, P. arenosus, P. pulmonis and P. phenylpyruvicus in the human intestinal tract [7].

Psychrobacter species are rare opportunistic human pathogens. Only a Psychrobacter spp subsetare considered opportunistic pathogens that medically relevant and were isolated from samples collected from brain tissue, human blood, urine, vulvae, ears, eyes, cerebrospinal fluid, wounds and other skin sources [8]. Clinical characteristics rely on the site of the infectionand include meningitis [9], and bacteremia, of all these cases, just one was related to marine environment[10].

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Case report

Sixty –five years old women admitted to AL-Yarmuk Teaching Hospital in Baghdad, Iraq. History of patients are with seizure and mild fever 37.9 °C and diabetic mellitus with no diabetic foot and stage 4 pressure ulcers (PU) at her back appeared before 7 month , patient didn't received any antibiotic treatment. WBC account was 15.1 (10 e9/L). Two sets of blood culture (Brian heart infusion broth) were drawn from two distinct vein sites, after a day of incubation the aerobic blood culture bottle was become turbid as a result of bacterial growth. The broth after that was subcultured into the macconky and blood agar and incubated at 37°C in aerobic conditions; the bacteria only grow in the blood agar after 48hr of aerobically incubation appeared gram negative coccobacilli in gram stain under the microscope. These coccobaccili bacteria were oxidase and catalase positive. The antimicrobial sensitivity test was used to show the resistant of isolate to antimicrobial drugs. The antibiotics used were Meropenem, Levofloxacine, Ceftazidime, Ceftriaxone, Azithromycin and Vancomycin. Although there is no antimicrobial break point to *Psychrobacter* sp.*,* the large size of inhibition zone was recorded to all antimicrobial drugs used in this study except Azithromycin which mean this isolate was sensitive.

The phenotypic identification by VITEK2 system gram negative card (bioMérieux) result was low discrimination organism of *Acinetobacter bumannii* complex, this result was not satisfy therefore the molecular method was followed to precisely identifying the bacterial isolate. For identification of the bacteria, molecular analysis was followed by the PCR amplification of 16S rRNA , the following Universal primer pair was used in the reaction of the PCR,8F : 5′- AGA GTT TGA TCC TGG CTC AG 3′ and reverse U1492R:5′- GGT TAC CTT ACG ACT T 3′[11] . The PCR reaction was carried out with 2µl of template DNA and 25µl as a total reaction volume using GoTaq® Green Master Mix, 2X as follow : Initial denaturation for 5min at 94°C ; 35 cycles of 94°C , 52°C , and 72°C for (94 sec,1min ,1min) for each of them respectively; and finishing with 7min at 72°C. The PCR product was sent to microgen incorporation for sequencing. Sequence of 1500base-pairs (bp) was obtained. The analysis of the sequence with the GenBank Basic Local Alignment Search Tool (BLAST) (http://www.ncbi.nlm.nih.gov/genbank)

The submission of the isolate 16S rRNA gene sequence by the GenBank BLAST revealed that the Iraqi isolate show that 100% homology with *Psychrobacter* sp. From china with gene bank accession number ID: MK205167.1, the next matches with *Psychrobacter pulmonis*(ID: MH550129.1, China), *Psychrobacter pulmonis* (ID: KU364058.1, India), Uncultured *Psychrobacter* sp. (ID: KF859544.1 China) with 99% similarity for each one. The similarity was 98% with *Psychrobacter faecalis* (ID: MK205166.1, China) and *Psychrobacter faecalis* (ID: MH712977.1, Chile). The isolate sequence was submitted in the NCBI as the first *Psychrobacter* sp. isolate in Iraq.

The neighboring method was used to construct the phylogenetic tree Based on sequences of 16S rRNA of the isolate and the similarity with 23 bacterial isolates as shown in figure (1). The software was used in phylogenetic analysis was Molecular Evolutionary Genetics Analysis (MEGA) software version 6.0 (http://www.megasoftware.net).

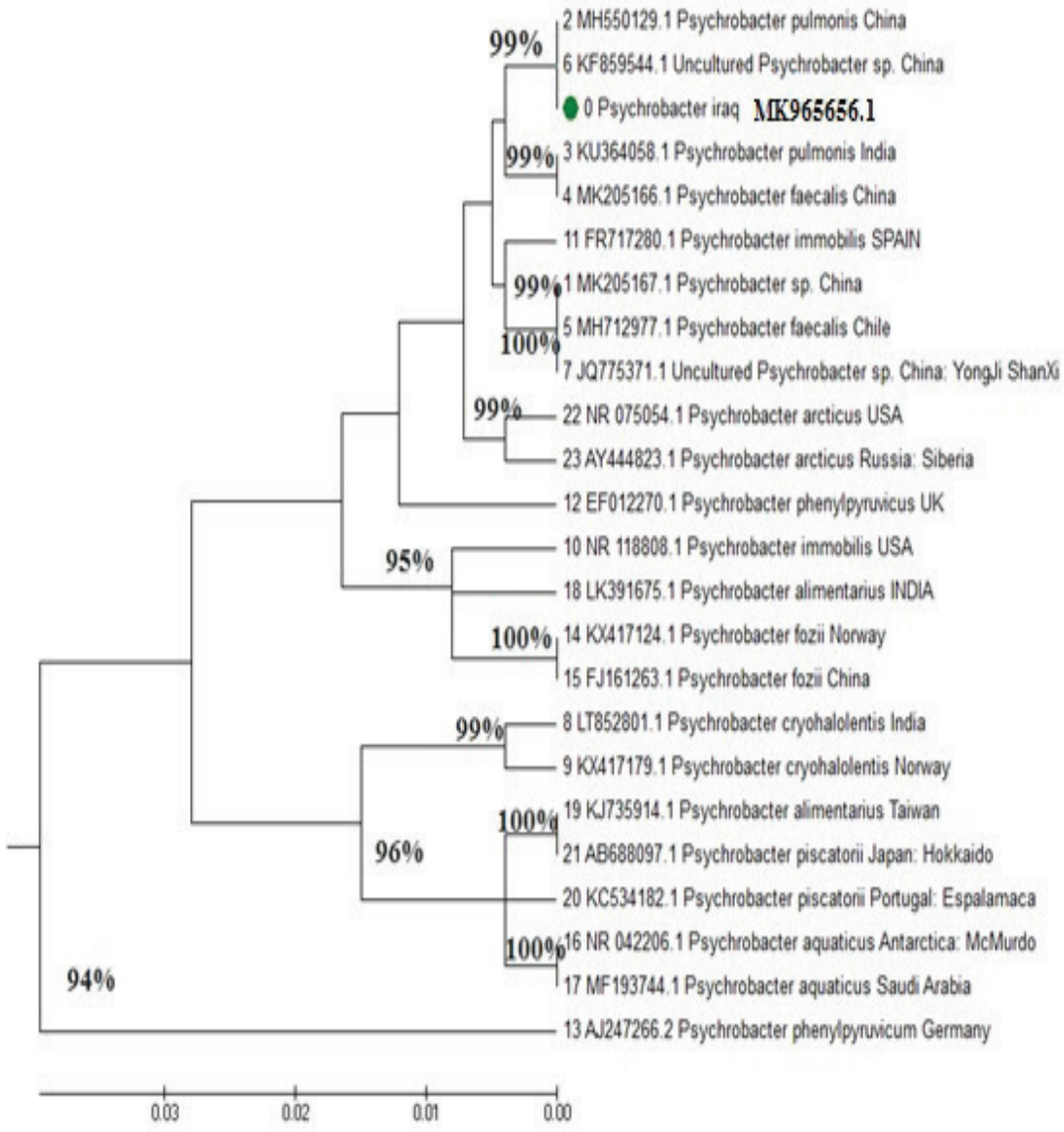

Figure 1: The relatedness of *Psychrobacter* sp. strain NA199151 to 23 other bacterial species

Discussion

Psychrobacter species are gram-negative coccobacilli, aerobic, nonpigmented, nonmotile[1] *Psychrobacter* species are rare opportunistic human pathogens and were isolated from samples collected from brain tissue , human blood, urine, vulvae, ears, eyes, cerebrospinal fluid, wounds and other skin sources [8]. Strains of *Psychrobacter spp.* are extremely susceptible to antimicrobial agents ; only one strain of *P. Phenylpyruvicus* was recorded to just be resistant to aztreonam and ampenicillin , while two strains of *P. immobilis* resistance to penicillin [12]. Disk diffusion testing was performed to antimicrobial sensitivity testing aerobicly at 35°C. Despite the lack of standardized breakpoints to *Psychrobacter spp.*, large zone sizes suggested the bacterial isolate was susceptible to penicillin, cefazolin, cefoxitin, tetracycline, ciprofloxacin, meropenem, cefepime and ceftriaxone. This isolate was tested negative to β-lactamase [13]. *P. sanguinis* isolate was susceptible to cefepime, amoxicillin, ceftazidime, meropenem, imipenem, ciprofloxacin, amikacin, ticarcillin and trimethoprim-sulfamethoxazole [14]. The isolate was resistant to lincomycin and susceptible to amoxicillin/clavulanate, amoxicillin, piperacillin, ticarcillin/clavulanate,

ceftazidime, cefotaxime, cefalotin, piperacillin/ tazobactam, cefepime, cefpirome, gentamicin, imipenem, netilmicin, tobramycin, trimethoprim/ sulfamethoxazole, pristinamycin, erythromycin, amikacin, polymyxin B, ofloxacin, nalidixic acid, fosfomycin and ciprofloxacin [4]. All these researches agree with the result of present study of antibiotic selectivity were the *Psychrobacter* sp. Isolate was sensitive to all antibiotics drugs except Azithromycin.

In 2016, one case of Psychrobacter associated with meningitis in a cerebrospinal fluid (CSF) of 13 year-old male patient's was revealed and isolate was recognized by metagenomics of the next generation sequencing (NGS) [9]. Four Psychrobacter isolates were obtained from human blood cultures at the Wadsworth Center (New York) from 2004 to 2008 that cannot identify correctly at the laboratory [5]. Scientific studies that identify microorganisms incorrectly by standard techniques are increasing [15]. As in this study the systems of phenotypic identification, Includes the VITEK2 system, couldn't identify psychrobacter sp. and misidentify as Acinetobacter bumanii. It is apparently that there is a increasing number of recently identified species of bacteria,in clinical microbiology the unmistakable identificatin of uncommon strains is increasingly required to enhance understanding of tissue reservoirs, transmission routes, susceptibility to antibiotic and treatment improvement and to help in the identifying of novel species of bacteria. The identification of unusual bacterial strain and bacteria with uncommon phenotypic profiles by standard techniques requires to be proven using a reliable method such as sequencing of 16S rRNA gene [15]. Additionally, M. Phenylpyruvica was reclassified to a genus Psychrobacter as (P. phenylpyruvica) by analysis of sequence of 16S rRNA genes [16].

In the present study, sequencing of the 16S rRNA gene was necessary for precise identification of bacterial isolate.Our research demonstrates that analyzes of 16S rRNA sequence and using different databases are helpful instruments for identifying unknown isolates.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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