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Short report

First report of *Ochrobactrum anthropi* isolated from bloodstream infection in Alexandria, Egypt

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ABSTRACT

Background: *Ochrobactrum anthropi* (*O. anthropi*) is a Gram-negative, nonfermenting bacillus, which has been recovered from clinical samples worldwide. However, it has never been reported from any clinical sample in Egypt. To the best of our knowledge, we report for the first time, the isolation of *O. anthropi* from bloodstream infection of a 75-year-old female patient. *Ochrobactrum anthropi* identification was performed using Vitek-2 system (BioMérieux, Marcy l'Etoile, France), and confirmed using 16S rRNA sequencing. The organism was further classified as *O. anthropi* subclade I, using *recA* sequence analysis. Susceptibility testing was carried out using broth microdilution method, and the isolate was found to be resistant to both ceftazidime and cefepime. Molecular investigation of genes conferring resistance to the third and fourth generation cephalosporins, showed that the isolate harbored *blaOCH-6* gene, and that *blaTEM*, *blaSHV* and *blaCTX-M* were not present. This work highlights the first isolation of *O. anthropi* from bloodstream infection in Alexandria, Egypt.

Introduction

Ochrobactrum anthropi (*O. anthropi*) is a nonfermenting, motile, Gram-negative bacillus. Genus *Ochrobactrum* belongs to family Brucellaceae, which includes other genera such as *Brucella*, *Pseudochrobactrum* and *Paenochrobactrum*. In 1988, *Ochrobactrum* was first described as a new genus for organisms previously known as centers for disease control and prevention (CDC) group Vd. *Ochrobactrum anthropi* was designated its type species [1-4]. *Ochrobactrum* species have been isolated from diverse environmental sites such as water, soil, plants, however, few species including *O. anthropi*,

O. intermedium, *O. pseudintermedium* and *O. haematophilu* have been recovered from human clinical samples [1-3].

Despite being closely related to genus *Brucella*, *O. anthropi* is considered a pathogen of low virulence. Genotypically, *O. anthropi* strains constitute a single clade that is further subdivided into six subclades designated (subclade I to subclade VI) [1,3,4]. The current work represents the first report of *O. anthropi* isolated from bloodstream infection in Alexandria, Egypt.

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Patient and Methods

Case presentation

A 75-year-old female patient presented to the emergency room. Her main complaints were fever, severe abdominal pain, nausea, and vomiting. The patient gave history of hypertension, diabetes, osteoarthritis, and the presence of a permanent pacemaker. Examination showed the following signs: fever ($>39^{\circ}\text{C}$), abdominal tenderness, bilateral lower-limb edema, elevated blood pressure ($>150/95$), tachycardia (80 B/min) and bed sores (grade 2) in sacral and both lower limbs. She was transferred to the intensive care unit (ICU) and the following investigations were conducted.

Laboratory investigations

Complete blood picture showed mild leukocytosis with absolute neutrophilia, C-reactive protein: 438.9 mg/L, D-dimer: 1998 ng/ml, brain natriuretic peptide (BNP): 17241 pg/ml and procalcitonin >100 ng/ml. Blood culture was withdrawn, and a positive signal was detected 18 hours post-inoculation on BacT/Alert 3D system (BioMérieux, Durham, NC, USA). It revealed the growth of a Gram-negative bacilli, which was identified as *Ochrobactrum anthropi* using Vitek-2 system (BioMérieux, Marcy l'Etoile, France). However, antimicrobial susceptibility could not be determined using this automated system because the card that was used was AST GN 222 (BioMérieux, Marcy l'Etoile, France), which was not the appropriate card for such organism. The patient was given meropenem empirically (loading dose: 1 gram/8 hours, maintenance dose: 1 gram/ 12 hours).

Ultrasound and radiologic investigations

Abdominal ultrasound revealed massive fluid collection at paracolic gutter. CT colonography revealed retro-pneumoperitoneum, rectosigmoid inflammatory process with upper rectal mural defect, para-umbilical hernia, enlarged liver and ascites as well as calculary gall bladder. Echocardiography revealed dilated ascending aorta, mild tricuspid regurgitation, pulmonary hypertension and that the ejection fraction was 50-55%. Lower limb doppler was free.

Ultrasound-guided aspiration from the abdominal fluid was carried out, and the aspirate was cultured on blood, MacConkey and Sabouraud dextrose agar (Oxoid, Cambridge, UK). Colonies grew on blood and Sabouraud Dextrose agar. Identification and antifungal susceptibility testing were performed using Vitek-2 system (BioMérieux, Marcy l'Etoile,

France), and showed that the organism was *Candida albicans*, which was susceptible to all the antifungal agents tested (fluconazole, voriconazole, micafungin and caspofungin). The patient was given fluconazole (loading dose: 800 mg once, maintenance dose: 200 mg/24 hours). The patient was diagnosed with intrabdominal sepsis and gastrointestinal tract (GIT) perforation.

Other procedures

The patient underwent exploration surgery. Evacuation of intraperitoneal stool collection as well as retroperitoneal abscesses was performed. Insertion of right and left drains and colostomy was done. Unfortunately, the patient condition deteriorated as shown by hemodynamic instability on vasopressors (norepinephrine and dobutamine). The patient was mechanically ventilated and died four days later.

Since this was the first time to encounter *O. anthropi* in a clinical sample from Egypt, further investigations were carried out.

Identification of the isolate using molecular techniques

Amplification of 16S rRNA and recA

16S rRNA gene and *recA* were amplified by PCR. The primers and their annealing temperatures are shown in **table (1)**. For the amplification we used: 95°C for 2 minutes, followed by 30 cycles at 95°C for 1 minute, 50°C for 1 minute, 72°C for 1 minute, and a final elongation step of 72°C for 7 min. The PCR reactions were performed on Veriti thermal cycler (Applied Biosystems, CA, USA), using DreamTaq Green PCR Master Mix (ThermoFischer, CA, USA).

16S rRNA and recA sequence analysis

The obtained amplicons of 16S rRNA and *recA* were sequenced using ABI 3730xl DNA sequencer (Applied Biosystems, CA, USA) using the forward and reverse primers listed in **table (1)**. Then, the obtained sequences were compared with the corresponding previously described sequences in GenBank using the BLASTN (<https://blast.ncbi.nlm.nih.gov>) tool.

The resulting nucleotide sequences of 16S rRNA showed 100% identity with 16S rRNA of *O. anthropi* and that of *O. lupini* as well as that of *O. cytisi* (GenBank accession number: MH281752.1, MT081281.1 and MF928100.1, respectively).

Using *recA* sequence analysis, the identification of the isolate was confirmed as *O. anthropi* and it was further classified as one of the members of subclade

I. The constructed phylogenetic tree is shown in **figure (1)**. The obtained 16S rRNA and *recA* nucleotide sequences were deposited in the GenBank database.

Susceptibility testing

Susceptibility testing of the isolate was performed using broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI) M-100 guidelines for non-Enterobacterales [5]. The antibacterial agents tested were meropenem, ceftazidime, cefepime, gentamycin, amikacin, and levofloxacin.

The isolate was found to be susceptible to meropenem, gentamycin, amikacin and levofloxacin. However, it was resistant to ceftazidime and cefepime. The obtained minimum inhibitory concentration (MIC) values are shown in **table (2)**.

Molecular detection of antimicrobial resistance genes

Genes which confer resistance to ceftazidime and cefepime (*blaTEM*, *blaSHV*, *blaCTX-M* and

blaOCH) were investigated. The primers and their annealing temperatures are shown in **table (1)** The amplification scheme was similar to that previously mentioned for 16S rRNA and *recA*.

Only *blaOCH* gene was detected. The amplicon (547 bp) was sequenced, and the resulting nucleotide sequence was deposited in the GenBank database. BLASTN (<https://blast.ncbi.nlm.nih.gov>) tool was used and the obtained sequence was identified as *blaOCH-6* gene. The constructed phylogenetic tree is shown in **figure (2)**.

Nucleotide sequence accession numbers

Nucleotide sequences of 16S rRNA, *recA* and *blaOCH-6* genes were deposited in the GenBank database under accession numbers MZ149997.1 (<https://www.ncbi.nlm.nih.gov/nuccore/MZ149997.1>), MZ209186.1 (<https://www.ncbi.nlm.nih.gov/nuccore/MZ209186.1>) and MZ242290.1 (<https://www.ncbi.nlm.nih.gov/nuccore/MZ242290.1>), respectively.

Table 1. Primers used in this study.

Primer	Nucleotide Sequence (5'-3')	Used for	Annealing temperature in °C	Reference
pr0R2 (F)	AGAGTTTGATCMTGGCTCAG	Amplification and sequencing of 16S rRNA gene	50	[1]
9REV (R)	AAGGAGGTGATCCAGCC			
<i>recA</i> (F)	ATGTCTCAAAATTCATTGCGAC	Amplification and sequencing of <i>recA</i>	51	[4]
<i>recA</i> (R)	AGCATCTTCTTCCGGTCCGC			
<i>blaOCH</i> (F)	CAGCTTCGACAAGATCACCA	Amplification and sequencing of <i>blaOCH</i>	53	[6]
<i>blaOCH</i> (R)	CTTGAGCGCAGTCGGATAG			
<i>blaSHV</i> (F)	GCAAAACGCCGGTTATTC	Amplification of <i>blaSHV</i>	50	[7]
<i>blaSHV</i> (R)	GGTTAGCGTTGCCAGTGCT			
<i>blaTEM</i> (F)	ATGAGTATTCAACATTTCCG	Amplification of <i>blaTEM</i>	46	[8]
<i>blaTEM</i> (R)	TTAATCAGTGAGGCACCTAT			
<i>blaCTX-M</i> (F)	CGCTTTGCGATGTGCAG	Amplification of <i>blaCTX-M</i>	50	[7]
<i>blaCTX-M</i> (R)	ACCGCGATATCGTTGGT			

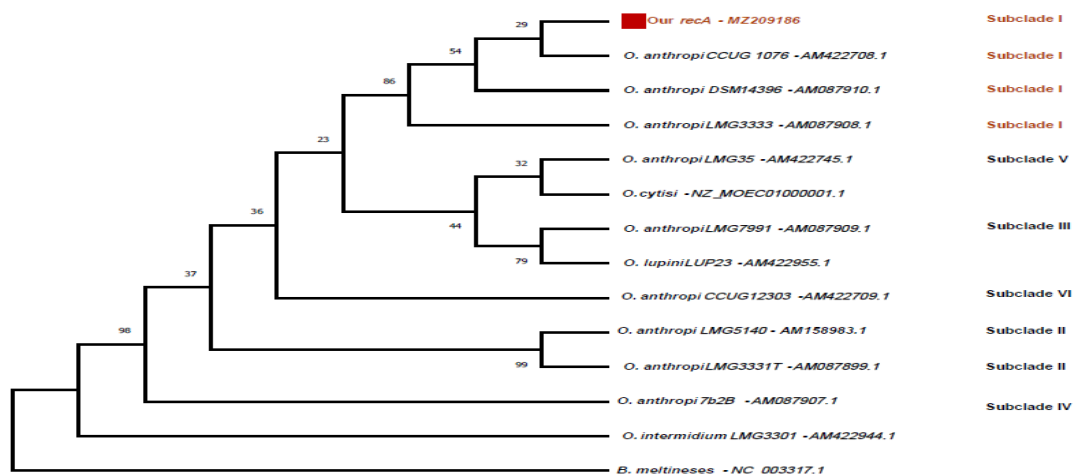
Table 2. Susceptibility pattern of our isolate.

Antibacterial Agent	MIC in $\mu\text{g/ml}$	Interpretation *
Meropenem	< 0.5	S
Ceftazidime	>256	R
Cefepime	256	R
Gentamycin	< 0.5	S
Amikacin	2	S
Levofloxacin	< 0.5	S

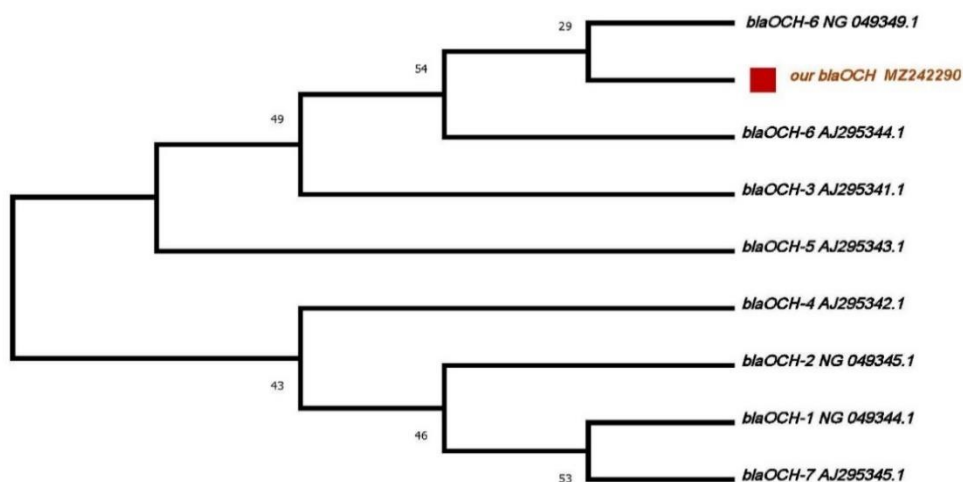
* Interpretation according to the CLSI guidelines for non-Enterobacterales [5].

Figure 1. Phylogenetic tree based on *recA* sequence analysis.

Phylogenetic tree was inferred by using the Maximum Likelihood method and Tamura 3-parameter model. The bootstrap values were calculated from 1000 replications. Evolutionary analysis was conducted in MEGA X.

**Figure 2.** Phylogenetic tree for the different variants of *blaOCH*.

Phylogenetic tree for the different variants of *blaOCH* was inferred by using the Maximum Likelihood method and Jukes-Cantor model. The bootstrap values were calculated from 1000 replications. Evolutionary analysis was conducted in MEGA X.



Discussion

Ochrobactrum anthropi has been regarded as a pathogen of low virulence. *O. anthropi* has been isolated from different clinical cases such as bacteremia, endocarditis, and others [1-3].

Ochrobactrum anthropi isolates in Egypt, were previously isolated from environmental samples such as hydrocarbon contaminated soil [9,10], plant–soil systems [11] as well as other environmental samples [12,13]. Here, we describe the isolation of *O. anthropi* from the bloodstream of an elderly female patient.

Identification of *O. anthropi* can be challenging; misidentification of *O. anthropi* as *Ralstonia paucula* or as *Brucella* spp. has been previously reported [1,14]. In the current study, the bacterium was correctly identified as *O. anthropi* using Vitek-2 system (BioMérieux, Marcy l’Etoile, France).

16S rRNA sequence analysis is one of the methods used for bacterial identification, interestingly *recA* sequencing has shown a higher discriminatory power between bacterial species and subspecies levels [4].

In the present study, 16S rRNA sequence analysis was used to confirm *O. anthropi* identification, however, it could not differentiate between *O. anthropi* and *O. cytisi*. Using *recA* sequence analysis, *O. anthropi* identification was confirmed and the isolate was classified as a member of subclade I

Ochrobactrum anthropi is characterized by being resistant to beta-lactams except for carbapenems. This trait is mediated via an AmpC-like beta-lactamase enzyme. This enzyme is encoded by *O. anthropi* ampC (*blaOCH*) gene [15], which has seven different variants: *blaOCH-1* to *blaOCH-7*. The isolated *O. anthropi* was resistant to ceftazidime and cefepime and the only gene that could be detected was *blaOCH-6*. The enzyme encoded by *blaOCH* gene is resistant to inhibition by clavulanic acid [1,15], and is also present in *non-anthropi* species, such as *O. intermedium* and *O. tritici* [6].

Conclusion

To the best of our knowledge, we report for the first time, the isolation of *O. anthropi* from bloodstream infection of a 75-year-old female patient in Alexandria, Egypt. Constant surveillance for emerging pathogens is desperately needed as different environmental organisms are being

recovered among clinical isolates worldwide. Robust infection control measures are crucial to prevent possible outbreaks due to these organisms. Effective antimicrobial programs play a fundamental role to attain judicious use of antimicrobial agents to pave the way for achieving best clinical outcomes.

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Conflict of interest

All authors declare no conflicts of interest in this paper

Ethical approval

Ethical approval was obtained from the Ethical Committee of the Medical Research Institute, Alexandria University.

References

- 1-Hagiya H, Ohnishi K, Maki M, Watanabe N, Murase T. Clinical characteristics of *Ochrobactrum anthropi* bacteremia. J Clin Microbiol 2013;51(4):1330-3.
- 2-Holmes MP, M. Kiredjian, K. Kersters. *Ochrobactrum anthropi* gen. nov., sp. nov. from Human Clinical Specimens and Previously Known as Group Vd. International Journal of Systematic and Evolutionary Microbiology 1988;38(4):406-16.
- 3-Chmelar D, Holy O, Kasakova I, Hajek M, Lazarova A, Gonzalez-Rey C, et al. Antibiotic susceptibility and production of endotoxin by *Ochrobactrum anthropi* isolated from environment and from patients with cystic fibrosis. Folia Microbiol (Praha) 2019;64(6):861-5.
- 4-Scholz HC, Al Dahouk S, Tomaso H, Neubauer H, Witte A, Schloter M, et al. Genetic diversity and phylogenetic relationships of bacteria belonging to the *Ochrobactrum-Brucella* group by *recA* and 16S

- rRNA gene-based comparative sequence analysis. *Syst Appl Microbiol* 2008;31(1):1-16.
- 5-**Clinical and Laboratory Standards Institute.** Performance Standards for Antimicrobial Susceptibility Testing, 30th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute, Wayne, PA, 2020.
- 6-**Alonso CA, Kwabugge YA, Anyanwu MU, Torres C, Chah KF.** Diversity of *Ochrobactrum* species in food animals, antibiotic resistance phenotypes and polymorphisms in the blaOCH gene. *FEMS Microbiol Lett* 2017;364(17).
- 7-**Grobner S, Linke D, Schutz W, Fladerer C, Madlung J, Autenrieth IB, et al.** Emergence of carbapenem-non-susceptible extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* isolates at the university hospital of Tübingen, Germany. *J Med Microbiol* 2009;58(Pt 7):912-22.
- 8-**Grimm V, Ezaki S, Susa M, Knabbe C, Schmid RD, Bachmann TT.** Use of DNA microarrays for rapid genotyping of TEM beta-lactamases that confer resistance. *J Clin Microbiol* 2004;42(8):3766-74.
- 9-**Abou-Shanab RA, Eraky M, Haddad AM, Abdel-Gaffar AB, Salem AM.** Characterization of Crude Oil Degrading Bacteria Isolated from Contaminated Soils Surrounding Gas Stations. *Bull Environ Contam Toxicol* 2016;97(5):684-8.
- 10-**Ibrahim HM.** Biodegradation of used engine oil by novel strains of *Ochrobactrum anthropi* HM-1 and *Citrobacter freundii* HM-2 isolated from oil-contaminated soil. *3 Biotech* 2016;6(2):226.
- 11-**Hanna AL, Youssef HH, Amer WM, Monib M, Fayez M, Hegazi NA.** Diversity of bacteria nesting the plant cover of north Sinai deserts, Egypt. *J Adv Res* 2013;4(1):13-26.
- 12-**Abou-Shanab RA, Angle JS, van Berkum P.** Chromate-tolerant bacteria for enhanced metal uptake by *Eichhornia crassipes* (Mart.). *Int J Phytoremediation* 2007;9(2):91-105.
- 13-**Sheraba NS, Diab MR, Yassin AS, Amin MA, Zedan HH.** Quality Control Testing for Tracking Endotoxin-Producing Gram-Negative Bacteria during the Preparation of Polyvalent Snake Antivenom Immunoglobulin. *PDA J Pharm Sci Technol* 2015;69(4):499-510.
- 14-**Aguilera-Arreola MG, Ostria-Hernandez ML, Albarran-Fernandez E, Juarez-Enriquez SR, Majalca-Martinez C, Rico-Verdin B, et al.** Correct Identification of *Ochrobactrum anthropi* From Blood Culture Using 16rRNA Sequencing: A First Case Report in an Immunocompromised Patient in Mexico. *Front Med (Lausanne)* 2018;5:205.
- 15-**Nadjar D, Labia R, Cerceau C, Bizet C, Philippon A, Arlet G.** Molecular characterization of chromosomal class C beta-lactamase and its regulatory gene in *Ochrobactrum anthropi*. *Antimicrob Agents Chemother* 2001;45(8):2324-30.