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Short Communication

Whole-genome analysis and description of an IMP-8-producing Ochrobactrum anthropi

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ABSTRACT

Objectives: To explore the genomic characterization of an IMP-8-producing Ochrobactrum anthropi and give suggestions for the application of antibiotics.

Methods: In 2021, the infection caused by CRKP was under control after nearly three months of using CAV, however, carbapenem-resistant O. anthropi isolates were collected from a rectal swab sample of a patient with Lumbar Disc Herniation Postoperative Infection. The rectal swab was then enriched in lysogenic broth overnight and inoculated onto China Blue agar plates containing 0.3μg/mL meropenem. And we investigated the characteristics of this carbapenem-resistant O. anthropi by MALDI-TOF MS. Immune colloidal gold technique, conjugation experiment, whole genome sequencing and antimicrobial susceptibility testing.

Results: Antimicrobial susceptibility testing showed that the O. anthropi were resistant to imipenem, cefmetazole, ceftazidime, cefotaxime, piperacillin/tazobactam, sulbactam/cefoprazone, cef-tazidime/avibactam, ceftipime, ciprofloxacin, aztreonam, and not susceptible to meropenem, ertapenem, polymyxin B, tigecycline, amikacin. Immune colloidal gold technique reflected that this strain produced IMP carbapenemases, and the presence of IMP-8 was verified by WGS, which was located in a 21,442 bp, nonconjugative plasmid.

Conclusion: Improper antibiotic treatment can cause intestinal flora imbalance and even bacteremia in patients, we should use antibiotics wisely and develop individualized treatment options.

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1. Introduction

Ochrobactrum anthropi, previously misidentified as Achromobacter species (CDC group Vd), is a rod-shaped and aerobic Gram-negative bacillus [1]. O. anthropi often causes catheter-associated infections in immunocompromised patients, and individual cases of meningitis, endocarditis, sepsis, bloodstream infections and intermittent low-grade fever have been reported [2–4], reflecting its most noteworthy feature of adhering to foreign bodies [5]. Furthermore, O. anthropi is widely distributed in environmental sources and a variety of clinical specimens, including blood, respiratory tract, urogenital tract, stool, pus and urine [1,2].

Most recent reported strains of O. anthropi were resistant to beta-lactams and groups of antibiotics such as penicillins, cephalosporins and aztreonam, but they were sensitive to aminoglycosides, fluoroquinolones, carbapenems, tetracyclines and trimethoprim/sulfamethoxazole. The aim of this study was to describe phenotypic and genotypic characteristics of an O. anthropi capable of producing IMP-8, obtained after more than two months of consecutive treatment with ceftazidime/avibactam (CAV) monotherapy, and to further highlight the necessity of rational use of antibiotics.

2. Material and methods

2.1. Bacterial isolates and identification

As part of a carbapenem resistance surveillance study, rectal swabs collected from an infected inpatient were suspended and

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enriched overnight in Luria-Bertani (LB) broth (Oxoid, UK) at 37 °C. Then broth suspensions of this rectal swab were inoculated onto China Blue agar plates containing 0.3 μg/ml meropenem and cultured overnight at 37 °C. After purification, the suspected colonies were identified by MALDI-TOF MS (MicroDIys, Shanghai, China).

2.2. Antimicrobial susceptibility testing and screening of resistance genes

The minimal inhibitory concentrations (MICs) of 15 commonly used antibiotics (include imipenem, meropenem, ertapenem, ceftazidime, cefotaxime, piperacillin/tazobactam, sulbactam/cephoperazone, ceftazidime/avibactam, cefepime, polymyxin B, tigecycline, ciprofloxacin, amikacin and aztreonam) for O. anthropi isolates were performed by broth microdilution method. The MIC of tigecycline was interpreted according to EUCAST breakpoints, and the other MICs were interpreted using the CLSI-M100 standard [6,7]. The presence of carbapenemases was detected by NG-Test® CARBA 5 (zhongshengzhongjie, Changsha, China) according to the manufacturer’s recommendation.

2.3. Conjugation experiment

The conjugation experiment was conducted by the filter-mating method as previously reported, using the rifampin-resistant Escherichia coli EC600 as the recipient [8]. In brief, O. anthropi and the recipient strain EC600 were cultivated separately in Luria-Bertani (LB) broth at 37 °C for 4 hours. Next, donor strain and EC600 were mixed gently on a 0.45-mm membrane positioned on Columbia Blood Agar at a volume ratio of 1:1. After overnight culture at 37 °C, the membrane was swirled in Luria-Bertani (LB) broth. Then the mixture was cultured on Mueller-Hinton (MH) agar plates supplemented with 0.8 μg/ml meropenem and 600 μg/ml rifampin to select for the transconjugant, and the sepsis was identified by MALDI-TOF MS.

2.4. Whole genome sequencing and bioinformatic analysis

Genomic DNA of O. anthropi was extracted using the PureLink Genomic DNA Mini Kit (Invitrogen, USA) according to the manufacturer’s instructions and sent to Novogene (Novogene, China) for whole genome sequencing using the Illumina NovaSeq 6000 platform. Genomic DNA was also subjected to the long-read Nanopore MinION platform after being treated with supplementary sequencing kit (Oxford Nanopore Technologies, Oxford, UK). MinION libraries were prepared using the SQK-RBK004 nanopore sequencing kit according to the manufacturer’s instructions. The library was then added to a MinION flow cell (R9.4.1) and sequenced. Both short and long reads were de novo hybrid assembled using Unicycler v0.4.8 [9]. Assembled genome sequences were annotated with RAST v2.0 [10]. Bacterial identification was conducted using Average Nucleotide Identity supported by Kostas Lab [11]. Acquired antibiotic resistance genes were identified by ResFinder 4.0. [12]. Alignment of plasmids with similar structures was generated by EasyFig_win_2.1.13.

3. Results

A strain T210003 was cultured from the rectal swab specimen and identified as O. anthropi by MALDI-TOF MS. The colonies are about 1 to 2 mm in diameter after overnight incubation on Columbia blood agar and presented as red, short, and rod-like under optical microscope (Fig. 1A, B).

Antimicrobial susceptibility testing was then performed on O. anthropi strain T210003, and the results showed that O. anthropi T210003 was resistant to ceftazidime, cefotaxime, cefepime, imipenem, ciprofloxacin, aztreonam and piperacillin/tazobactam, sulbactam/cefoprazone and cefazidime/avibactam, but susceptible to meropenem, ertapenem, polymyxin B, tigecycline and amikacin (Table 1), a similar antimicrobial susceptibility profile to most reported strains except for resistant to imipenem [3-5]. Further carbapenemases presence detection by NG-Test® CARBA 5 (zhongshengzhongjie, Changsha, China) indicated strain T210003 produced IMP-type carbapenemases.

The complete genome of O. anthropi strain T210003 was obtained by whole-genome sequencing. Strain T210003 was found to harbour two chromosomes and two plasmids with size of 2 865 725 bp, 1 984 998 bp, 188 867 bp and 21 442 bp, respectively (BioSample SAMN23500291, GenBank accession no. CP088964-CP088967, respectively). Analysis of antimicrobial resistance identified four antimicrobial resistance genes, bla<sub>QDCI-2</sub>, bla<sub>IMP-8</sub>, aac(6’)-Ib-cr and tet(C), encoding resistance to β-lactam, aminoglycoside, quinolone and tetracycline. The resident beta-lactamase bla<sub>QDCI-2</sub> and tet(C) genes were located in the 2 865 725 bp chromosome, and the bla<sub>IMP-8</sub> and aac(6’)-Ib-cr genes were located in the 21 442 bp plasmid, designated as pT210003-IMP, which is nonconjugative according to conjugation assay. Plasmid carried genes that encode conjugation related proteins, including Ti-type conjugative transfer relaxase TraA, mobilisation protein MobC and type IV secretory system conjugative DNA transfer family protein. However, plasmid
pT210003-IMP was not classifiable according to Inc-type, MOB and MFP typing schemes due to insufficient components. Gene blaIMP-8 was located in the Tn5053-like transposon, which comprises intI1, blaIMP-8, aac(6’)-Ib-cr, tinR, tinQ, tinB and tinA genes. A pair of left and right inverted repeats (IRL and IRR) (16 bp: CCTGATTTTTGC-GACA) have been identified boarding this element in plasmid pT210003-IMP, indicating transposition of the whole genetic element. Thus it was hypothesised that plasmid pT210003-IMP was formed by transposition of the transposon carrying blaIMP-8, from plasmid-like pT17285-IMP (GenBank accession no. KX784503.1) into plasmid-like pFDAARGOS_362 (GenBank accession no. CP024589.1) (Fig. 1C).

4. Discussion

Infection caused by multidrug-resistant (MDR) bacteria has been a global problem that poses great threats to public health. Since the discovery of carbapenems, this kind of antibiotic has been widely used in clinical practice as the last-resort antibiotic. However, along with the antibiotic abuse and the emergence of carbapenemases, including Ambler class A (e.g., KPC types), class B (e.g., IMP types) and class D (e.g., OXA types), carbapenem resistance has become increasingly widespread [14]. In the majority of cases, carbapenem was used successfully in patient treatment; however, carbapenemase-producing O. anthropi had been reported successively [2,15]. Although CAV is a new β-lactam/β-lactamase combination antibiotic with a wide antibacterial spectrum and excellent therapeutic effect against Gram-negative bacteria, the combination is inactivated on metallo-β-lactamases, like IMP types [16].

We presented the first report of (to the best of our knowledge) an IMP-8-producing O. anthropi. Although the blaIMP-8 located on a conjugative plasmid, we should remain concerned about the potential clonal dissemination of carbapenem-resistant O. anthropi and conduct bacterial surveillance when uncommon opportunistic pathogens are present to prevent more serious risks for public health.

In conclusion, we demonstrate the importance of using antibiotics judiciously to treat infection, as long-term use of CAV not only eliminates susceptible bacteria but also selects resistant isolates, which can lead to intestinal flora imbalance and, in severe cases, sepsis. More effective and safe treatments are needed to prevent complications arising from intestinal flora imbalance.

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Table 1

<table>
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<th>Strain</th>
<th>MIC (mg/L)</th>
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<tr>
<td></td>
<td>IPM</td>
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<td>T210003</td>
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IPM, imipenem; MEM, meropenem; ETP, ertapenem; CMZ, cefmetazole; CAZ, ceftazi dime; CTX, cefotaxime; TZP, piperacillin/tazobactam; SCF, sulbactam/ceftoprazone; CAV, ceftazidime/avibactam; FEP, cephepine; PB, polymyxin B; TGC, tigecycline; CIP, ciprofloxacin; AK, amikacin; ATM, aztreonam.

Declaration of Competing Interests

The authors declare no conflict of interests.

Ethical Approval

This study was approved by the Ethics Committee of Second Affiliated Hospital, Zhejiang University School of Medicine (2018-039). The subjects gave written informed consent in accordance with the Declaration of Helsinki.

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