ORIGINAL PAPER

Polish Journal of Microbiology
2022, Vol. 71, No 1, 73–81
https://doi.org/10.33073/pjm-2022-010

# Detection by Whole-Genome Sequencing of a Novel Metallo-β-Lactamase Produced by *Wautersiella falsenii* Causing Urinary Tract Infection in Tunisia

RAOUAA MAAROUFI[1, 2, 3], OLFA DZIRI[2, 3, 4], LINDA HADJADJ[1], SEYDINA M. DIENE[1], JEAN-MARC ROLAIN[1] and CHEDLY CHOUCHANI[2, 3, 4]*

[1] Aix-Marseille Université MEPHI, AP-HM, IRD, IHU Méditerranée Infection, Marseille, France
[2] Laboratoire des Microorganismes et Biomolécules Actives, Faculté des Sciences de Tunis, Université Tunis El-Manar, Tunis, Tunisie
[3] Laboratoire de Recherche Sciences et Technologies de l'Environnement, Institut Supérieur des Sciences et Technologies de l'Environnement de Borj-Cedria, Hammam-Lif Université de Carthage, Tunisie
[4] Unité de Service en Commun pour la Recherche « Plateforme Génomique », Institut Supérieur des Sciences et Technologies de l'Environnement de Borj-Cedria, Hammam-Lif, Université de Carthage, Tunisie

Submitted 20 August 2021, accepted 22 November 2021, published online 27 February 2022

Abstract

*Wautersiella falsenii* is a rarely non-fermenting Gram-negative bacterium and belongs to the *Flavobacteriaceae* family. This nosocomial pathogen can cause several human infections, especially among immunocompromised patients. Here, we describe the whole genome sequence of a clinical *W. falsenii* strain isolated from a urine sample of a 35-year-old woman with a urinary tract infection in Tunisia. We investigated its phenotype and genotype.

After bacterial identification by the MALDI-TOF method, the whole-genome sequencing of this strain was performed. This isolate was not susceptible to various antibiotics, including β-lactams, aminoglycosides, and quinolones. However, it remains susceptible to imipenem (MIC = 0.25 mg/l), ertapenem (MIC = 0.75 mg/l), and meropenem (MIC = 0.19 mg/l). Interestingly, the E-TEST® (MP/MPI) showed a reduced MIC of meropenem +/– EDTA (0.064 µg/ml). Besides, the color change from yellow to red in the β CARBA test only after 24 hours of incubation can be interpreted in two ways. On the one hand, as a likely low expression of the gene encoding metallo-β-lactamase. On the other hand, and more likely, it may be a false-positive result because, according to the test manufacturer's recommendations, the test should be read after 30 minutes. Perhaps, therefore, this gene is not expressed in the tested strain. Moreover, the whole-genome sequence analysis demonstrated the presence of a novel chromosomally located subclass B1 metallo-β-lactamase EBR-like enzyme, sharing 94.92% amino acid identity with a previously described carbapenemase produced by *Empedobacter brevis*, EBR-1. The results also showed the detection of other antibiotic resistance genes and the absence of plasmids. So far, this study is the first report on the detection of *W. falsenii* in Tunisia. These findings prove that *W. falsenii* could be a potential reservoir of antibiotic resistance genes, e.g., β-lactamases. Collaborative efforts and effective hygiene measures should be established to prevent the emergence of this species in our health care settings.

Keywords: MALDI-TOF, *Wautersiella falsenii*, whole-genome sequencing, metallo-β-lactamase, urinary tract infection, Tunisia

## Introduction

*Wautersiella falsenii*, also known as *Empedobacter falsenii*, was first described in 2006 (Kämpfer et al. 2006; Zhang et al. 2014). This non-fermentative Gram-negative species belongs to the *Flavobacteriaceae* family and is characterized as a non-motile rod-shaped bacterium, positive for indole and urease production (Kämpfer et al. 2006). In addition, it grows aerobically at 37°C on standard media, such as blood agar, tryptic soy agar, or MacConkey agar (Kämpfer et al. 2006; Zhang et al. 2014). To our knowledge, this species is the only member of the *Wautersiella* genus, and it is most closely related to *Empedobacter brevis*, showing 94–95% similarity of the 16S rRNA gene sequences (Kämpfer et al. 2006). However, a previous study reported the detection of three bacterial isolates obtained from poultry, showing 97.2% of similarity with the 16S rRNA

* Corresponding author: Ch. Chouchani, Laboratoire de Recherche Sciences et Technologies de l'Environnement, Institut Supérieur des Sciences et Technologies de l'Environnement de Borj-Cedria, Hammam-Lif Université de Carthage, Tunisie;
**e-mail: chedly.chouchani@gmail.com**

gene of *W. falsenii*. These findings exhorted researchers to propose that these related isolates could be classified as new species of *Wautersiella* genus associated with poultry (Christensen and Bisgaard 2010). *W. falsenii* was rarely detected in clinical isolates that could be explained by the difficulty of its identification (Van der Velden et al. 2012). Indeed, the choice of the identification method for this species is very important. As previously described, the use of the Phoenix automated identification system could misidentify the isolate as *Sphingomonas paucimobilis*, unlike the MALDI-TOF MS, which identifies it correctly (Van der Velden et al. 2012).

Few data are describing the detection of *W. falsenii* species on an international scale. Even if it was rarely reported, it was recovered from various clinical specimens like blood, ear discharges, oral cavity, pleural fluid, pus, respiratory tract, vaginal swabs, wounds, urine, and cervical neck abscess samples (Kämpfer et al. 2006; Van der Velden et al. 2012; Traglia et al. 2015). It was also recovered from other origins, noting the metalworking aerosols and fluids (Perkins and Angenent 2010), the tiled carpet surface of hospitals (Harris et al. 2010), soils, polluted sediment, and rodent skin (Maleki-Ravasan et al. 2015). This species presents a potential risk to human health since it has been considered a reservoir of antibiotic resistance genes (Traglia et al. 2015). In fact, *W. falsenii* isolates are generally identified as resistant to several antibiotics, including those of the last resort, carbapenems, and colistin (Van der Velden et al. 2012; Traglia et al. 2015). It could also contribute to the spread of resistance genes to other pathogens, especially those coding for β-lactamases (Collins et al. 2018). In addition, recent studies have reported the detection of genes coding for novel subclass B1 metallo-β-lactamases among this uncommon nosocomial pathogen, named EBR-2 (Collins et al. 2018) and EBR-3 (WP_150823468.1).

A detailed analysis of the whole genome of *W. falsenii*, using the high-throughput sequencing technologies, is needed to decipher the genome of this species, explain its phenotype, and understand the different molecular mechanisms involved in antibiotic resistance. Thus, the main objectives of our study were as described above. The whole-genome sequencing seems to be a key to the detection of novel antibiotic resistance features (Collins et al. 2018). In the present study, we report for the first time the detection of a novel chromosomally located metalloenzyme in *W. falsenii* isolate recovered in 2016 from the urine sample of a Tunisian woman that suffered from a urinary tract infection. To our knowledge, this is the first case reported in Tunisia and the third in the world after the two previously reported cases in Netherlands and India (Van der Velden et al. 2012; Zaman et al. 2017).

## Experimental

### Materials and Methods

**Clinical data and bacterial identification.** In 2016, a 35-year-old woman was presented in a private medical office with symptoms of the urinary tract infection (UTIs). She had a fever, pain in the lower abdomen, and a burning sensation in the urinary tract. This non-hospitalized patient had not have a previous UTI in her medical history. Her doctor requested a cyto-bacteriological urine examination to check the causes of these symptoms.

The urine sample was submitted to a private medical analysis laboratory in El Kram, Tunisia. The sample was cultured on a nutrition agar medium and was incubated for 24 hours at 37°C in order to isolate the microorganism causing the urinary tract infection. The isolate was then identified using the matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) method. Moreover, there is no information about the antibiotic treatment taken by this infected patient.

**Antimicrobial susceptibility testing.** The antimicrobial susceptibility testing of the isolated bacterium was performed using the standard disk diffusion method on Mueller-Hinton E agar medium (MHE BioMérieux, Marcy-l'Etoile, France). The results were interpreted using Scan 4000® (Interscience, Saint Nom la Breteche, France), according to the Antibiogram Committee of the French Society for Microbiology (Société Française de Microbiologie 2019) and the Clinical and Laboratory Standards Institute guidelines (CLSI 2019). Thus, a total of 34 antibiotics were tested as follow: amoxicillin (AX); amoxicillin/clavulanic acid (AMC); ticarcillin (TIC); ticarcillin/clavulanic acid (TIM); cefepime (FEP); colistin (CS); amikacin (AK); tobramycin (TOB); streptomycin (S); spectinomycin (SPT); ciprofloxacin (CIP); pefloxacin (PEF); nalidixic acid (NA); furans (FF); nitrofurantoin (F); doxycycline (DO); tigecycline (TGC); piperacillin/tazobactam (TPZ); temocillin (TEL); cefoxitin (FOX); cephalothin (KF); ceftriaxone (CRO); cefotaxime (CTX); aztreonam (ATM); ertapenem (ETP); meropenem (MEM); imipenem (IPM); gentamicin (CN), kanamycin (K), ofloxacin (OFX), minocycline (MI), trimethoprim/sulfamethoxazole (SXT); rifampicin (RA) and sulfadiazine (SD).

**Whole-genome sequencing and genomic analysis.** Genomic DNA was extracted using the EZ1 DNA Kit (Qiagen, Courtaboeuf, France). Then, the whole-genome sequencing (WGS) was performed for *W. falsenii* isolate using Illumina MiSeq sequencer. The obtained sequences were assembled by Spades assembler and annotated using the PROKKA program. In

addition, the research of the presence of antibiotic resistance genes and plasmids was carried out using ARGANNOT and PlasmidFinder programs, respectively. Besides, for phylogenetic analysis, the conserved proteins sequences of different carbapenemases, detected among the *Flavobacteriaceae* family and other Gram-negative bacteria, were downloaded from the NCBI database and were independently aligned using MUSCLE to cluster homologous sequences.

Moreover, the screening of virulence genes was performed using the Virulence Factor Database (VFDB, http://www.mgc.ac.cn/VFs/) and the virulence Finder Database available at the Center for Genomic Epidemiology server (https://cge.cbs.dtu.dk/services/VirulenceFinder/). Besides, the bacteria's pathogenicity towards human hosts was also investigated (https://cge.cbs.dtu.dk/services/PathogenFinder/).

**Phenotypic screening of carbapenemase production.** The Minimal Inhibitory Concentrations (MICs) were determined for only imipenem, meropenem, and ertapenem, using the E-TEST® method (Biomérieux, Marcy l'Etoile, France). The MIC results were interpreted using the CLSI guidelines (CLSI 2019).

The detection of carbapenemase production was carried out using the β CARBA test. This colorimetric test is based on the change of color of a chromogenic substrate in the presence of carbapenemase-producing isolate. Thus, the presence of carbapenem-hydrolyzing activity is justified by the color change from yellow to orange-red, or even to purple after 30 minutes of incubation at 37°C using an NDM producing *Escherichia coli* as a positive control (Meier and Hamprecht 2019). The results were also interpreted after 1 hour and 24 hours of incubation at 37°C of *W. falsenii* isolate.

Moreover, an E-TEST® gradient strip (MP/MPI) was used to investigate the production of metallo-β-lactamases (MBLs). This reagent strip contains increasing concentrations of meropenem antibiotic (MP) on one end and meropenem supplemented with a constant level of EDTA (MPI) on the other. The EDTA inhibits MBLs' activity by chelating their active site zinc ions. Thus, a difference between the two MIC results reveals the production of a metallo-β-lactamase by the tested isolate (Girlich et al. 2013).

## Results

**Bacterial isolate.** The cyto-bacteriological urine examination of the patient concerned showed a high number of leukocytes and epithelial cells, thus proving the urinary tract infection. The primary phenotypic tests of the urine culture showed the presence of a non-lactose fermenting Gram-negative rod, characterized as positive for oxidase production. This isolate was then identified as *W. falsenii* using MALDI-TOF MS, showing a high score value equal to 2.230.

**Antimicrobial susceptibility testing.** According to the antimicrobial susceptibility results, *W. falsenii* isolate showed a multi-drug resistance profile. Thus, it was resistant to several antibiotics (n = 13) that belong to different families, noting β-lactams (amoxicillin, amoxicillin/clavulanic acid, ticarcillin, and ticarcillin/clavulanic acid), aminoglycosides (amikacin, tobramycin, streptomycin, and spectinomycin), quinolones (ciprofloxacin, pefloxacin, and nalidixic acid), furans, and nitrofurantoin as shown in Table I. It was intermediate resistant to two antibiotics, doxycycline, and tigecycline. However, it was sensitive to the other 19 tested antibiotics listed above in the Material and Methods section, including carbapenems, third-generation cephalosporins, gentamicin, rifampicin, and colistin. This isolate had low minimal inhibitory concentrations (MIC) for imipenem (0.25 mg/l), ertapenem (0.75 mg/l), and meropenem (0.19 mg/l).

**Genomic analysis.** The whole-genome sequence of this rare bacterium was obtained using the Illumina MiSeq sequencer (Illumina, San Diego, CA, USA). The complete genome showed a total of 3,215,391-bp. Thus, it was assembled into 129 contigs with a minimum contig size of 58,696-bp and a maximum contig size of 206,832-bp. The genome annotation predicts 2,982 coding sequences (CDS). The obtained whole-genome sequence of this isolate has been submitted at the DDBJ/ENA/GenBank under the accession number JAIKTW000000000. As expected, the genome analysis of the WGS revealed the presence of three genes, $bla_{EBR}$, *tetX*, and *aadS*. No plasmid has been identified within the genome sequences. Besides, no virulence genes have been detected in our isolate. Also, no pathogenicity factors were found. Indeed, this isolate was predicted as a non-human pathogen, noting a very low probability of being (0.217).

Despite being susceptible to carbapenems (imipenem, meropenem, and ertapenem), this isolate exhibits a chromosomally located gene coding for a subclass-B1 metallo-β-lactamase. This enzyme presented 94.92% protein sequence similarity with a previously described carbapenemase produced by *E. brevis*, the EBR-1 enzyme (AF416700). Also, it showed 95.74% protein sequence similarity with the EBR-3 produced by *E. falsenii* (WP_150823468.1).

Furthermore, the phylogenetic tree, based on the protein sequences of the serine β-lactamases (Class A, C, and D) as well as the metallo-β-lactamases (Class B), showed that our obtained protein sequence was clustered in the group of the metallo-β-lactamases, particularly in the subclass B1 (Fig. 1). Thus, it was closely related to the three metallo-β-lactamases produced by *Flavobacteriaceae* (*E. brevis* and *E. falsenii*), noting

Table I
Comparison between all reported *Wautersiella falsenii* bacterial infections and that of the present study.

| Reference | Number of isolates | Years of isolation | Country | Gender and age of infected patients | Patient suffered from | Clinical specimens | Phenotypic resistance pattern | Detected genes | Detected plasmids |
|---|---|---|---|---|---|---|---|---|---|
| Kämpfer et al. 2006 | 26 | 1980–2004 | Belgium | – | – | blood, wounds, pus, respiratory tract, ear discharge, oral cavity, vaginal swab, pleural fluid, and other from an unknown origin | – | – | – |
| Van der Velden et al. 2012 | 1 | 2012 | Netherlands | one-year-old girl | pyelonephritis | urine | nitrofurantoin, amoxicillin-clavulanic acid, piperacillin-tazobactam, ceftriaxone, ceftazidime, meropenem, tobramycin, and colistin | – | – |
| Traglia et al. 2015 | 1 | 2013 | Argentina | 18-year-old woman | acute otitis media | cervical neck abscess | ampicillin, ampicillin-sulbactam, cephalothin, meropenem, colistin | 29 genes coding for efflux pumps, and tripartite multidrug resistance systems, class a β-lactamase, metallo-β-lactamase (ebr-2), three class c β-lactamases, resistance to streptogramin trimethoprim, bacitracin, and macrolide | none |
| Giordano et al. 2016 | 1 | 2016 | Italy | 32-year-old man | lymphoblastic leukemia | the respiratory tract | amikacin, amikacin-clavulanic acid, ampicillin-sulbactam, cefotaxime, ceftazidime, doripenem, gentamicin, imipenem, piperacillin-tazobactam | – | – |
| Zaman et al. 2017 | 1 | 2017 | India | five-year-old boy | bladder cancer | urine | ampicillin, ampicillin-sulbactam, and colistin. | – | – |
| This study | 1 | 2016 | Tunisia | 35-year-old woman | UTI | urine | amoxicillin, amoxicillin-clavulanic acid, ticarcillin, ticarcillin-clavulanic acid, amikacin, tobramycin, streptomycin, spectinomycin, ciprofloxacin, pefloxacin, nalidixic acid, furans, nitrofurantoin | $bla_{\text{EBR-like}}$, *TetX* and *aadS* | none |

– – not studied

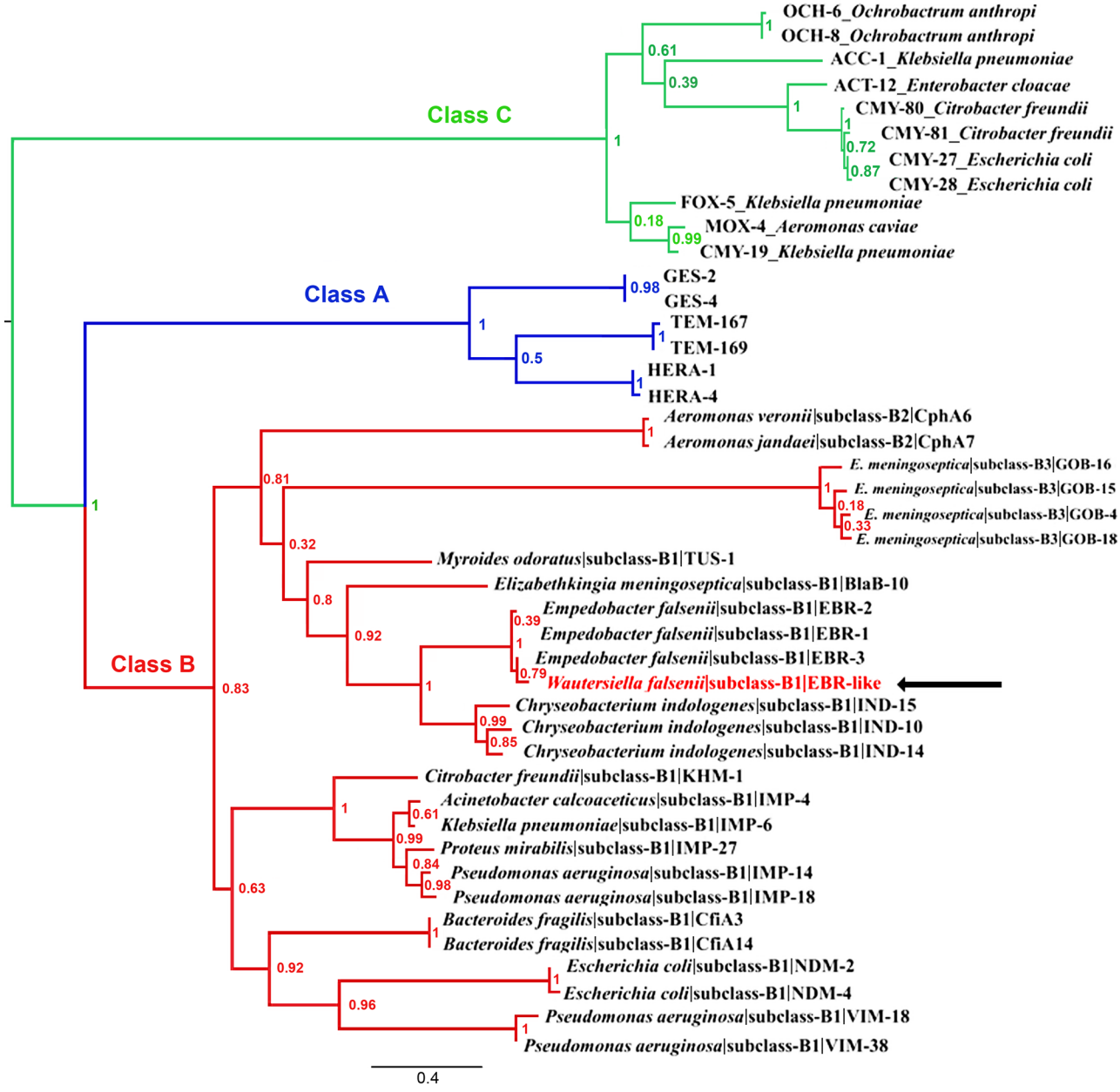


Fig. 1. Phylogenetic tree based on the protein sequences of; serine β-lactamase (Class A and Class C) and metallo-β-lactamase (Class B).

EBR-1, EBR-2, and EBR-3, showing a Bootstrap percentage greater than 70% based on maximum likelihood analyses of 1,000 replications, as presented at the node (Fig. 1).

Moreover, the alignment of our concerned protein sequence with those of other metallo-β-lactamases was presented in Fig. 2. Indeed, the conserved motif of the MBL active site (HxHxDH) and the residues of metallo-β-lactamases were highlighted by yellow color. These results confirmed that *W. falsenii* isolate produced a novel subclass-B1 metallo-β-lactamase, belonging to the EBR-like family enzyme.

**Phenotypic detection of carbapenemase production.** To verify the obtained WGS result, we tested the minimal inhibitory concentration (MIC) for carbapenems by E-TEST® MBL strip (Biomérieux, Marcy l'Etoile, France). This isolate had a low minimal inhibitory concentration for; imipenem (0.25 mg/l) and ertapenem (0.75 mg/l). Using the β CARBA NP test, a change of color from yellow to red was observed only after 24 hours of incubation of *W. falsenii* isolate, contrary to the positive control isolate, which showed a color shift after only thirty minutes of incubation. The color change from yellow to red in the β CARBA test only after 24 hours of incubation can be interpreted in two ways. On the one hand, as a likely low expression of the gene encoding metallo-β-lactamase. On the other hand, and more likely, it may be a false-positive result because, according to the test manufacturer's recommendations, the test should be read after 30 minutes. Perhaps,

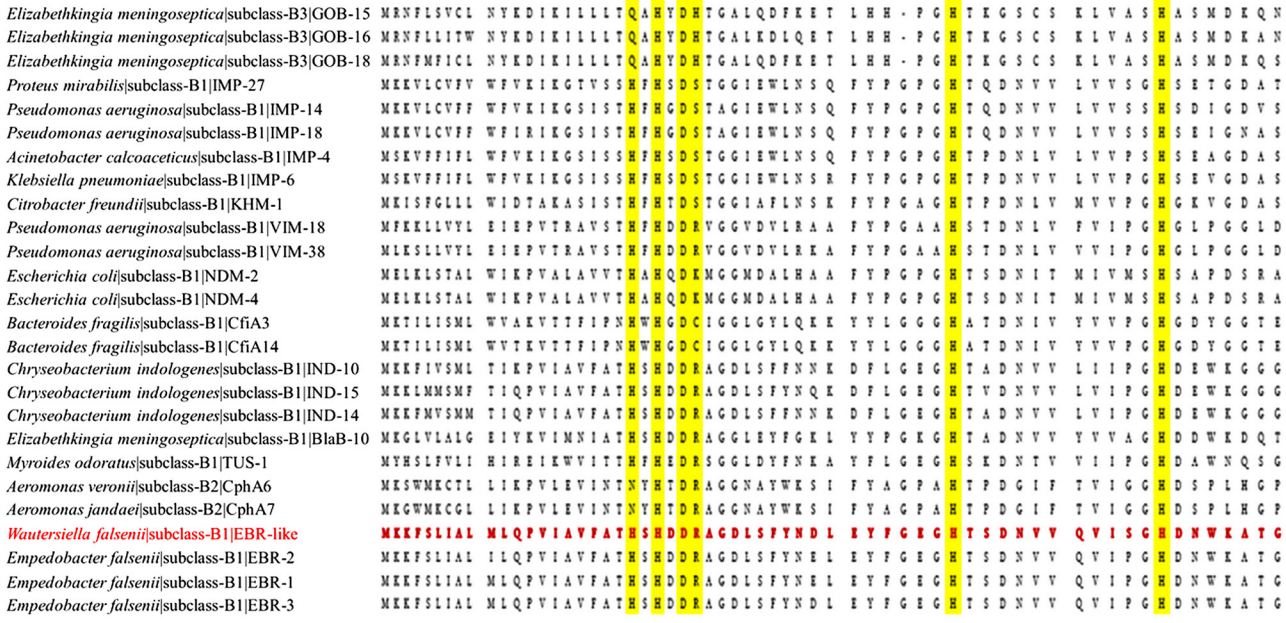


Fig. 2. Protein alignment among the metallo-β-lactamase type B genes: Our sequence was represented with red color. The conserved motif of the MBL "HxHxDH" presented in their active site, and residues of bacterial metallo-β-lactamase enzymes were represented with a yellow color.

therefore, this gene is not expressed in the tested strain. It is noteworthy that these results were confirmed using the E-TEST® strip (MP/MPI). Besides, a significant difference between the two MICs for meropenem (0.19 mg/l) and meropenem + EDTA (0.064 mg/l) was observed. Indeed, this result proved the production of a metallo-β-lactamase by our isolate.

## Discussion

Since its first description in 2006, *W. falsenii* has become a real concern and a severe nosocomial pathogen, even if it has been rarely reported (Kämpfer et al. 2006). Indeed, it was isolated from various clinical origins (blood, wounds, pus, respiratory tract, ear discharge, oral cavity, vaginal swab, pleural fluid, cervical neck abscess sample, and urine), causing thus several infections types (Kämpfer et al. 2006; Van der Velden et al. 2012; Traglia et al. 2015). Few studies are describing the detection of *W. falsenii* isolates throughout the globe. Besides, all reported *W. falsenii* bacterial infections were presented in Table I and compared with the isolate detected. Moreover, we noticed the lack of information about *W. falsenii* species, including detected genes and plasmids. In-depth studies of this species are very required in order to understand its basis genomic. To date, only one study detailing the whole-genome analysis of this non-fermentative Gram-negative bacterium was reported (Collins et al. 2018). The present study is the second report deciphering the genome of *W. falsenii* in the world.

As previously reported, all *W. falsenii* isolates were detected among hospitalized patients, especially among the immunosuppressed patients of a young age (Christensen and Bisgaard 2010; Giordano et al. 2016). However, our isolate was detected among a non-hospitalized patient suffering from a urinary tract infection. To the best of our knowledge, our study is the first report describing the detection of *W. falsenii* causing UTI in Tunisia and the third in the world after the two reported cases in Netherlands and India (Van der Velden et al. 2012; Meier and Hamprecht 2019). Thus, the first case was detected among a one-year-old child with a complicated UTI in 2012 (Van der Velden et al. 2012) and the second among a five-year-old child with bladder cancer (Meier and Hamprecht 2019). The detection of such isolate has not been hitherto detected in Tunisia.

What is more, studying the whole-genome sequence of this isolate allowed us to detect a novel chromosomally located gene coding for a subclass-B1 metallo-β-lactamase. The obtained results of the β CARBA test and the E-TEST® (MP/MPI), which showed a metallo-β-lactamase at a low level, confirmed these findings. Besides, the color change from yellow to red in the β CARBA test only after 24 hours of incubation can be interpreted in two ways. On the one hand, as a likely low expression of the gene encoding metallo-β-lactamase EBR-1. On the other hand, and more likely, it may be a false-positive result because, according to the test manufacturer's recommendations, the test should be read after 30 minutes. Perhaps, therefore, this gene is not expressed in the tested strain. Thus,

the comparison of the protein sequences revealed that our detected enzyme was closely related to the three metallo-β-lactamases produced by *Flavobacteriaceae* (EBR-1, EBR-2, and EBR-3). Indeed, the analysis of this sequence by the ARGANNOT-database showed that it presented a 94.89 % protein sequence similarity with the EBR-1 enzyme produced by *E. brevis* (AF416700) (Bellais et al. 2002a). Moreover, high similarity percentages equal to 95.74% and 94.04% were recorded when compared, by the NCBI database, our obtained protein sequence with EBR-3 (WP_150823468.1), and EBR-2 produced by *E. falsenii* (Collins et al. 2018), respectively. However, it shared only 57.69% amino acid identity with the IND-1 enzyme produced by *Chryseobacterium indologenes* (Bellais et al. 1999; Bellais et al. 2000b). Besides, other bacterial species of *Flavobacteriaceae* family produced several carbapenem-hydrolyzing Ambler class B enzymes, including EBR-1 from *E. brevis* (Perkins and Angenent 2010), BlaB and GOB from *Chryseobacterium meningoseptica* (Rossolini et al. 1998; Bellais et al. 2000a), IND from *C. indologenes* (Bellais et al. 1999; Bellais et al. 2000b), CGB-1 from *Chryseobacterium gleum* (Bellais et al. 2002b), a β-lactamase from *Flavobacterium odoratum* (Sato et al. 1985).

In addition, the phylogenetic analysis, based on the comparison of the published protein sequences, revealed that our obtained protein sequence was clustered in the same group of those detected among the *Flavobacteriaceae* family. Besides, we have also identified the conserved domain of the metallo-β-lactamases. All these findings confirmed that the detected enzyme belonged to the subclass-B1 metallo-β-lactamase, especially the EBR-like family. This study is the first to report the detection of the EBR-like enzyme in Tunisia.

The whole-genome sequencing analysis also revealed the presence of several other genes associated with other antibiotic resistance. Indeed, we noted the detection of the tetracycline-inactivating monooxygenase encoding gene, *tetX*, with a 99.74 % identity. This chromosomally encoded *tetX* gene confers a low level of tigecycline resistance in *W. falsenii* isolate with a MIC value equal to 3 mg/l. The tetracycline destructase, such as Tet(X), represents a unique enzymatic tetracycline inactivation mechanism (Forsberg et al. 2015). Indeed, this mechanism has been confirmed for in vitro activity, showing the degradation of all tetracyclines, including tigecycline (Moore et al. 2005).

Additionally, we described another gene, named *aadS* and showed a 99.77% identity. This gene is associated with streptomycin-resistance in *W. falsenii* isolate, and codes for the aminoglycoside 6-adenylyltransferase. The aadS-encoded peptide demonstrated a significant homology to Gram-positive streptomycin-dependent adenyltransferases (Smith et al. 1992). It was phenotypically silent in the wild-type *Bacteroides* (Smith et al. 1992). The aminoglycosides are known as broad-spectrum antibacterial compounds and are used extensively to treat bacterial infections (Davies and Wright 1997; Ramirez and Tolmasky 2010). Thus, the genes, coding for resistance to this antibiotic family, are prevalent among Gram-positive bacteria (Vakulenko and Mobashery 2003; Tolmasky 2007), noting that enzymatic modification like streptomycin-modifying enzymes is considered as the most prevalent mechanism conferring resistance to aminoglycosides in the clinical settings (Ramirez and Tolmasky 2010).

Moreover, the *aadS* gene was identified in many organisms; *Bacteroidetes* (WP_003013318.1), *Elizabethkingia anophelis* (WP_009086755.1), *Elizabethkingia meningoseptica* (WP_016169991.1), *Flavobacteriaceae* (WP_010257826.1) and *Chryseobacterium* (WP_007842749.1): *C. indologenes* (ID: 41187294), *Chryseobacterium scophthalmum* (ID: 41008694), *Chryseobacterium vrystaatense* (ID: 35824473). Otherwise, we have noted the absence of plasmids in this isolate as previously described (Collins et al. 2018). These findings suggest that *W. falsenii* species is naturally resistant to several antibiotics, and it could be a potential reservoir of the antibiotic resistance encoding genes.

## Conclusions

The emergence of infections caused by the uncommon Gram-negative bacteria such as *W. falsenii* constitutes a real concern. Although this species was rarely detected, it should not be ignored. To date, the MALDI-TOF method represents the best way to identify this species. Thus, it was very required to decipher its genomic basis using the high throughput sequencing technologies to explain its phenotype and understand its genotype. Indeed, the characterization of the different molecular mechanisms involved in antibiotic resistance could orient the therapeutic decisions. We report in this work a novel chromosomally located metallo-β-lactamase, EBR-like enzyme in *W. falsenii* isolate, causing the urinary tract infection. Interestingly, we have noted a color change from yellow to red in the β CARBA test only after 24 hours of incubation that can be interpreted in two ways. On the one hand, as a likely low expression of the gene encoding metallo-β-lactamase. On the other hand, and more likely, it may be a false-positive result because, according to the test manufacturer's recommendations, the test should be read after 30 minutes. Perhaps, therefore, this gene is not expressed in the tested strain. To the best of our knowledge, this is the first detection of *W. falsenii* isolate in Tunisia. Besides, effective hygiene measures were also required to avoid the spread of this species in healthcare settings.

**ORCID**
Chedly Chouchani https://orcid.org/0000-0002-8294-8289

**Accession numbers**

This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession JAIKTW000000000. The version described in this paper is version JAIKTW010000000.

**Funding**

This work was partly funded by IHU Méditerranée Infection, Aix-Marseille-University in France. This work was supported by the Tunisian Ministry of Higher Education and Scientific Research and Campus France Under the PHC-Utique project (Code 18G0819), which offered a scholarship to Miss Raouaa MAAROUFI to attend the IHU Méditerranée Infection, Aix-Marseille-University in France.

**Conflict of interest**

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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