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RESEARCH ARTICLE

Analysis of NDM-1 and IMP-8 carbapenemase producing *Raoultella planticola* clinical isolates

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ABSTRACT

Object of our study was to analyze the carriage of resistance genes in carbapenem-resistant *Raoultella planticola* (CRRP) by whole genome sequencing (WGS). Three strains of CRRP (named WF0027, WF3597 and WF3648) were collected for clinical analysis and susceptibility of antimicrobial agents was determined. The WGS of three strains was done by Illumina platform and strain identification was performed by average nucleotide identity, and the antibiotic resistance genes carried by the three strains were detected by ABRicate software. Whole genome data of 46 CRRP strains were downloaded from the National Center for Biotechnology Information (NCBI) database, and the evolutionary tree was constructed by genomic single nucleotide polymorphism together with this study strains. Antimicrobial susceptibility testing revealed that WF3597 and WF3648 were susceptible to tigecycline and colistin, while exhibited resistance to 24 antimicrobial agents. WF0027 was resistant to 18 antimicrobial agents. A total of 25 resistance genes were identified using ABRicate software. WF0027 carried $bla_{\text{IMP-8}}$, whereas WF3597 and WF3648 carried $bla_{\text{NDM-1}}$ carbapenem resistance gene. As predicted by the PlasmidFinder, WF3597 and WF3648 carried one plasmid IncFII(p14)_1_p14, whereas WF0027 carried five plasmids. Evolutionary tree results show all strains are clustered into six groups, the strains WF3597 and WF3648 belonged to the same evolutionary group (E clade) and WF0027 belonged to the F clade. Three CRRP strains in our study carried carbapenem resistance genes ($bla_{\text{NDM-1}}$ or $bla_{\text{IMP-8}}$) and were resistant to multiple antimicrobial agents, posing a significant challenge for clinical treatment.

KEYWORDS

carbapenem-resistant *Raoultella planticola*, antibiotic resistance genes, homology analysis, whole genome sequencing

INTRODUCTION

Raoultella planticola is a gram-negative bacterium of the *Enterobacteriaceae* family, which is usually found in soil, plant, and aquatic environments [1–2]. *R. planticola* is considered a relatively harmless bacterium in the environment, which is rarely associated with human infections and generally harmless to humans [2]. The first two cases of *R. planticola* infections were reported in 1984 [3]. Recently, an increased incidence of *R. planticola* infections in humans has been reported in the literature. Its pathogenicity is similar to that of *Klebsiella* spp. and can cause urinary tract infections, pneumonia, and bacteremia [4–6].

Cephalosporins, fluoroquinolones, and carbapenems are the main antimicrobial agents used in the treatment of *Enterobacteriaceae* infections. Carbapenems are among the most effective agents mainly used for the treatment of multidrug resistant gram-negative bacteria and are commonly used in clinical settings owing to their stability against β-lactamases and

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low toxicity. However, the production of various carbapenem hydrolyzing enzymes, so called 'carbapenemases' poses a serious threat to carbapenem therapy [7–8]. *R. planticola* has recently been reported to be resistant to various antimicrobial agents, including carbapenems, and it carries various resistance genes, including bla_{KPC-2} and bla_{KPC-3} [9], bla_{IMP-8} [10], extended-spectrum β-lactamases (ESBLs) $bla_{CTX-M-9}$ [11], and New Delhi metallo-β-lactamase-1 (bla_{NDM-1}) [12].

In this study, we analyzed the distribution characteristics of clinical infections, antibiotic resistance, and antibiotic resistance genes of *R. planticola* using whole genome sequencing (WGS) to provide a theoretical basis for clinical treatment and basic information for epidemiological research.

MATERIALS AND METHODS

Collection of clinical isolates

All carbapenem-resistant *R. planticola* (CRRP) strains were retrospectively collected from a hospital in the north of China between January 2012 and December 2022. All strains were identified using the VITEK MS system (Sysmex-bio-Merieux, Marcy l'Etoile, France) with a fully automated bacterial identifier.

Antimicrobial susceptibility testing

We evaluated the susceptibility of each isolate to the following 26 antimicrobial agents, including β-lactams (ampicillin/sulbactam, piperacillin/tazobactam, cefoperazone/sulbactam, amoxicillin/clavulanic acid, cefuroxime, ceftazidime, ceftriaxone, cefepime, cefoxitin, cefazolin, cefotaxime, aztreonam), carbapenems (imipenem, meropenem, ertapenem), aminoglycosides (gentamicin, tobramycin, amikacin), fluoroquinolones (ciprofloxacin, levofloxacin), sulfonamides (cotrimoxazole), tetracyclines (tetracycline, minocycline, tigecycline), polymyxin (colistin) and chloramphenicol. The susceptibility testing results were interpreted after the 2022 Clinical and Laboratory Standards Institute guidelines (https://clsi.org). *Escherichia coli* ATCC 25922 was used as the quality control strain.

Whole genome sequencing and assembly

Bacterial DNA was extracted using the Omega Bio-Tek Bacterial DNA Kit (Doraville, GA, USA) following an overnight culture of the strains at 37 °C. DNA libraries were prepared using the Illumina Nextera XT DNA library preparation kit (Illumina, USA) according to the manufacturer's instructions prior to sequencing on the Illumina HiSeq or MiSeq platform. Raw sequences were trimmed for quality, followed by assembly and annotation. Low-quality reads and adapters were trimmed using TrimGalore (v0.4.5, https://github.com/FelixKrueger/TrimGalore) and assembled via SPAdes (v3.13.0) (http://bioinf.spbau.ru/spades) using default parameters [13].

Whole genome sequencing analysis

Genome sequences of CRRP in this study were compared with those of type strains of *Raoultella* species using the average nucleotide identity (ANI) based on BLAST and *in silico* DNA-DNA hybridization (isDDH). The pairwise ANI with a ≥96% cutoff and isDDH with a ≥70% cutoff were used for accurately identifying species. ANI analysis was performed using the Orthologous ANI tool 0.93.1 [14], whereas isDDH analysis was performed using Genome-Genome Distance Calculator 3.0 [15]. The National Center for Biotechnology Information (NCBI) AMRFinder database was used to detect antimicrobial resistance genes using ABRicate (v0.9.8, https://github.com/tseemann/abricate) [16]. PlasmidFinder was used to search for plasmids [17]. Except for the data of the three CRRP in this study, those of the other CRRP strains required for constructing the phylogenetic tree were downloaded from the NCBI database. Consequently, the complete genomic data of 46 CRRP strains were obtained from the NCBI database (accessed on 31. January 2023). Based on the core genomes of *Raoultella planticol*, phylogeny was used for single nucleotide polymorphism (SNP) analysis. MEGAX 10.1.8 was used to generate unrooted maximum-likelihood phylogenetic trees with a bootstrap iteration of 1,000 [18]. The phylogenetic tree was visualized using the Interactive Tree of Life [19].

Nucleotide accession numbers

The sequences of the three CRRP strains were submitted to GenBank under BioProject PRJNA987540.

RESULTS

Clinical case analysis and antimicrobial susceptibility testing

Patient 1 (WF0027) was an elder female admitted to our emergency department for various underlying diseases such as heart disease and emphysema. The patient was treated with cefuroxime, levofloxacin, and piperacillin/tazobactam following admission, after CRRP was isolated patient treated with cefoperazone/sulbactam and discharged in good condition. Patients 2 (WF3597) and 3 (WF3648) were from the urology department, both of whom presented with renal failure and underwent allogeneic kidney transplantation on the same day, and CRRP was isolated from pus specimens of both patients postoperatively. Following CRRP isolation, patient 2 was treated with ceftizoxime and tigecycline and discharged in good condition. To contrast, patient 3 was treated with cephazolin and ceftizoxime and discharged in good condition (Table 1).

The antimicrobial sensitivity results revealed (Table 2) that CRRP was resistant to a variety of antimicrobial agents. WF0027 was resistant to 18 antimicrobial agents but was sensitive only to tigecycline, colistin, amikacin, and levofloxacin. WF3597 and WF3648 were resistant to 24

Table 1. Case information about infections caused by carbapenem-resistant *R. planticola*

Patient	Isolate number	Gender	Age	Department	Specimen types	Diagnosis	Isolates date	Invasive operation before isolation of strains	Antimicrobial agents used before isolation of strains	Antimicrobial agents used after isolation of strains	Outcome
1	WF0027	Female	75	Emergency Department	Sputum	An acute attack of chronic bronchitis, heart disease	2012.02.25	No	cefuroxime (02.18–19) levofloxacin (02.19, 02.21) piperacillin/tazobactam (02.21)	cefoperazone/ sulbactam (02.26)	recovery
2	WF3597	Male	22	Urology Department	Pus	Uremia, allogeneic kidney transplantation	2021.02.25	Yes	/	tigecycline (02.26) ceftizoxime (02.25–26)	recovery
3	WF3648	Male	48	Urology Department	Pus	Uremia, allogeneic kidney transplantation	2021.02.25	Yes	/	cephazolin (02.25–26) ceftizoxime (02.25–26)	recovery

*/means no antimicrobial agent has been used.

antimicrobial agents, sensitive to only tigecycline and colistin, and resistant to all three carbapenems.

Antibiotic resistance gene and plasmid analysis

A total of 25 resistance genes were detected (Table 2). WF0027 carried 16 resistance genes, including the carbapenem resistance gene $bla_{\text{IMP-8}}$, whereas WF3597 and WF3648 carried 19 resistance genes, including the carbapenem resistance gene $bla_{\text{NDM-1}}$. WF0027 carried five plasmids, and both WF3597 and WF3648 carried one plasmid, IncFII(p14)_1_p14.

Homology analysis

In this experiment, the phylogenetic tree was constructed by co-constructing the three CRRP strains in this study and 46 CRRP strains downloaded from NCBI (Fig. 1). The findings revealed that the 49 CRRP strains were divided into six clusters, with WF3597 and WF3648 having high homology, in the E clade, with isolates from China and Germany, and WF0027 in the F clade exhibiting higher homology with isolates from the USA in 2019.

DISCUSSION

R. planticola belongs to the family *Enterobacteriaceae*. It was originally named *Klebsiella planticola* and classified under the genus *Klebsiella*. In 2001, the genus *R. planticola* was created and classified under a new genus after it was discovered that the 16S rRNA genes and rpoB genes did not match those of the *Klebsiella* genus. This new species was renamed as *R. planticola*. [20]. It has rarely been associated with serious infections in humans. However, the number of *R. planticola* infections has increased in recent years. The incidence of *R. planticola* infections might have been previously underestimated owing to challenges in isolation and confusion with other bacteria, including *Klebsiella* spp. [3]. *R. planticola* has been poorly reported in the domestic and international literature, and most cases are reported on a case-by-case basis. In this study, we aimed to analyze the antibiotic resistance of *R. planticola* in our hospital, analyze the WGS data of carbapenem-resistant strains, elucidate their resistance gene carriage, and investigate homologous evolutionary relationships of prevalent nosocomial strains. This study provides data support for the clinical selection of antimicrobial therapy and the prevention and control of nosocomial infections.

R. planticola infections are most common in elder patients with underlying diseases or those who have undergone traumatic surgery or invasive treatment [21]. Patients with trauma, malignancies, and gastroenteritis are susceptible to *R. planticola* infection following immunocompromised and invasive medical examinations [1, 22–24]. Consistent with Hong G's study, two of the three patients in this study had undergone allogeneic kidney transplantation, and other elderly patient with multiple underlying diseases (chronic bronchitis, heart disease, emphysema, etc.) [21].

Table 2. Information of *R. planticola* strains: antibiotic resistance and plasmid analysis

	Antimicrobial agents			Antibiotic resistance genes	Plasmid
	R	I	S		
WF0027	SAM, AMC, CXM, CAZ, CRO, FEP, CSL, ETP, FOX, GEN, TOB, AMK, CHL, SXT, TCY, MNO, CZO, IPM, CTX	TZP, ATM, MEM, CIP	TGC, COL, AMK, LVX	*aac3-Iid, aadA16, strA, strB, qnrB4, mphA, catA2, arr3, sul1, sul2, tetD, dfrA27,* $bla_{\text{DHA-1}}$, $bla_{\text{TEM-1D}}$, $bla_{\text{IMP-8}}$, $bla_{\text{CTX-M-14}}$	IncFII_1_pKP91 IncFIB(K) _1_Kpn3 repA_1_pKPC-2 Col440I_1 ColRNAI_1
WF3597	SAM, TZP, AMC, CXM, CAZ, CRO, FEP, CSL, ATM, ETP, FOX, GEN, TOB, AMK, CHL, SXT, TCY, MNO, CZO, IPM, MEM, CTX, CIP, LVX	/	TGC, COL	*aac3-Iid, aadA2, armA, qnrB4, mphA, mphE, msrE, catA2, catB3, arr3, sul1, sul2, tetD, dfrA12,* $bla_{\text{DHA-1}}$, $bla_{\text{OXA-1}}$, $bla_{\text{TEM-1D}}$, $bla_{\text{NDM-1}}$, $bla_{\text{CTX-M-3}}$	IncFII(p14) _1_p14
WF3648	SAM, TZP, AMC, CXM, CAZ, CRO, FEP, CSL, ATM, ETP, FOX, GEN, TOB, AMK, CHL, SXT, TCY, MNO, CZO, IPM, MEM, CTX, CIP, LVX	/	TGC, COL	*aac3-Iid, aadA2, armA, qnrB4, mphA, mphE, msrE, catA2, catB3, arr3, sul1, sul2, tetD, dfrA12,* $bla_{\text{DHA-1}}$, $bla_{\text{OXA-1}}$, $bla_{\text{TEM-1D}}$, $bla_{\text{NDM-1}}$, $bla_{\text{CTX-M-3}}$	IncFII(p14) _1_p14

AMC, amoxicillin/clavulanic acid; AMK, amikacin; ATM, aztreonam; CAZ, ceftazidime; CHL, chloramphenicol; CIP, ciprofloxacin; COL, colistin; CRO, ceftriaxone; CSL, cefoperazone/sulbactam; CTX, cefotaxime; CXM, cefuroxime; CZO, cefazolin; ETP, ertapenem; FEP, cefepime; FOX, cefoxitin; GEN, gentamicin; I, intermediate; IPM, imipenem; LVX, levofloxacin; MEM, meropenem; MNO, minocycline; R, resistant; S, susceptible; SAM, ampicillin/sulbactam; SXT, trimethoprim-sulfamethoxazole; TCY, tetracycline; TGC, tigecycline; TOB, tobramycin; TZP, piperacillin/tazobactam.

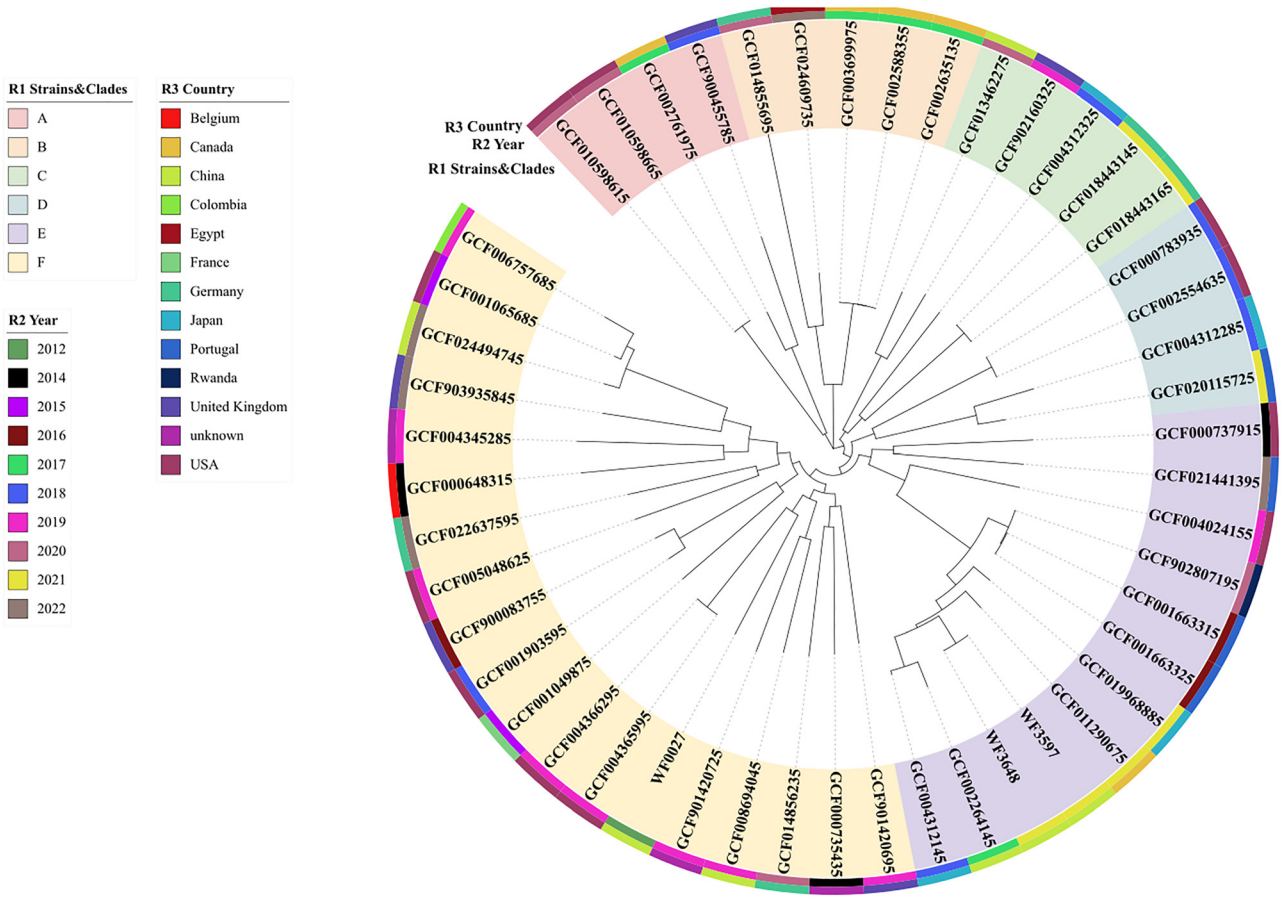

Fig. 1. Homology analysis of CRRP

Carbapenems are the most effective β-lactams for the treatment of gram-negative bacillary infections, especially those caused by *Enterobacteriaceae*. However, since the number of carbapenem-resistant *Enterobacteriaceae* has been increasing in recent years, the selection of therapeutic agents has become a challenge for physicians treating such infections in clinical settings. The findings of the antimicrobial susceptibility testing in this study revealed that the three strains of CRRP were highly resistant to 26 antimicrobial agents and sensitive to only tigecycline and colistin. Owing to the high resistance rate, the number of antibiotic classes available for clinical selection remains limited, which poses a major challenge to the clinical treatment of CRRP. The known mechanism of carbapenem resistance is the production of carbapenemase, including class A β-lactamases (KPC, reported by SENTRY surveillance program of USA in 2009 [9]), class B metallo-β-lactamase (IMP-8, reported from China Taiwan in 2014 [10]; NDM-1, reported from China in 2014 [12]), and class D β-lactamase (OXA-48, reported from Finland in 2012 [25]). Notably, KPC, NDM-1, and IMP-8 are usually detected on plasmids or transposons, which can easily cause the transfer of resistance genes among different *Phytophthora* species and even *Enterobacteriaceae*, making treatment extremely difficult [26]. All strains carried carbapenem resistance genes, however, owing to the small number of strains, determining whether carbapenem resistance was entirely dependent on the carbapenemase genes, is not possible. Therefore, we will study the resistance mechanisms to provide an important theoretical basis for the clinical treatment of CRRP strains in the future.

Analysis of homology results has demonstrated high homology between WF3597 and WF3648, and both strains were isolated at the same unit where patients had allogeneic renal transplantation, suggesting a potential risk of nosocomial infection. WF0027 was isolated in 2012 and is distantly related to WF3597 and WF3648, which were isolated in 2021.

Although *R. planticola* is an opportunistic pathogen with low pathogenicity, the emergence of CRRP under prolonged antibiotic pressure poses a significant risk to patients with underlying disease. CRRP is resistant to multiple antimicrobial agents and carries multiple resistance genes, and it is critical to focus on the rational use of antimicrobial agents, the prevalence of multidrug resistant strains, and the prevention of the spread of resistance genes.

Ethics approval and consent to participate: Given that all strains of this experiment were bacteria isolated from routine samples of the patients, and this study did not involve the patient's private information or data on animal subjects, this study was exempted from the requirement of ethical approval by the Ethics Committee of Weifang People's Hospital.

Consent for publication: Not applicable.

Competing interests: There is no competing interest between the authors.

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Author's contributions: K.D. designed the study Q.W., X.W., and C.C. conducted the study, collected the data, and prepared the article. L.Z. and J.M. provided valuable advice and edited the manuscript. All authors approved the final version of the manuscript.

REFERENCES

1. Ershadi A, Weiss E, Verduzco E, Chia D, Sadigh M. Emerging pathogen: a case and review of *Raoultella planticola*. Infection 2014; 42(6): 1043–6.
2. Ferragut C, Izard D, Gavini F, Kersters K, Deley J, Leclerc H. *Klebsiella trevisanii*: a new species from water and soil. Int J Syst Bacteriol 1983; 33: 133–42.
3. Freney J, Fleurette J, Gruer LD, Desmonceaux M, Gavini F, Leclerc H. *Klebsiella trevisanii* colonisation and septicaemia. Lancet 1984; 1(8382): 909.
4. Olson DS Jr, Asare K, Lyons M, Hofinger DM. A novel case of *Raoultella planticola* urinary tract infection. Infection 2013 Feb; 41(1): 259–61.
5. Cho YJ, Jung EJ, Seong JS, Woo YM, Jeong BJ, Kang YM, et al. A case of pneumonia caused by *Raoultella planticola*. Tuberc Respir Dis (Seoul) 2016 Jan; 79(1): 42–5.
6. Lam PW, Salit IE. *Raoultella planticola* bacteremia following consumption of seafood. Can J Infect Dis Med Microbiol 2014 Jul; 25(4): e83–4.
7. Zeng M, Xia J, Zong Z, Shi Y, Ni Y, Hu F, et al. Guidelines for the diagnosis, treatment, prevention and control of infections caused by carbapenem-resistant gram-negative bacilli. J Microbiol Immunol Infect 2023; S1684-1182(23): 00036-1.
8. Armin S, Fallah F, Karimi A, Karbasiyan F, Alebouyeh M, Rafiei Tabatabaei S, et al. Antibiotic susceptibility patterns for carbapenem-resistant Enterobacteriaceae. Int J Microbiol 2023; 2023: 8920977.
9. Castanheira M, Deshpande LM, DiPersio JR, Kang J, Weinstein MP, Jones RN. First descriptions of bla_{KPC} in Raoultella spp. (*R. planticola* and *R. ornithinolytica*): report from the SENTRY antimicrobial surveillance program. J Clin Microbiol 2009 Dec; 47(12): 4129–30.
10. Tseng SP, Wang JT, Liang CY, Lee PS, Chen YC, Lu PL. First report of bla(IMP-8) in *Raoultella planticola*. Antimicrob Agents Chemother 2014; 58(1): 593–5.
11. Tufa TB, Fuchs A, Feldt T, Galata DT, Mackenzie CR, Pfeffer K, et al. CTX-M-9 group ESBL-producing *Raoultella planticola* nosocomial infection: first report from sub-Saharan Africa. Ann Clin Microbiol Antimicrob 2020 Aug 17; 19(1): 36.
12. Li J, Lan R, Xiong Y, Ye C, Yuan M, Liu X, et al. Sequential isolation in a patient of *Raoultella planticola* and Escherichia coli bearing a novel ISCR1 element carrying bla_{NDM-1}. PLoS One 2014 Mar 3; 9(3): e89893.
13. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 2012; 19(5): 455–77.
14. Lee I, Ouk Kim Y, Park SC, Chun J. OrthoANI: an improved algorithm and software for calculating average nucleotide identity. Int J Syst Evol Microbiol 2016 Feb; 66(2): 1100–3.
15. Meier-Kolthoff JP, Carbasse JS, Peinado-Olarte RL, Göker M. TYGS and LPSN: a database tandem for fast and reliable genome-

based classification and nomenclature of prokaryotes. Nucleic Acids Res 2022 Jan 7; 50(D1): D801–d807.
16. Feldgarden M, Brover V, Haft DH, Prasad AB, Slotta DJ, Tolstoy I, et al. Validating the AMRFinder tool and resistance gene database by using antimicrobial resistance genotype-phenotype correlations in a collection of isolates. Antimicrob Agents Chemother 2019; 63: e00483-19.
17. Carattoli A, Zankari E, Garcia-Fernandez A, Voldby Larsen M, Lund O, Villa L, et al. PlasmidFinder and pMLST: in silico detection and typing of plasmids. Antimicrob Agents Chemother 2014; 58(7): 3895–903.
18. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. Mol Biol Evol 2018; 35: 1547–9.
19. Letunic I, Bork P. Interactive tree of life (iTOL) v4: recent updates and new developments. Nucleic Acids Res 2019; 47: W256–9.
20. Drancourt M, Bollet C, Carta A, Rousselier P. Phylogenetic analyses of *Klebsiella* species delineate *Klebsiella* and *Raoultella* gen. nov., with description of *Raoultella ornithinolytica* comb. nov., *Raoultella terrigena* comb. nov. and *Raoultella planticola* comb. nov. Int J Syst Evol Microbiol 2001; 51: 925–32.
21. Hong G, Yong HJ, Lee D, Kim DH, Kim YS, Park JS, et al. Clinical characteristics and treatment outcomes of patients with pneumonia caused by *Raoultella planticola*. J Thorac Dis 2020 Apr; 12(4): 1305–11.
22. Kim SW, Kim JE, Hong YA, Ko GJ, Pyo HJ, Kwon YJ. *Raoultella planticola* peritonitis in a patient on continuous ambulatory peritoneal dialysis. Infection 2015; 43: 771–5.
23. de Campos FP, Guimarães TB, Lovisolo SM. Fatal pancreatic pseudocyst co-infected by *Raoultella planticola*: an emerging pathogen. Autops Case Rep 2016; 6: 27–31.
24. Puerta-Fernandez S, Miralles-Linares F, Sanchez-Simonet MV, Bernal-Lopez MR, Gomez-Huelgas R. *Raoultella planticola* bacteraemia secondary to gastroenteritis. Clin Microbiol Infect 2013; 19: E236–7.
25. Osterblad M, Kirveskari J, Hakanen AJ, Tissari P, Vaara M, Jalava J. Carbapenemase-producing Enterobacteriaceae in Finland: the first years (2008–11). J Antimicrob Chemother 2012; 67: 2860–4.
26. Xu M, Xie W, Fu Y, Zhou H, Zhou J. Nosocomial pneumonia caused by carbapenem-resistant *Raoultella planticola*: a case report and literature review. Infection 2015 Apr; 43(2): 245–8.