Whole genome sequencing of multi-drug resistant Psychrobacter sp. SIT isolated from a sheep Hydatid Cyst fluid in Mosul City

By Talal S. Salih

WORD COUNT 3473
TIME SUBMITTED 10-JUN-2024 03:06PM
PAPER ID 109544434

Original Article

Whole genome sequencing of multi-drug resistant *Psychrobacter* sp. SIT isolated from a sheep Hydatid Cyst fluid in Mosul City

Saba A. Hamid [1], Inam A. Abdulhameed [2], Talal S. Salih[3]

[1]Department of Microbiology, College of Medicine, University of Ninevah, Iraq

[2]Department of Anatomy, College of Medicine, University of Mosul, Iraq

[3]Department of Medical Physics, College of Science, University of Mosul, Iraq

CORRESPONDING AUTHOR: Talal Sabhan Salih,

Email: talal.salih@uomosul.edu.iq

ABSTRACT

Background and Objectives. Secondary infections of hydatid cysts are most commonly caused by bacteria such as *Escherichia coli, Staphylococcus aureus*, *Pseudomonas* species and *Salmonella* species. Yet, no study has been reported the isolation of bacterial strains belong to the genus *Psychrobacter* from hydatid cysts. This study aimed to present the first whole genome sequence of a multidrug-resistant *Psychrobacter* sp. SIT isolated from a sheep hydatid cyst fluid in Mosul city, Iraq.

Materials and Methods. The whole-genome of *Psychrobacter* sp. SIT was sequenced using MiSeq Sequencing System (Illumina, USA). The comprehensive ResFinder 4.0 and CARD tools were used to identify the acquired antibiotic and the quaternary ammonium compounds resistance genes in the genome of *Psychrobacter* sp. SIT.

Results. The sequence genome of *Psychrobacter* sp. SIT yielded a total of 3,331,920 bp with a DNA GC content of 42.82% and a total of 2,955 protein-coding sequences (CDSs). The results revealed the presence of six genes that corresponded to the predicted resistance phenotypes against five different antibiotic classes, including aminoglycoside (*aph(3'')*-Ib and *aph(6)*-Id), aminocyclitol (*aadA*14),

amphenicol (*flo*R), tetracycline (*tet*(H)), and beta-lactam ($bla_{CARB-16}$). Moreover, *qac*G and *qac*J genes which confer resistance to benzalkonium chloride (BKC) and cetyltrimethylammonium bromide (CTAB) disinfectants were also reported. The results of *is*DDH revealed that *Pscychrobacter* sp. SIT was closely related to *Psychrobacter cibarius* DSM 16327 with with *is*DDH value of 64.9%. The whole genome sequence of *Psychrobacter* sp. SIT has been deposited at DDBJ/ENA/GenBank under the accession number JAQGEL000000000.

Conclusions. In the current study, an Iraqi bacterium, *Psychrobacter* sp. SIT, was whole genome sequenced. The ResFinder 4.0 and CARD tools revealed that there were eight antibiotic and quaternary ammonium compounds resistance genes in the genome of *Psychrobacter* sp. SIT. The *is*DDH values <70% suggest that *Psychrobacter*. sp. SIT is not only a new strain, but also a new species of the genus *Psychrobacter*.

Keywords: Acquired ABR genes, hydatid cysts, *Psychrobacter*, *qac* resistance genes, whole genome sequencing

INTRODUCTION

Hydatid disease, one of the most common zoonotic diseases worldwide, is a parasitic infection caused by the larvae of a tapeworm *Echinococcus granulosus*. It can cause cysts to grow in various organs including the liver, lungs, and brain of dogs and livestock such as sheep, cattle, goats, and pigs [1]. Humans become accidently infected through the ingestion of the parasite eggs [2].

While secondary bacterial infection of the hydatid cysts is rare, it can occur and lead to severe complications and even be life-threatening if left untreated [3]. Studies have reported several bacterial species such as *Escherichia coli*, *Proteus vulgaris*, *Citrobacter freundii*, *Aeromonas hydrophila*, *Staphylococcus* spp., *Enterococcus* spp., *Pseudomonas* spp., and *Salmonella* spp. were isolated from hydatid cyst fluids [4,5]. To the best of our knowledge, *Psychrobacter pulmonis* is the only species within *Psychrobacter* genus reported from lung clinical sample of a lamb [6].

Psychrobacter is an opportunistic pathogen found in a wide range of environments. Moreover, some species belong to *Psychrobacter* have been found to cause serious infections in humans, including arthritis, foot abscess, surgical wound infections, nosocomial ocular infections, bacteraemia and meningitis in infants [7,8]. Very recently, a case of cerebrospinal fluid shunt infection of an ex-premature infant girl caused by *Psychrobacter piechaudii* in the UK has been reported [9].

The genus *Psychrobacter* was first isolated from poultry and reported in 1986 [10]. The type species of *Psychrobacter* is *Psychrobacter inmobilis*. The type strain of *Psychrobacter inmobilis* is ATCC 43116, CIP 102557, DSM 7229, JCM 20442 and NBRC 15733; and the GenBank accession number of its 16S rRNA gene is AJ309942. The genus *Psychrobacter* currently includes 44 species with validly published names [11]. The most two recent novel species of the genus *Psychrobacter* are *Psychrobacter halodurans* and *Psychrobacter coccoides* were isolated from marine sediment samples in China [12]. In this study, we presented the whole genome sequence of *Psychrobacter* sp. SIT, which was isolated from a sheep hydatid cyst fluid in Mosul city, Iraq, together with the description of acquired antibiotic and quaternary ammonium compounds resistance genes, general genome features, and the detailed of genome annotation using comprehensive bioinformatics tools.

MATERIALS AND METHODS

Isolation of *Psychrobacter* sp. SIT from a sheep hydatid cyst fluid

The study initially aimed to isolate common bacteria associated with hydatid diseases caused by parasitic infections in sheep. However, 100 μl of a hydatid liver fluid from a sheep was collected using a sterile syringe, cultured on nutrient agar medium and incubated at temperatures ranged from 10 °C to 37 °C for 72 h. The *Psychrobacter* sp. SIT strain was selected for whole genome sequencing based on its unusual culture growth shape and its ability to grow at low temperatures. Moreover, the preliminary antibiotic susceptibility testing using the disk diffusion assay [13] on the Muller-Hinton plates showed that *P*sychrobacter sp. SIT resistance to the following

antibiotics: streptomycin (S-25), ampicillin (AM-10), amoxicillin (AX-10), piperacillin (PRL-100), chloramphenicol (C-10) and tetracycline (TE-10).

Whole genome sequencing, assembly and annotation

The genome of *Psychrobacter* sp. SIT was sequenced at the Leibniz Institute (DSMZ-German Collection of Microorganisms and Cell Cultures GmbH, Germany). Genomic DNA was extracted using MasterPureTM Complete DNA Purification Kit (Epicentre Biotechnologies, Germany). Nextera XT DNA Library Preparation Kit (Illumina, USA) was applied to prepare the genomic DNA library. Prepared DNA library was sequenced on a MiSeq Sequencing System (Illumina, USA) using MiSeq Reagent Kit v2. The sequence reads were de novo assembled to draft genome via SPAdes 3.5 tool [14]. After genome assembly, the obtained contigs were annotated using the RASTtk server [15] and the SEED tool [16] was used for predicting functional genes in subsystem categories.

Whole- genome-based phylogenetic tree analysis and *in silico* whole genome DNA-DNA hybridization (*is*DDH) comparisons

The Type Strain Genome Server (TYGS) [17] was applied to infer the whole-genome-based phylogenetic tree of *Psychrobacter* sp. SIT and the most related strains. *is*DDH values between *Psychrobacter* sp. SIT and the most closely related strains was measured using GGDH bioinformatics tool [11] which is integrated within the TYGS.

Detection of the acquired antibiotic resistance genes (ABR) and resistance phenotypes prediction

The platform of ResFinder 4.0 tool [18] was used to detect the antibiotic resistance genes in the genome sequence. The ResFinder 4.0 contains a database that translate the sequence genotypes into the resistance phenotypes which displays the results in created tables. As default, all the antibiotic databases were selected with a threshold of 80% identity and a minimum hit length of 80% for the best matching resistance antimicrobial genes in the ResFinder 4.0 database and the input *Psychrobacter* sp. SIT sequence as well as the most closely strains.

Detection of the quaternary ammonium compounds (*qac*) resistance genes

The *qac* resistance genes in the *Psychrobacter* sp. SIT genome were detected using the Comprehensive Antibiotic Resistance Database (CARD) program version 3.2.6 [19], through the resistance gene identifier (RGI) tool version 6.0.1 [20].

RESULTS AND DISCUSSION

General genome features of *Psychrobacter* sp. SIT

A detailed summary of the genome features of *P*. sp. SIT is shown in Table 1. The assembled genome sequence of *P*. sp. SIT yielded a total of 3,331,920 bp with a DNA GC content of 42.82% distributed within 38 contigs with N50 value of 155953, and with a largest contig of 370,318 bp. RASTtk annotated a total of 2,955 protein-coding sequences (CDSs), 46 tRNA genes and 275 subsystems. Most of the predicted genes in the subsystem categories were involved in amino acids and derivatives synthesis (265), cofactors, vitamins, prosthetic groups and pigments production (212), carbohydrates metabolism (201), protein metabolism (149) and fatty acids, lipids, and Isoprenoids (86) (Figure 1).

TABLE 1. General features of the *Psychrobacter* sp. SIT genome

Feature	Value
Total genome size (pb)	3,331,920
DNA GC content (%)	42.82
Number of contigs	38
Largest contig size (bp)	370,318
Smallest contig size (bp)	1203
N50	155,953
Total of protein-coding sequences (CDSs)	2,955
Number of tRNA genes	46

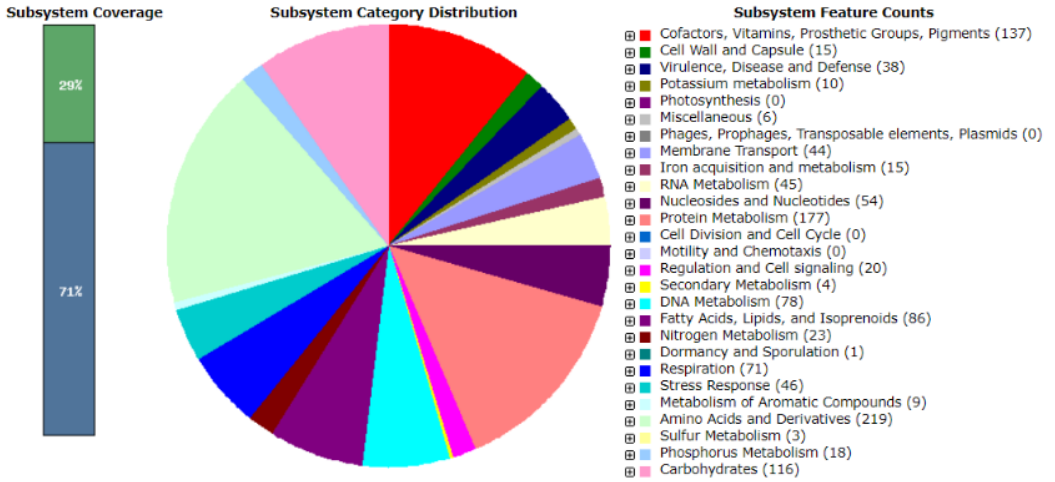

FIGURE 1: Subsystem category distribution statistics of *Psychrobacter* sp. SIT. The pie chart showed the count of each subsystem feature and the subsystem coverage were displayed using SEED viewer tool. The green bar of the subsystem coverage corresponds to the percentage of the proteins included in the subsystems while the blue bar corresponds to the percentage of the proteins that are not included in the subsystems.

Whole-genome-based phylogenetic tree and *is*DDH comparison values

The TYGS analysis identified thirteen strains of the genus *Psychrobacter* that are most closely related to *Psychrobacter* sp. SIT (Figure 2). The results have displayed that the nearest relative to *Psychrobacter* sp. SIT were *Psychrobacter cibarius* DSM 16327, *Psychrobacter immobilis* DSM 722 and *Psychrobacter aquimaris* DSM 16329 with *is*DDH values of 64.9%, 64.7% and 39.7% respectively. All other related strains have shown *is*DDH values ≤ 24.9%. The TYGS addresses the gold standard methods and state-of-the-art estimates in the genomic era to determine nearest bacterial genome sequences with valid names [11]. Values obtained from *is*DDH method can estimate species boundaries based on whole genome sequence data by applying formula of probability that DDH > 70% is same species [21,22]. Results of *is*DDH values <70% have revealed sufficient dissimilarity of *Psychrobacter* sp. SIT to their closest relative species within the genus of *Psychrobacter* and might indicate that *Psychrobacter* sp. SIT was not only a new strain, but also a new species of the genus *Psychrobacter*.

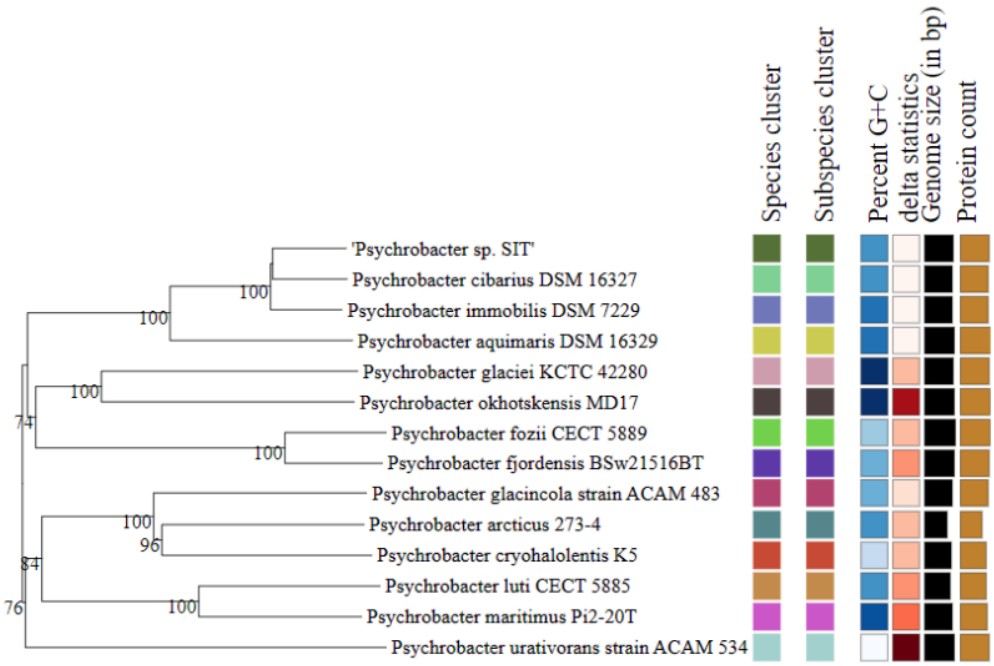

FIGURE 2: Whole-genome-based phylogenetic tree constructed from the thirteen whole genome sequences of the genus *Psychrobacter* that are most related to *P*. sp. SIT. The tree generated via the TYGS based on balanced minimum evolution method (100X bootstrap support values). Labels on leaves were indicated by association to species and subspecies clusters, genomic GC percent, δ values, overall genome size and total number of proteins

Whole-genome-based phylogenetic tree and *is*DDH comparison values

The ResFinder 4.0 tool analysis indicated that *Psychrobacter* sp. SIT was harboring genes mediated to five different antibiotic classes: aminoglycoside, aminocyclitol, amphenicol, tetracycline and beta-lactam. A total of six resistance genes were identified in the genome sequence of *Psychrobacter* sp. SIT (Table 2). The resistance *aph(3'')*-Ib and *aph(6)*-Id genes that identified in *P*. sp. SIT were homologous to *aph(3'')*-Ib and *aph(6)*-Id genes that found in *Shigella flexneri* (accession no. AF321551) and *Escherichia coli* (accession no. M28829) with identities of 99.8% and 100% respectively. Studies have revealed that the occurrence of *aph(3'')*-

Ib and *aph(6)*-Id genes conferring resistance to streptomycin antibiotic [23]. These two genes are among the most predominant aminoglycosides observed in Gram negative pathogens in particularly the family Enterobacteriaceae [24,25]. Florfenicol antibiotic has been widely used in farm animals, as a veterinary medicine and feed additive. The occurrence of *flo*R gene in a bacterial genome confers resistance to chloramphenicol and florfenicol antibiotics [26]. Currently, *flo*R resistance gene is widespread not only in veterinary pathogens but also in various human-originated clinical isolates such as *Escherichia coli* [27], *Klebsiella pneumoniae* [28] and *Pseudomonas aeruginosa* [29]. On the other hand, the *tet*(H) resistance gene detected in *Psychrobacter* sp. SIT was highly similar to *tet*(H) gene that was found in *Pasteurella aerogenes* (accession no. AJ245947), an animal pathogen associated with pigs and hamsters, [30]. However, the *tet*(H) gene has also been identified in some clinical bacterial isolates [31]. Interestingly, the *bla*$_{\text{CARB-16}}$ gene sequence that identified in *Psychrobacter* sp. SIT was identical (100%) to the *bla*$_{\text{CARB-16}}$ gene sequence that identified in *Psychrobacter maritimus* MR29-12 which was isolated from an ancient permafrost [32]. This result might suggest that this gene is within the intrinsic genes in *Psychrobacter* species. However, recent studies have reported that the presence of *bla*$_{\text{CARB-16}}$ gene in *Acinetobacter baumannii* clinical isolates conferring resistance to carbapenem antibiotics including ampicillin, amoxicillin and piperacillin [33,34].

TABLE 2. Antibiotic resistance genes detected in the genome sequence of *Psychrobacter* sp. SIT by ResFinder 4.0 tool with ID ≥80%.

Resistance class	**Resistance gene**	**Identity**	**Predicted phenotype**	**PubMed ID**	**Accession No.**
Aminoglycoside	*aph(3'')*-Ib	99.8%	Streptomycin	12029529	AF321551
	aph(6)-Id	100%	Streptomycin	2653965	M28829
Aminocyclitol	*aad*A14	80.9%	Spectinomycin, streptomycin	15980396	AJ884726
Amphenicol	*flo*R	98.2%	florfenicol	10339826	AF118107
Tetracycline	*tet*(H)	100%	Doxycycline, tetracycline	10913704	AJ245947

Beta-lactam	*bla*$_{CARB-16}$	100%	Ampicillin, amoxicillin, piperacillin	25063046	HF953351

CONCLUSION

In conclusion, this study has shown that new species of bacteria could be isolated from hydatid cysts. The new strain *Psychrobacter* sp. SIT of the genus *Psychrobacter* will attribute in studying of secondary bacterial infections of hydatid cysts. Compared with other *Psychrobacter* genomes, *Psychrobacter* sp. SIT might be a new species of the genus *Psychrobacter*. The genome analysis using the comprehensive RestFinder and CARD bioinformatics tools conferred that *Psychrobacter* sp. SIT is a multi-drug resistant bacterium where found to harbor six antibiotic resistance genes belong to five different antibiotic classes. In addition, *qac* genes which confer resistance to the quaternary ammonium compounds were also found in *Psychrobacter* sp. SIT genome. Finally, the current study highlights the rise of multidrug-resistant bacteria among environmental bacterial isolates, which can make them difficult to treat and control.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHOR'S CONTRIBUTIONS

Conceptualization, Saba A. Hamid, Inam A. Abdulhameed and Talal S. Salih; methodology, Saba A. Hamid, Inam A. Abdulhameed and Talal S. Salih; software, Talal S. Salih; validation, Saba A. Hamid and Inam A. Abdulhameed; formal analysis, Talal S. Salih; investigation, Saba A. Hamid and Inam A. Abdulhameed; resources, Saba A. Hamid; data curation, Saba A. Hamid; writing—original draft preparation, Saba A. Hamid and Inam A. Abdulhameed; writing—review and editing, Talal S. Salih;

visualization, Inam A. Abdulhameed. All authors have read and agreed to the published version of the manuscript.

ACKNOWLEDGEMENTS

The authors would like to acknowledge both Ninevah and Mosul universities for their support and for providing facilities needed to complete this study.

REFERENCES

1. Pakala T, Molina M, Wu GY. Hepatic echinococcal cysts: a review. Journal of clinical and translational hepatology. 2016 Mar 3;4(1):39. doi: 10.14218/JCTH.2015.00036
2. Wen H, Vuitton L, Tuxun T, Li J, Vuitton DA, Zhang W, McManus DP. Echinococcosis: advances in the 21st century. Clinical microbiology reviews. 2019 Mar 20;32(2):10-128. doi: 10.1128/CMR.00075-18
3. Brik K, Hassouni T, Youssir S, Baroud S, Elkharrim K, Belghyti D. Epidemiological study of Echinococcus granulosus in sheep in the Gharb plain (North-West of Morocco). Journal of parasitic diseases. 2018 Dec;42:505-10. doi: 10.1007/s12639-018-1026-7
4. Ziino G, Giuffrida A, Panebianco A, Bilei S. Bacteria isolated from 25 hydatid cysts in sheep, cattle and goats. Veterinary Record. 2009 Aug;165(8):234-6. doi.org/10.1136/vr.165.8.234
5. Ahmed AB, Ras R, Mahmoud AF, El-Ghazaly E, Widmer G, Dahshan H, Elsohaby I. Prevalence and bacterial isolation from hydatid cysts in dromedary camels (Camelus dromedarius) slaughtered at Sharkia abattoirs, Egypt. Journal of Parasitic Diseases. 2021 Mar;45:236-43. doi: 10.1007/s12639-020-01300-x
6. Vela AI, Collins MD, Latre MV, Mateos A, Moreno MA, Hutson R, Dominguez L, Fernandez-Garayzabal JF. Psychrobacter pulmonis sp. nov., isolated from the lungs of lambs. International journal of systematic and evolutionary microbiology. 2003 Mar;53(2):415-9. doi.org/10.1099/ijs.0.02413-0
7. Deschaght P, Janssens M, Vaneechoutte M, Wauters G. Psychrobacter isolates of human origin, other than Psychrobacter phenylpyruvicus, are predominantly Psychrobacter faecalis and Psychrobacter pulmonis, with emended description of P. faecalis. International journal of systematic and evolutionary microbiology. 2012 Mar;62(Pt_3):671-4. doi.org/10.1099/ijs.0.032631-0

8. Bonwitt J, Tran M, Droz A, Gonzalez A, Glover WA. Psychrobacter sanguinis wound infection associated with marine environment exposure, Washington, USA. Emerging Infectious Diseases. 2018 Oct;24(10):1942. doi: 10.3201/eid2410.171821
9. Kumaria A, Crusz SA, Lister M, Kirkman MA, Macarthur DC. Psychrobacter piechaudii shunt infection: first report of human infection. Child's Nervous System. 2022 Jul 1:1-4. doi.org/10.1007/s00381-021-05401-7
10. Juni E, Heym GA. Psychrobacter immobilis gen. nov., sp. nov.: genospecies composed of gram-negative, aerobic, oxidase-positive coccobacilli. International Journal of Systematic and Evolutionary Microbiology. 1986 Jul;36(3):388-91. doi.org/10.1099/00207713-36-3-388
11. 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 research. 2022 Jan 7;50(D1):D801-7. doi: 10.1093/nar/gkab902
12. Shang DD, Yang GJ, Chen GJ, Du ZJ. Psychrobacter halodurans sp. nov. and Psychrobacter coccoides sp. nov., two new slightly halophilic bacteria isolated from marine sediment. International Journal of Systematic and Evolutionary Microbiology. 2022 Jan 17;72(1):005173. doi.org/10.1099/ijsem.0.005173
13. Bauer AW. Antibiotic susceptibility testing by a standardized single disc method. Am. J. of Clinc. Path.. 1996;45:149-58.
14. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. Journal of computational biology. 2012 May 1;19(5):455-77. doi: 10.1089/cmb.2012.0021
15. Brettin T, Davis JJ, Disz T, Edwards RA, Gerdes S, Olsen GJ, Olson R, Overbeek R, Parrello B, Pusch GD, Shukla M. RASTtk: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. Scientific reports. 2015 Feb 10;5(1):1-6. doi: 10.1038/srep08365

16. Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M, Vonstein V. The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). Nucleic acids research. 2014 Jan 1;42(D1):D206-14. doi: 10.1093/nar/gkt1226
17. Meier-Kolthoff JP, Göker M. TYGS is an automated high-throughput platform for state-of-the-art genome-based taxonomy. Nature communications. 2019 May 16;10(1):2182. doi.org/10.1038/s41467-019-10210-3
18. Bortolaia V, Kaas RS, Ruppe E, Roberts MC, Schwarz S, Cattoir V, Philippon A, Allesoe RL, Rebelo AR, Florensa AF, Fagelhauer L. ResFinder 4.0 for predictions of phenotypes from genotypes. Journal of Antimicrobial Chemotherapy. 2020 Dec;75(12):3491-500. doi.org/10.1093/jac/dkaa345
19. Alcock BP, Huynh W, Chalil R, Smith KW, Raphenya AR, Wlodarski MA, Edalatmand A, Petkau A, Syed SA, Tsang KK, Baker SJ. CARD 2023: expanded curation, support for machine learning, and resistome prediction at the Comprehensive Antibiotic Resistance Database. Nucleic acids research. 2023 Jan 6;51(D1):D690-9. doi.org/10.1093/nar/gkac920
20. Jia B, Raphenya AR, Alcock B, Waglechner N, Guo P, Tsang KK, Lago BA, Dave BM, Pereira S, Sharma AN, Doshi S. CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database. Nucleic acids research. 2016 Oct 25:gkw1004. doi: 10.1093/nar/gkw1004
21. Auch AF, von Jan M, Klenk HP, Göker M. Digital DNA-DNA hybridization for microbial species delineation by means of genome-to-genome sequence comparison. Standards in genomic sciences. 2010 Jan;2:117-34. doi.org/10.4056/sigs.531120
22. Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M. Genome sequence-based species delimitation with confidence intervals and improved distance functions. BMC bioinformatics. 2013 Dec;14:1-4. doi.org/10.1186/1471-2105-14-60

23. Krause KM, Serio AW, Kane TR, Connolly LE. Aminoglycosides: an overview. Cold Spring Harbor perspectives in medicine. 2016 Jun 1;6(6):a027029. doi: 10.1101/cshperspect.a027029

24. Azimi L, Armin S, Samadi Kafil H, Abdollahi N, Ghazvini K, Hasanzadeh S, Shahraki Zahedani S, et al. Evaluation of phenotypic and genotypic patterns of aminoglycoside resistance in the Gram-negative bacteria isolates collected from pediatric and general hospitals. Molecular and cellular pediatrics. 2022 Feb 4;9(1):2. doi.org/10.1186/s40348-022-00134-2

25. Haley BJ, Kim SW, Salaheen S, Hovingh E, Van Kessel JA. Virulome and genome analyses identify associations between antimicrobial resistance genes and virulence factors in highly drug-resistant Escherichia coli isolated from veal calves. Plos one. 2022 Mar 17;17(3):e0265445. doi.org/10.1371/journal.pone.0265445

26. Li P, Zhu T, Zhou D, Lu W, Liu H, Sun Z, et al. Analysis of resistance to florfenicol and the related mechanism of dissemination in different animal-derived bacteria. Frontiers in Cellular and Infection Microbiology. 2020 Jul 31;10:369. doi: 10.3389/fcimb.2020.00369

27. Fernandez-Alarcon C, Singer RS, Johnson TJ. Comparative genomics of multidrug resistance-encoding IncA/C plasmids from commensal and pathogenic Escherichia coli from multiple animal sources. PloS one. 2011 Aug 12;6(8):e23415. doi.org/10.1371/journal.pone.0023415

28. Lu J, Zhang J, Xu L, Liu Y, Li P, Zhu T, et al. Spread of the florfenicol resistance floR gene among clinical Klebsiella pneumoniae isolates in China. Antimicrobial Resistance & Infection Control. 2018 Dec;7:1-9. doi.org/10.1186/s13756-018-0415-0

29. Qian C, Liu H, Cao J, Ji Y, Lu W, Lu J, et al. Identification of floR Variants Associated With a Novel Tn 4371-Like Integrative and Conjugative Element in Clinical Pseudomonas aeruginosa Isolates. Frontiers in Cellular and Infection Microbiology. 2021 Jun 21;11:685068. doi.org/10.3389/fcimb.2021.685068

30. Frederiksen W. Ecology and significance of Pasteurellaceae in man—an update. Zentralblatt für Bakteriologie. 1993 Jun 1;279(1):27-34. doi.org/10.1016/S0934-8840(11)80488-3

31. Alaygut D, Engin A. Pasteurella aerogenes as an asymptomatic bacteriuria agent. Journal of Tropical Pediatrics. 2018 Feb;64(1):82-4. doi.org/10.1093/tropej/fmx028

32. Petrova M, Kurakov A, Shcherbatova N, Mindlin S. Genetic structure and biological properties of the first ancient multiresistance plasmid pKLH80 isolated from a permafrost bacterium. Microbiology. 2014 Oct;160(10):2253-63. doi.org/10.1099/mic.0.079335-0

33. Moyo SJ, Manyahi J, Hubbard AT, Byrne RL, Masoud NS, Aboud S, et al. Molecular characterisation of the first New Delhi metallo-β-lactamase 1-producing Acinetobacter baumannii from Tanzania. Transactions of The Royal Society of Tropical Medicine and Hygiene. 2021 Sep;115(9):1080-5. doi.org/10.1093/trstmh/traa173

34. Zhao F, Liu H, Yao Y, Zhang L, Zhou Z, Leptihn S, Yu Y, Hua X, Fu Y. Description of a rare pyomelanin-producing carbapenem-resistant Acinetobacter baumannii strain coharboring chromosomal OXA-23 and NDM-1. Microbiology Spectrum. 2022 Aug 31;10(4):e02144-22. doi: 10.1128/spectrum.02144-22