Functional Metagenomics Reveals Novel Antibiotic Resistomes in Polar Soils

Xiuqin Xie[1#], Weibin Cheng[2,3#], Zhaohong Li[1], Rong He[1], Ke Yuan[1, 4],
Qinghua Zhang[5], Ruiqiang Yang[5], LiLi Ming[6], Ke Yu[7],
Tiangang Luan[4,8], Baowei Chen[1, 4*]

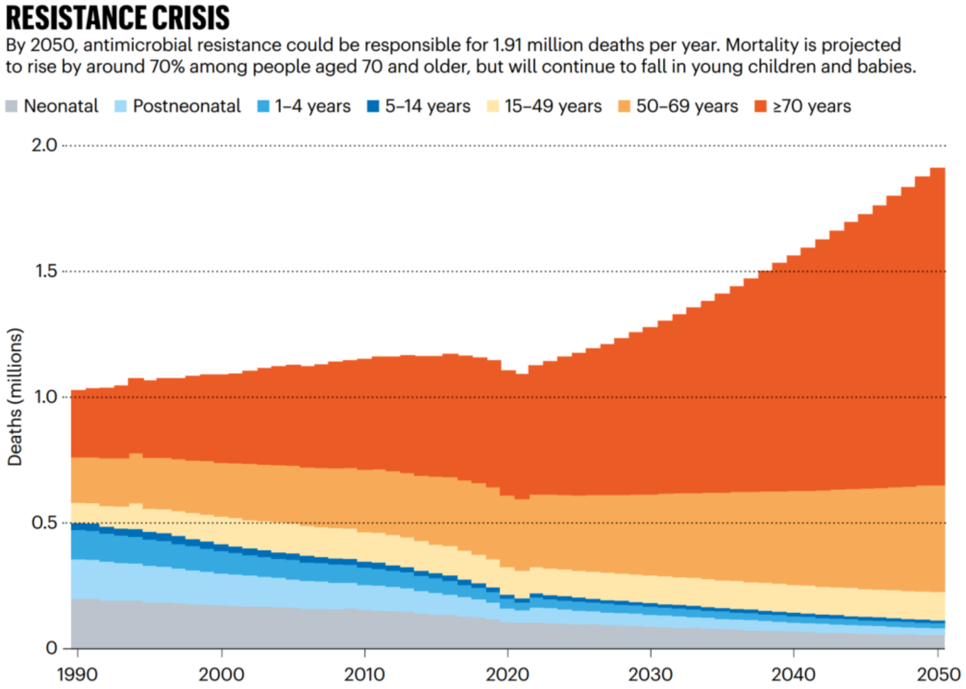
[1]Guangdong Provincial Key Laboratory of Marine Resources and Coastal Engineering, School of Marine Sciences, Sun Yat-sen University, Zhuhai, China
[2]Institute for Healthcare Artificial Intelligence Application, The Affiliated Guangdong Second Provincial General Hospital of Jinan University, Guangzhou, China
[3]Faculty of Health Sciences, City University of Macau, Macao SAR, China
[4]Guangdong Provincial Laboratory of Chemistry and Fine Chemical Engineering Jieyang Center, Jieyang, China
[5]State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing, China
[6]Technical Center of Gongbei Customs District, Zhuhai, China
[7]Eco-environment and Resource Efficiency Research Laboratory, School of Environment and Energy, Shenzhen Graduate School, Peking University, Shenzhen, China

[8]State Key Laboratory of Biocontrol, School of Life Sciences, Sun Yat-sen University, Guangzhou, China

Xiuqin Xie, Weibin Cheng, Zhaohong Li, Rong He, Ke Yuan, Qinghua Zhang, Ruiqiang Yang, et al. 2025. Functional Metagenomics Reveals Novel Antibiotic Resistomes in Polar Soils. ***iMeta*** 4: e70069. https://doi.org/10.1002/imt2.70069

Background

The risks of antibiotic resistance

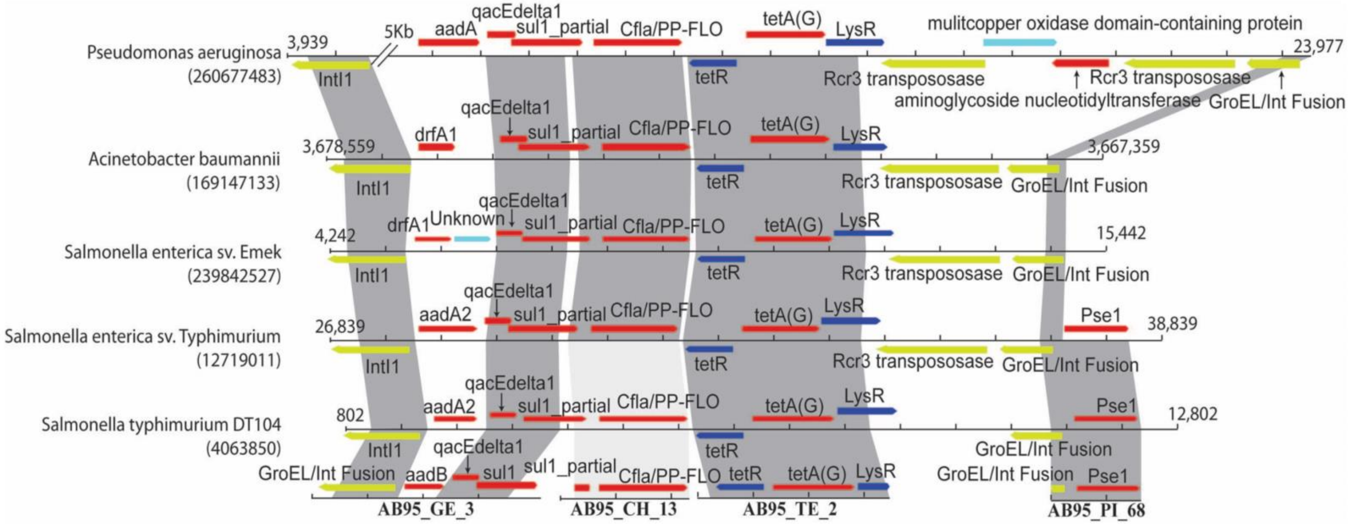

RESISTANCE CRISIS

By 2050, antimicrobial resistance could be responsible for 1.91 million deaths per year. Mortality is projected to rise by around 70% among people aged 70 and older, but will continue to fall in young children and babies.

By 2050, antimicrobial resistance (AMR) is projected to directly cause 1.91 million deaths annually worldwide.

(Naddaf M. *Nature*. 2024.)

Natural environments serve as reservoirs for ARB and ARGs

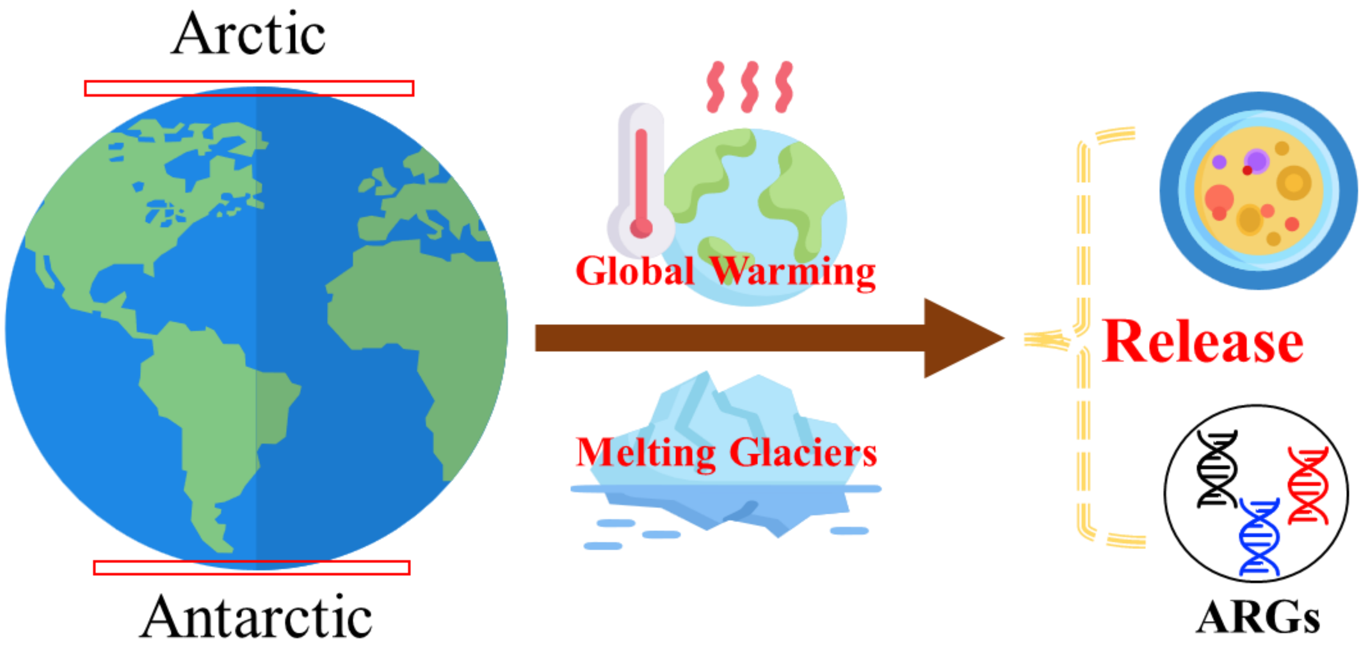


Soil microbiota are recognized as one of the reservoirs for ARGs that undergo exchange with clinical pathogens.

(Forsberg., et al. *Science*. 2012.)

Clinically relevant pathogens carrying ARGs have been identified in natural environments, *e.g.*, ***Serratia liquefariens*、*Yersinia enterorolztira*.** (Mogrovejo-Arias, D.C., et al. *Environmental Earth Sciences*, 2020. Dancer, S.J., et al. *Journal of Applied Microbiology*, 1997.)

Background



Arctic

Antarctic

Global Warming

Melting Glaciers

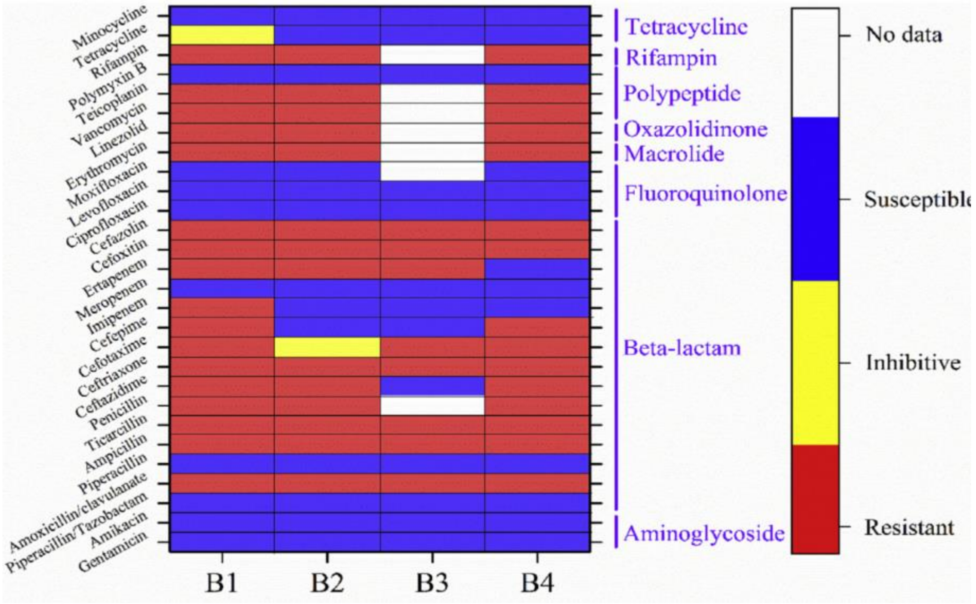
Release

ARGs

- It is estimated that global warming could release approximately **4.0×10^{21}** microbial cells annually from thawing cryospheric environments. (Edwards, A., Frontiers in Earth Science, 2015.)
- In 2016, an anthrax outbreak occurred in Russia's Yamal region, and the source strain *Bacillus anthracis* was identical to isolates obtained from tissues of deceased animals in Siberian permafrost. (Timofeev, V., et al., Plos One, 2019.)
- East Siberian permafrost isolates harbor functional resistance genes to chloramphenicol, gentamicin, tetracycline, and mercury compounds. (Mindlin SZ., et al., Russian Journal of Genetics, 2008.)

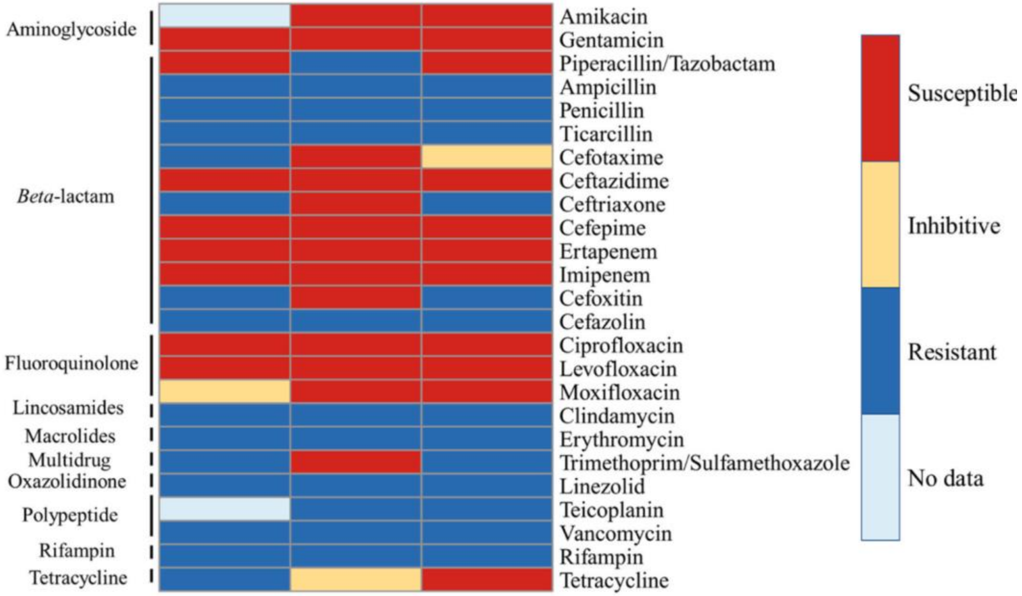
Preliminary Findings and Implications

Antibiotic resistance phenotypes of culturable bacteria in Antarctic soils



Yuan, K., et al., *Ecotoxicology and Environmental Safety*, 2019.

Antibiotic resistance phenotypes of culturable bacteria in Arctic soils



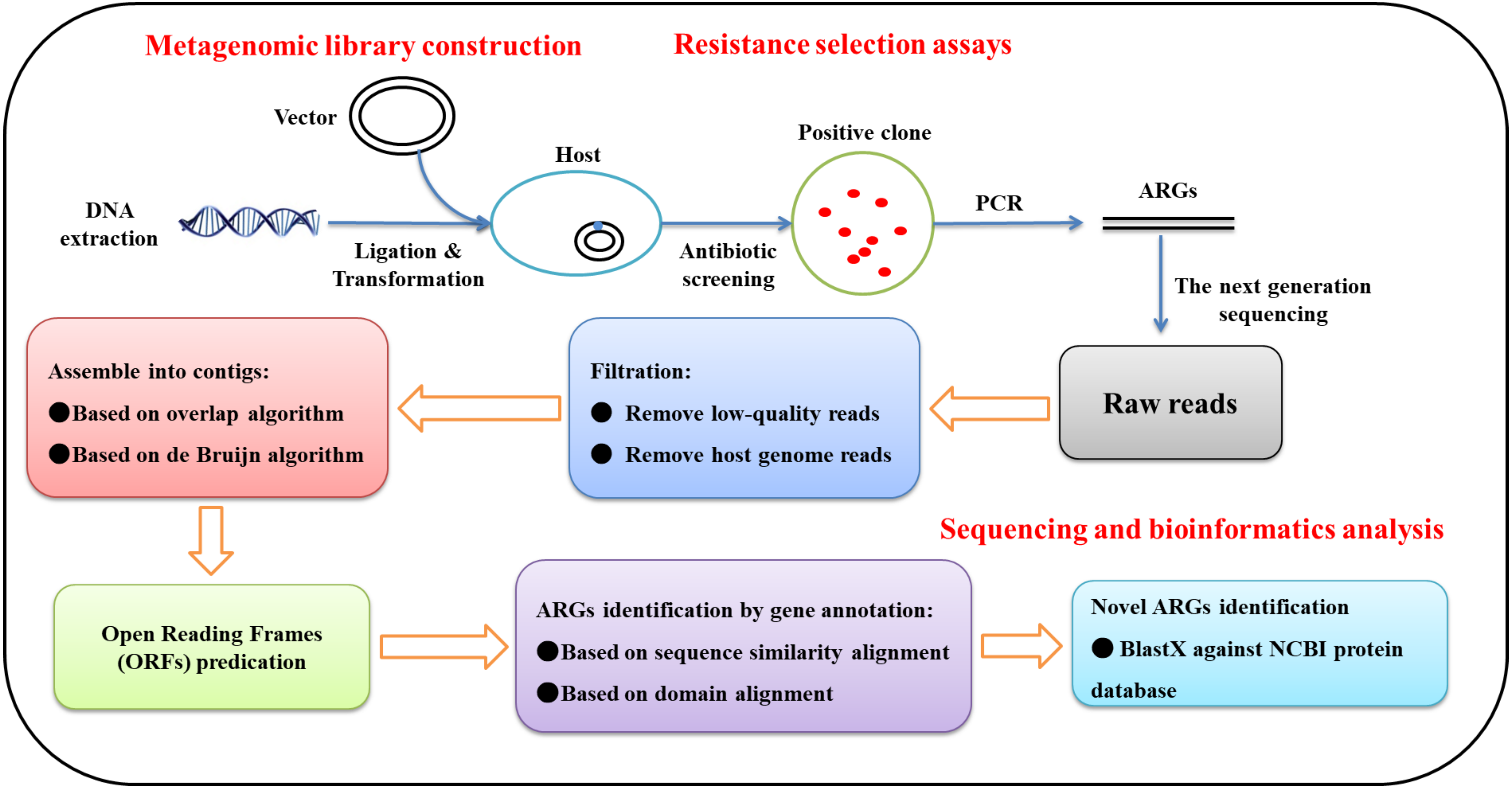
Xie, X., et al., *Journal of Hazardous Materials*, 2024.

Inconsistence between antibiotic resistance phenotype and genotype

Scientific hypothesis

Polar environments may harbor novel, uncharacterized antibiotic resistance genes

Methodology——Functional metagenomic approach



Metagenomic library construction

Resistance selection assays

Raw reads

Filtration:

- Remove low-quality reads
- Remove host genome reads

Assemble into contigs:

- Based on overlap algorithm
- Based on de Bruijn algorithm

Sequencing and bioinformatics analysis

Open Reading Frames (ORFs) predication

ARGs identification by gene annotation:

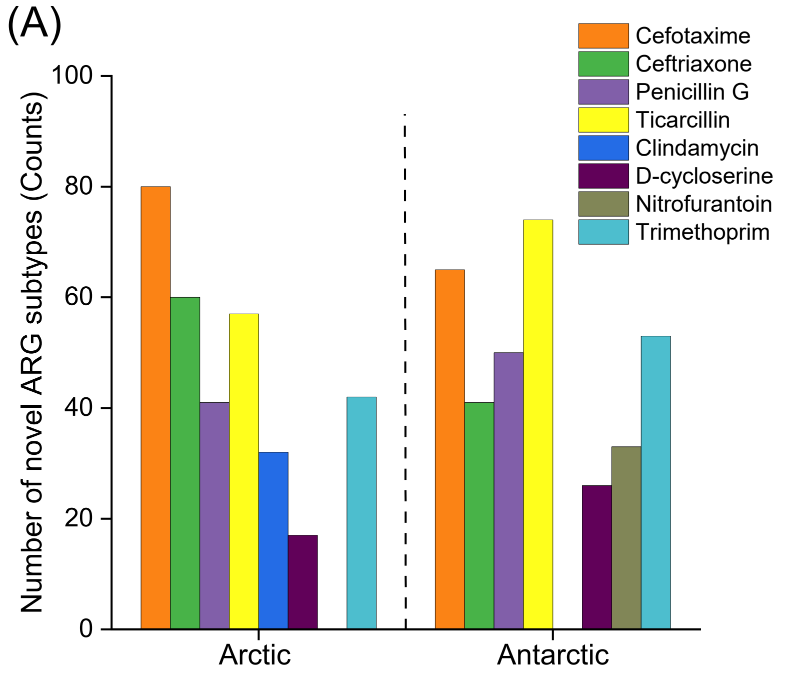
- Based on sequence similarity alignment
- Based on domain alignment

Novel ARGs identification

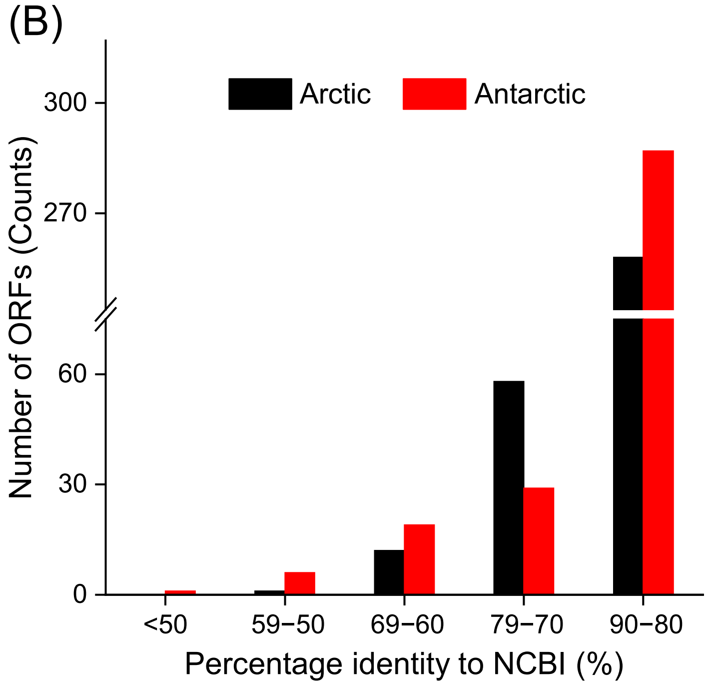
- BlastX against NCBI protein database

Results and discussion

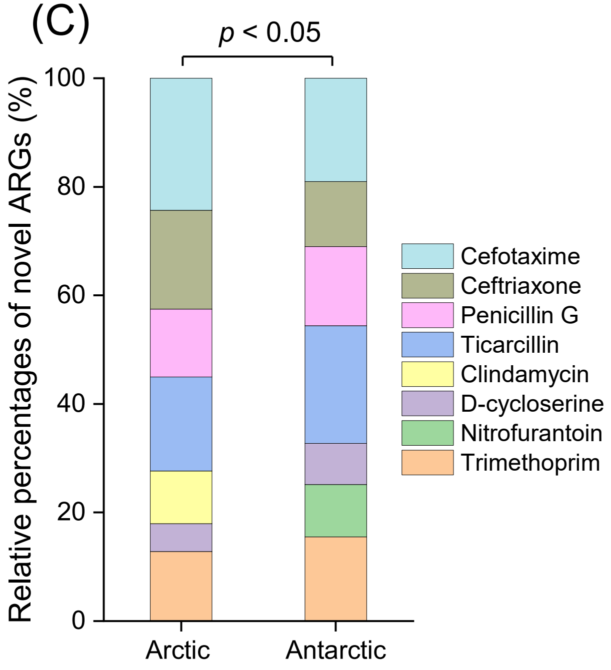
Identification and diversity of novel ARGs in polar soils



Number of novel ARGs categorized by antibiotic types



Identity of novel polar ARGs to the top hit in the NCBI protein database

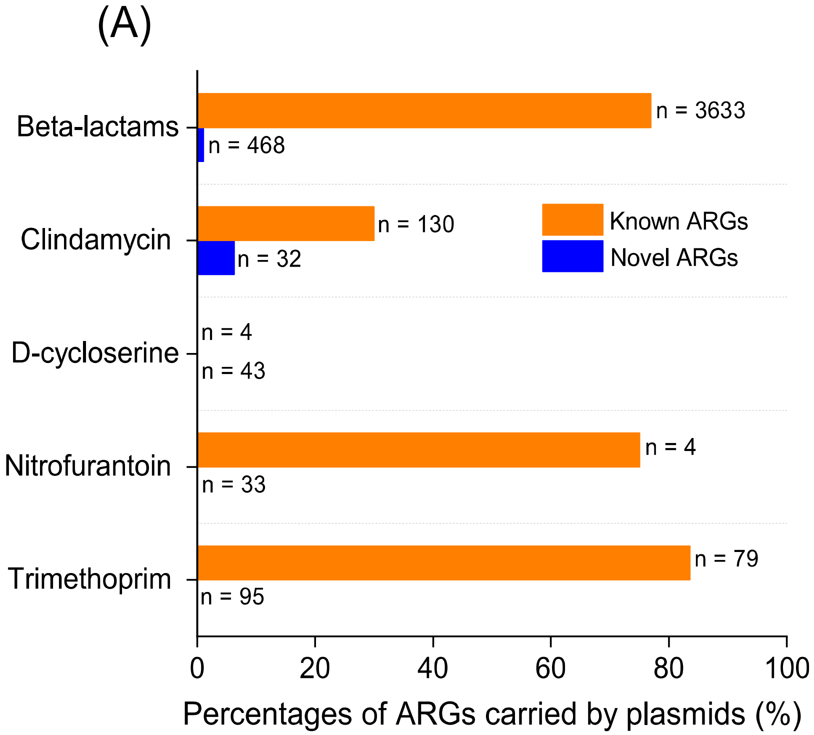


Comparative composition profiles of novel ARGs identified in polar soils

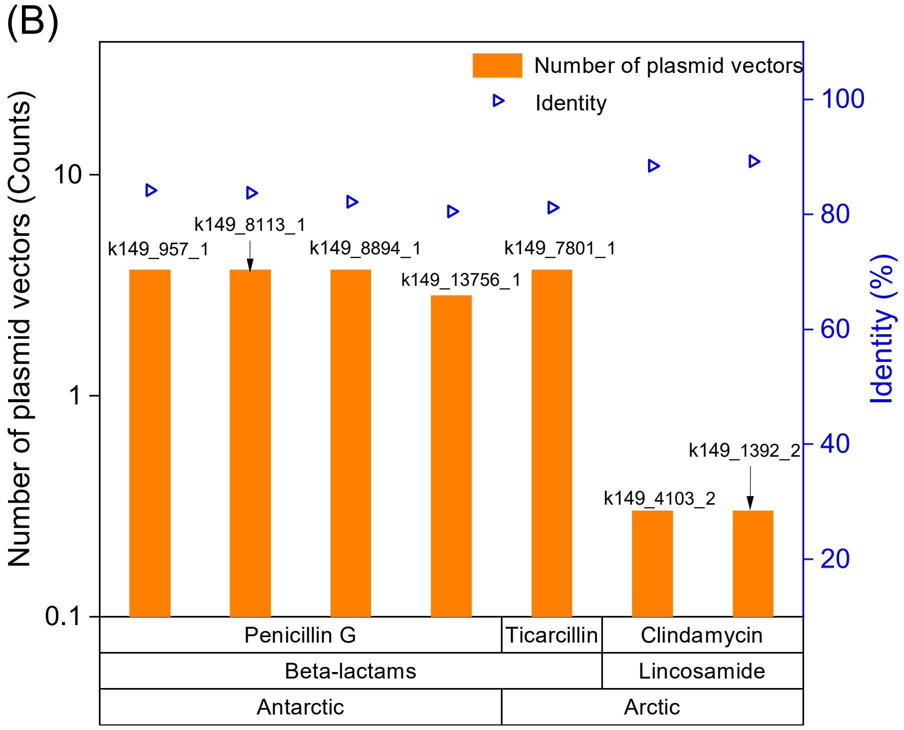
- **A total of 342 and 329 novel ARGs were identified in Arctic and Antarctic soils, respectively.**
- **Approximately 70.0% of the novel ARGs conferred resistance to beta-lactams, followed by folate synthesis inhibitors (~14.2%), D-cycloserine (~6.4%), nitrofuran (~4.9%), and clindamycin (~4.8%).**
- **Approximately 20.0% of novel polar ARGs showed < 80.0% identity to their closest NCBI homologs.**
- **Novel ARG composition differed significantly between Arctic and Antarctic soils ($p < 0.05$)。**

Results and discussion

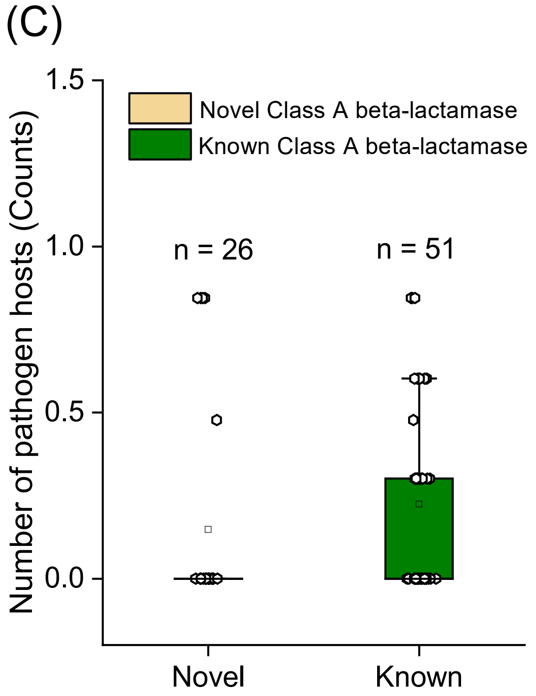
Horizontal Transfer Potential of Novel ARGs in Polar Soils



Percentages of plasmid-carrying ARGs over the total ARGs between novel and known ARGs



Summary of novel polar ARGs carried by plasmids

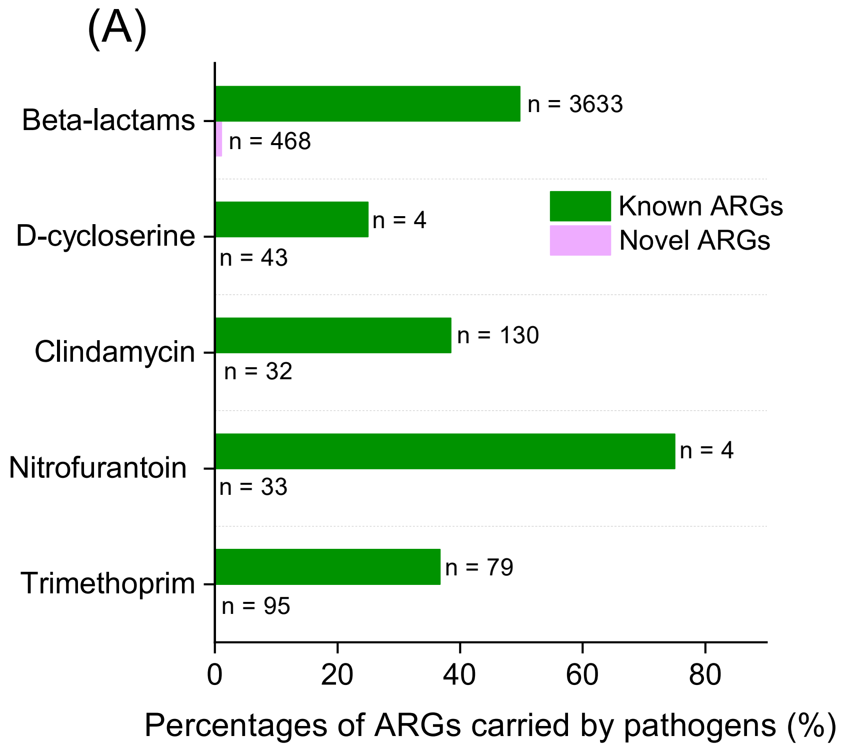


Number of plasmid vectors for novel and known ARGs related to Class A beta-lactams

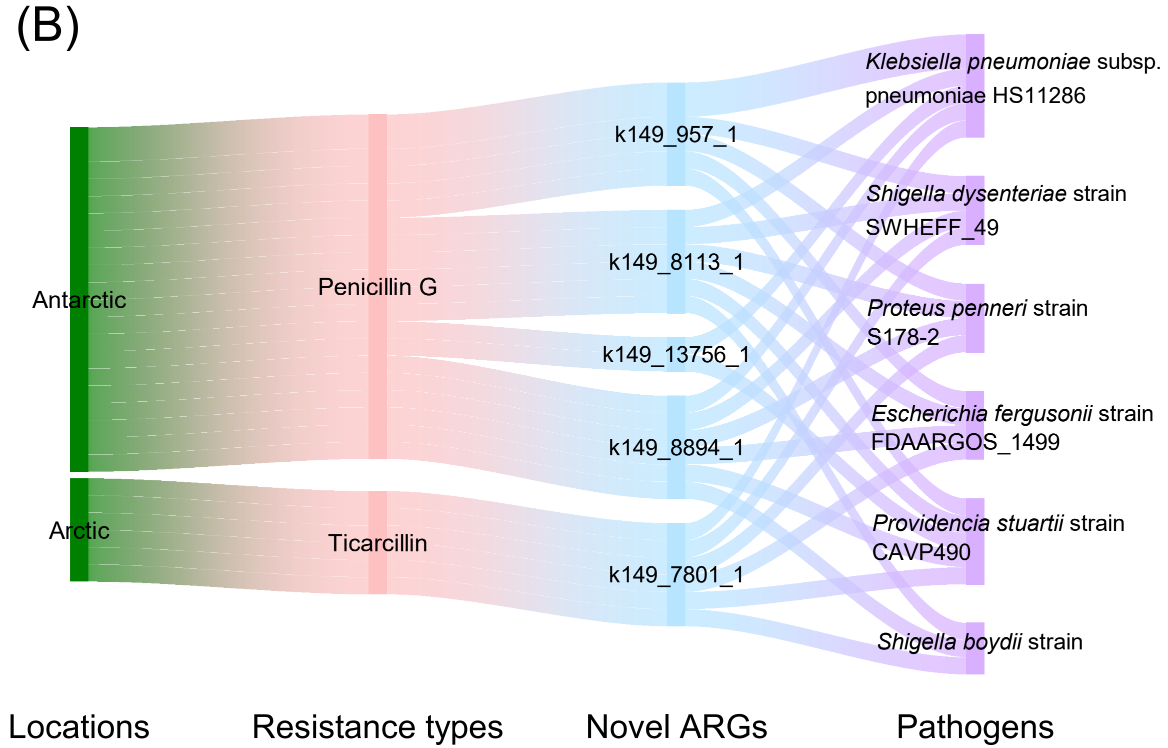
- **Greater than 75.0% of known ARGs related to beta-lactams, nitrofurantoin, and trimethoprim were plasmid-associated.**
- **Plasmid-borne novel ARGs in polar regions accounted for only approximately 1.0% of the total novel ARGs, with 3 identified in the Arctic and 4 in the Antarctic.**
- **The occurrence of known ARGs encoding Class A beta-lactamases on plasmids was statistically significantly higher than that of novel polar ARGs ($p < 0.05$).**

Results and discussion

Novel polar ARGs in the genomes of human pathogenic bacteria



Relative percentages of human pathogenic bacteria-carrying ARGs over the total ARGs between novel and known ARGs

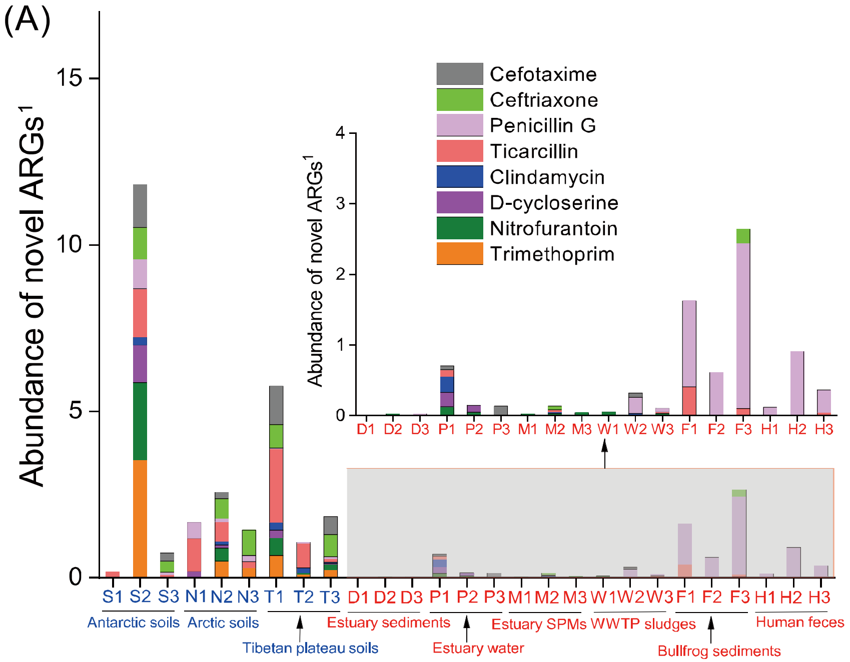


Predicted human pathogenic hosts of novel ARGs identified in Arctic and Antarctic soils

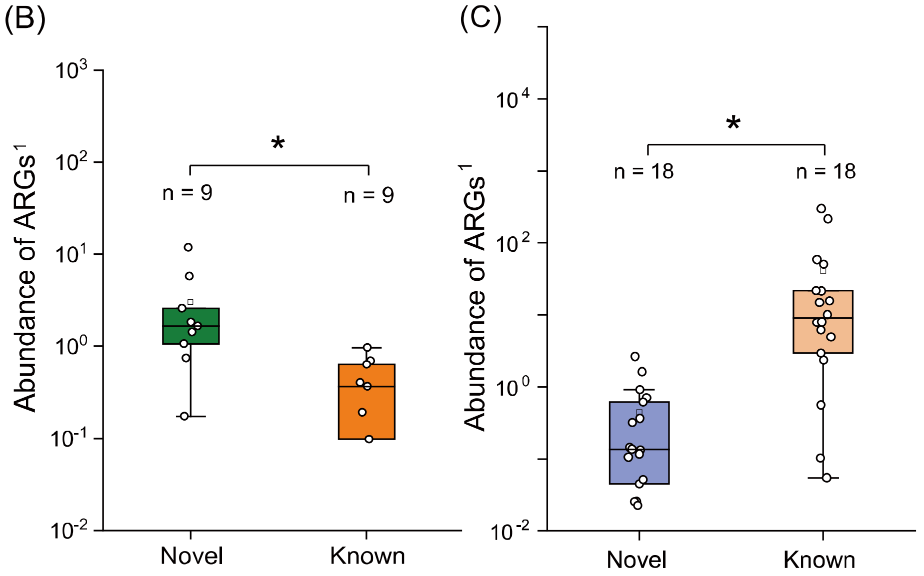
- **Known ARGs carried by human pathogenic bacterial hosts accounted for approximately 25.0% of the total known ARGs.**
- **Five novel polar ARGs were identified in the genomes of human pathogenic bacteria, representing only 0.75% of the total novel polar ARGs.**
- **The novel polar ARGs carried by human pathogenic bacteria were also detectable on plasmids.**

Results and discussion

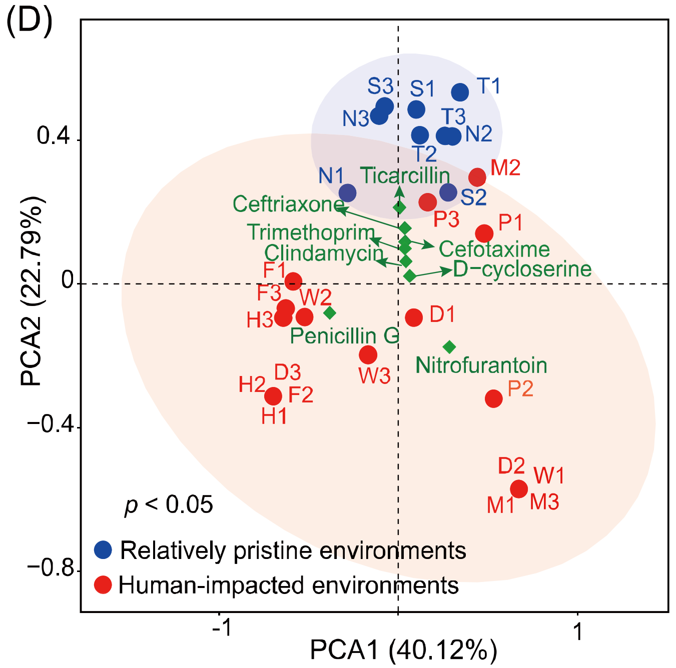
Distribution of novel polar ARGs across various environments



Abundance of novel polar ARGs in different environments with varying human impacts



Comparison in the total abundance between novel and known ARGs in the relatively pristine (B) and human-impacted (C) environments



Categorization of sampling sites according to the abundance of novel polar ARGs

- **A total of 138 novel polar ARGs (mainly β-lactam and trimethoprim-related) were found across nine environments.**
- **Sixty novel polar ARGs were identified in Tibetan Plateau soils, versus 29 in seven human-impacted environments.**
- **Novel polar ARGs outnumbered known ARGs in pristine environments ($p < 0.05$), and the reverse pattern was observed in human-impacted settings.**
- **Novel ARG profiles differed significantly ($p < 0.05$) between natural and human-impacted environments.**

Summary

- Using functional metagenomics, we discovered novel ARGs conferring resistance to clinical antibiotics in polar soils, demonstrating that polar environments constitute significant reservoirs for novel ARGs.
- Novel polar ARGs exhibit lower mobility and host pathogenic risk than known ARGs.
- Novel polar ARGs serve as biomarkers for effectively discriminating antibiotic resistomes in environments subjected to varying degrees of human impacts.

Xiuqin Xie, Weibin Cheng, Zhaohong Li, Rong He, Ke Yuan, Qinghua Zhang, Ruiqiang Yang, et al. 2025. Functional Metagenomics Reveals Novel Antibiotic Resistomes in Polar Soils. ***iMeta*** 4: e70069. https://doi.org/10.1002/imt2.70069

iMeta: To be top journals in biology and medicine

WILEY

"***iMeta***" launched in 2022 by iMeta Science Society, impact factor (IF) **33.2**, ranking top 65/22249 in world and 2/161 in the microbiology. It aims to publish innovative and high-quality papers with broad and diverse audiences. **Its scope is similar to *Cell*,** *Nature Biotechnology/Methods/Microbiology/Medicine/Food*. Its unique features include video abstract, bilingual publication, and social media with 600,000 followers. Indexed by **SCIE/ESI**, **PubMed**, **Google Scholar** etc.

"***iMetaOmics***" launched in 2024, with a **target IF>10, and its scope is similar to *Nature Communications*, *Cell Reports***, *Microbiome, ISME J, Nucleic Acids Research, Briefings in Bioinformatics,* etc.

"***iMetaMed***" launched in 2025, with a **target IF>15, similar to *Med, Cell Reports Medicine***, *eBioMedicine, eClinicalMedicine* etc.

Society: http://www.imeta.science
Publisher: https://wileyonlinelibrary.com/journal/imeta
iMeta: https://wiley.atyponrex.com/journal/IMT2
Submission: iMetaOmics: https://wiley.atyponrex.com/journal/IMO2
iMetaMed: https://wiley.atyponrex.com/journal/IMM3

iMetaScience

iMetaScience

office@imeta.science
imetaomics@imeta.science

Promotion Video

Update
2025/7/6