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Emergence of fosfomycin resistance gene *fosZ* in carbapenem and fosfomycin-resistant *Pseudomonas juntendi* isolates in ChinaYanan Wang^{1,2#}, Ziru Yang^{1#}, Xuebin Xu³¹International Joint Research Center of National Animal Immunology, College of Veterinary Medicine, Henan Agricultural University, Zhengzhou, Henan, China²Beijing Key Laboratory of Antimicrobial-Resistant Pathogen Microbiology and AI-Empowered Containment, CAS Key Laboratory of Pathogen Microbiology and Immunology, Institute of Microbiology, Chinese Academy of Sciences (CAS), Beijing, China³Division of Pathogen Testing and Analysis, Shanghai Municipal Center for Disease Control and Prevention, Shanghai, China

With the emergence and spread of plasmid-mediated fosfomycin resistance genes[1–3], fosfomycin has been moved up to the highest priority critically important antimicrobials in the 2024 *World Health Organization list of medically important antimicrobials*[4,5]. Due to the reduction in treatment options for multidrug resistant bacterial infections, fosfomycin has been reintroduced to treat infections caused by multidrug-resistant *Pseudomonas* (*P.*) *aeruginosa* and carbapenem-resistant Enterobacteriaceae. Carbapenem-resistant-*P. aeruginosa* have been listed in the high-priority pathogens in the 2024 *World Health Organization Bacterial Priority Pathogens List*. The increasing prevalence of fosfomycin resistance genes has become a serious challenge in both animals and clinical environments[1–3,6,7]. Recently, a novel plasmid-mediated fosfomycin resistance mechanism, *fosZ*, has been described in a conjugative *IncP*–2 plasmid recovered from a carbapenem-resistant *P. aeruginosa* strain in China[8]. It shows less than 70% amino acid identity with known *fosA* proteins and displays a fosfomycin minimum inhibitory concentration (MIC) of 128 mg/L. Although the *fosZ* gene has been identified in six species, including *P. aeruginosa*, *P. putida*, *P. fulva*, *P. asiatica*, *P. oleovorans*, and *P. defluvi*, there are gaps in our understanding of the host range of *fosZ*.

Here, we report the first identification of *fosZ* from a *P. juntendi* isolate of human origin in China. A *Pseudomonas* spp. strain DS24IMWYN200_XXB469 was isolated from the urine sample of a 79-year-old male in a nursing home in Qingpu District, Shanghai, on July 2, 2024, using a Columbia blood agar plate. The species was identified by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) and whole-genome sequencing. Antimicrobial susceptibility testing was performed using broth microdilution and interpreted according to the Clinical and Laboratory Standards Institute 2023 standards and

the European Committee on Antimicrobial Susceptibility Testing. DS24IMWYN200_XXB469 was resistant to ampicillin, ceftazidime, cephalixin, cefoxitin, cefazolin, cefotaxime, cefuroxime, ceftazidime/avibactam, ampicillin/sulbactam, amoxicillin/clavulanate, ertapenem, meropenem, imipenem, chloramphenicol, florfenicol, nalidixic acid, ciprofloxacin, fosfomycin, gentamicin, streptomycin, trimethoprim/sulfamethoxazole, tetracycline, and tigecycline, while non-susceptible to polymyxin, colistin, and cefepime, but susceptible to amikacin, and azithromycin (Figure 1A and Supplementary Table 1). The exact MIC values and corresponding CLSI 2023 and EUCAST interpretive criteria were determined for imipenem, meropenem, and tigecycline (Supplementary Table 1).

The genomic DNA of DS24IMWYN200_XXB469 was extracted and sequenced by the DNBSEQ-T7 platform and assembled by Unicycler v0.5.0 (<https://github.com/rrwick/Unicycler>), and then generated a total length of 6070833 bp with an N50 of 59046 bp and GC content of 62%. The species was identified by both gtdb-Tk v2.6.1 (<https://github.com/Ecogenomics/GTDBTk>) and MetaPhlAn v4.2.4 (<https://github.com/biobakery/MetaPhlAn>), indicating that DS24IMWYN200_XXB469 belongs to *P. juntendi*. DS24IMWYN200_XXB469 harbored one chromosome and one

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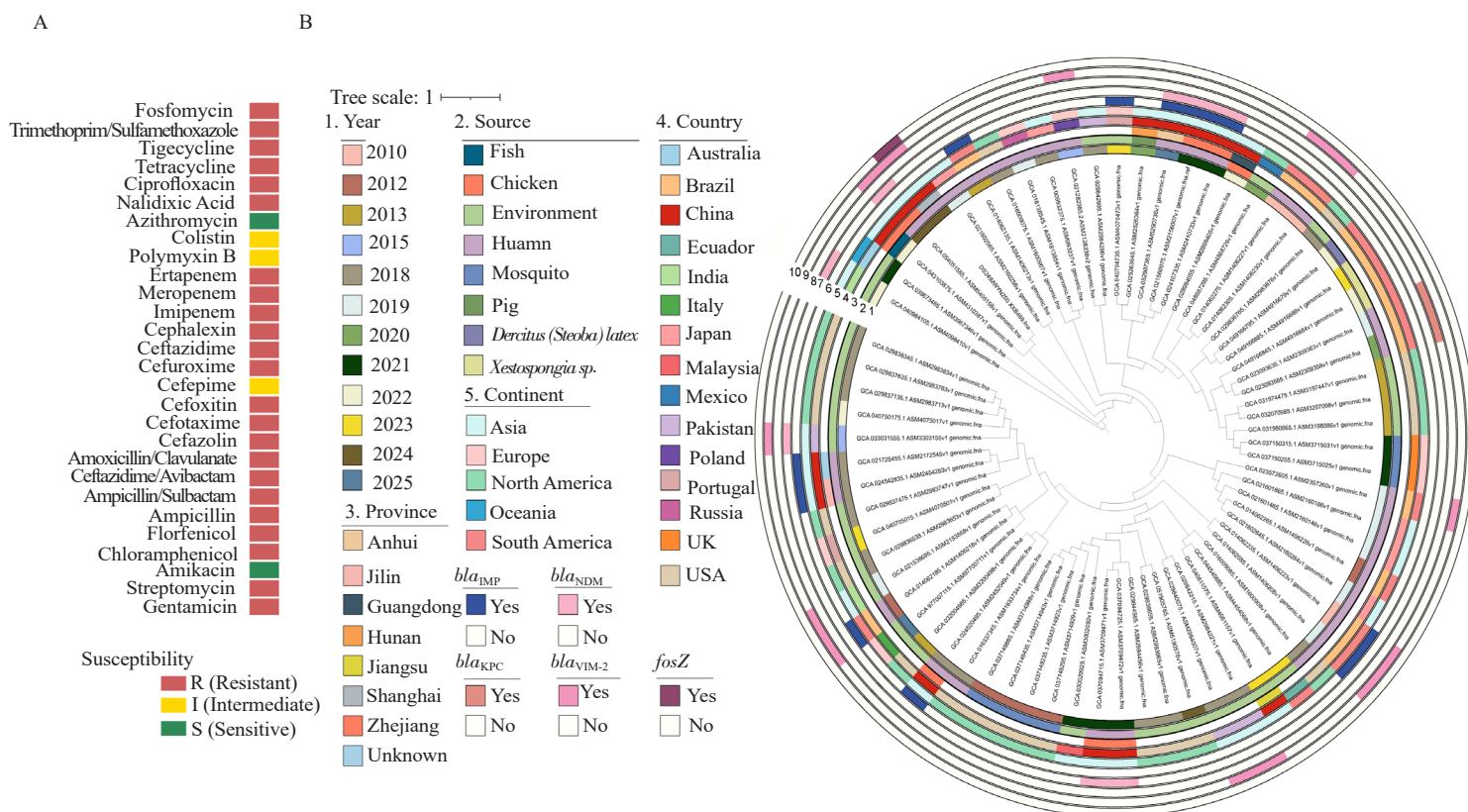
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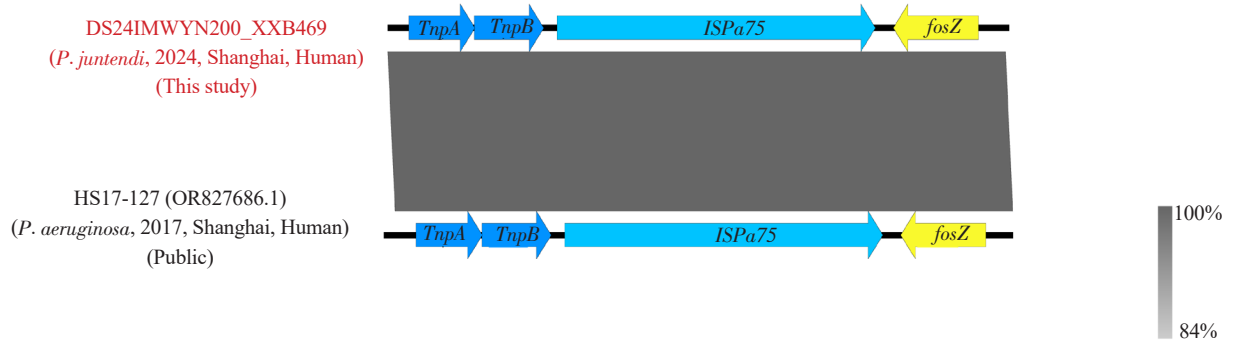
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Phylogenetic analysis indicated that the *fosZ*-positive strain was clustered together with a genome from human urine in Jinhua, Zhejiang, in 2024, and a genome from a fish intestinal isolate in Wenzhou, Zhejiang, in 2022 (Figure 1B and Supplementary Table 2). The strains from different regions of China have significant genetic differences and are distributed on different branches, clustering separately from other regions (for example, Europe and Australia). Among these 70 *P. juntendi* isolates used in this work, only one fosfomycin resistance gene, *fosZ*, was identified in DS24IMWYN200_XXB469. To the best of our knowledge, this is the first report of the identification of new discovered fosfomycin resistance gene, *fosZ*, in a carbapenem- and fosfomycin-resistant *P. juntendi* isolate. In addition, a total of 35 carbapenem resistance

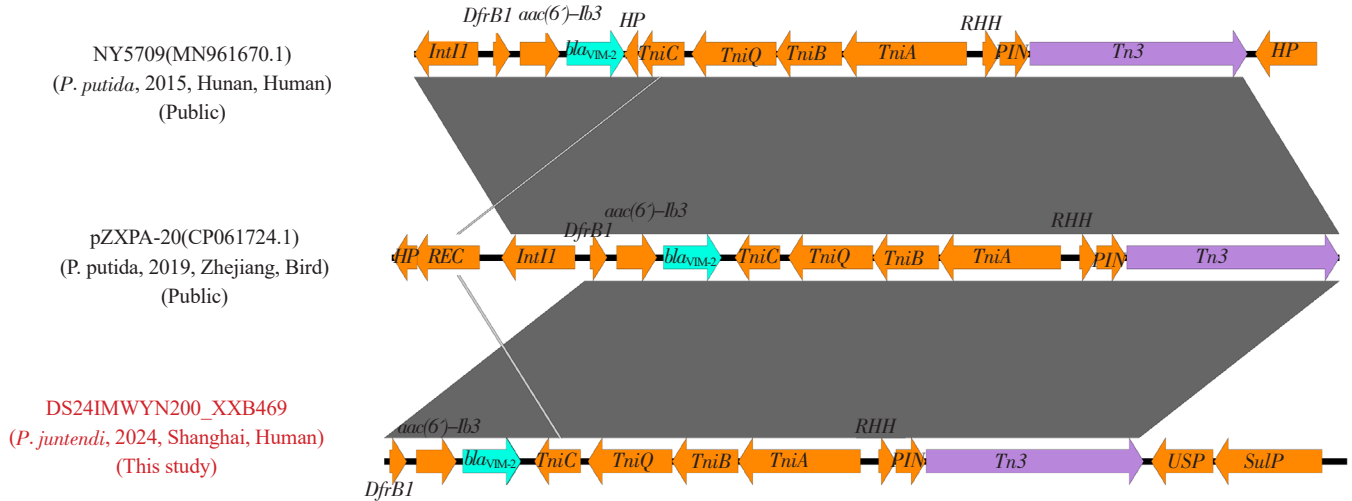
In summary, to our knowledge, this is the first report of the convergence of *fosZ*, *bla*_{VIM-2}, and *tmexC3-tmexD3-toprJ3* in *P. juntendi* isolated from a human, which expanded our knowledge of the host reservoir of *fosZ*. To better protect human, animal, and environmental health worldwide, a continuous surveillance of carbapenem, tigecycline, and fosfomycin resistance in different environments is urgently needed to fight the emergence and



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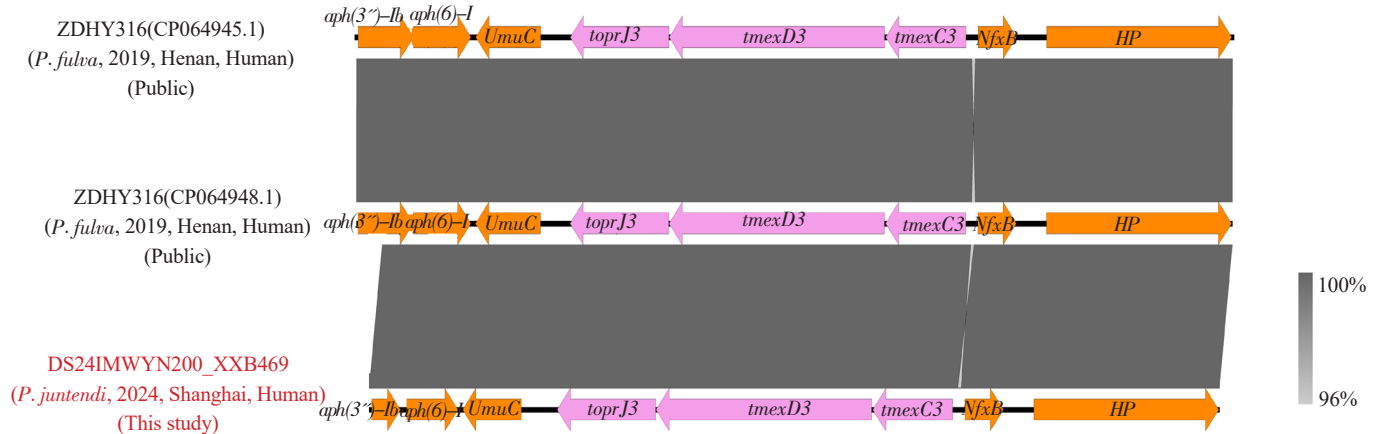


Figure 1. Comparative analysis of *fosZ*-harbor *P. juntendi* genomes and comparison of the genetic context of *fosZ*. A: Susceptibility of the *fosZ*-positive strain of *P. juntendi* to commonly used antibiotics. R: resistant; I: intermediate; S: sensitive. B: Phylogenetic analysis of *fosZ*-harbor *P. juntendi* and 69 publicly available genomes. From the inner to outer circles are year, source, province (only isolates in China), country, continent, *bla_{VIM-2}*, *bla_{NDM}*, *bla_{KPC}*, *bla_{VIM-2}*, and *fosZ*. The *P. juntendi* PP_2463 (GCA_021560075.1) was used as the reference genome. C: Genetic environment of the *fosZ* gene in *P. juntendi* and *P. aeruginosa* HS17-127 (OR827686.1). Different genes are shown in different colors. Arrows indicate: insertion sequence (light blue), transposase genes *tnpA/tnpB* (dark blue), resistance gene *fosZ* (yellow). Regions of >84% identity are indicated by dark grey shading. D: Genetic environment of the *bla_{VIM-2}* gene in *P. juntendi* genome. *P. putida* NY5709 (MN961670.1) and *P. putida* ZXA-20 (CP061724.1). Different genes are shown in different colors. Arrows indicate: *bla_{VIM-2}* (green), *Tn3* transposon (purple), and other genes (orange). E: Genetic context of *tmexC3-tmexD3-toprJ3* in the *fosZ*-positive *P. juntendi* genome. *P. fulva* ZDHY316 (CP064945.1), and *P. fulva* ZDHY414 (CP064948.1). Different genes are shown in different colors. Arrows indicate: *tmexC3-tmexD3-toprJ3* (pink), and other genes (orange).

dissemination of these resistant pathogens. Overall, our study highlights the emergence of *bla*_{VIM-2}, *fosZ*, and *tmexC3–tmexD3–toprJ3*, which are predicted to be associated with resistance to the last-line antibiotics, carbapenem, fosfomycin, and tigecycline, and may pose a huge challenge to clinical therapy in the future.

Ethical approval

The participant provided written informed consent and allowed analysis of strains and data for medical case report.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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Data availability statement

The data supporting the findings of this study are available from the corresponding author upon request.

Authors' contributions

Wang YN and Xu XB conceived and designed the project. Xu XB collected samples, strains, and epidemiological data. Yang ZR performed bioinformatics analysis, created the figures and tables.

Wang YN, Yang ZR, and Xu XB drafted and revised the manuscript.

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Supplementary Table 1. MIC values and resistance phenotypic interpretation according to CLSI M100-S33 (2023) and EUCAST.

Antibiotic	MIC (µg/mL)	Susceptibility Interpretation	Note
Fosfomycin	200 µg DISC	R	Kirby-Bauer (K-B) disk diffusion met
Gentamicin	8	R	
Streptomycin	32	R	
Amikacin	2	S	
Chloramphenicol	64	R	
Florfenicol	32	R	
Ampicillin	64	R	
Ampicillin/Sulbactam	64	R	
Ceftazidime/Avibactam	8	R	
Amoxicillin/Cavulanic Acid	64	R	
Cefazolin	32	R	
Cefotaxime	16	R	
Cefoxitin	64	R	
Cefepime	8	I	
Cefuroxime	32	R	
Ceftazidime	32	R	
Ceftiofur	16	R	
Imipenem	8	R	
Meropenem	4	R	
Ertapenem	8	R	
Polymyxin B	1	I	
Colistin	1	I	
Nalidixic Acid	64	R	
Ciprofloxacin	16	R	
Azithromycin	4	S	
Tetracycline	32	R	
Tigecycline	8	R	
Sulfamethoxazole/Trimethoprim	8	R	

Supplementary Table 3. Virulence factors identified in *P. juntendi* strains.

Sample ID	alga8	algaA	algaB	algaC	algaD	algaE	algaF	algaG	algaH	algaI	algaJ	algaK	algaL	algaM	algaN	algaO	algaP	algaQ	algaR	algaS	algaT	algaU	algaV	algaW	algaX	algaY	algaZ	algaAA	algaAB	algaAC	algaAD	algaAE	algaAF	algaAG	algaAH	algaAI	algaAJ	algaAK	algaAL	algaAM	algaAN	algaAO	algaAP	algaAQ	algaAR	algaAS	algaAT	algaAU	algaAV	algaAW	algaAX	algaAY	algaAZ	algaBA	algaBB	algaBC	algaBD	algaBE	algaBF	algaBG	algaBH	algaBI	algaBJ	algaBK	algaBL	algaBM	algaBN	algaBO	algaBP	algaBQ	algaBR	algaBS	algaBT	algaBU	algaBV	algaBW	algaBX	algaBY	algaBZ	algaCA	algaCB	algaCC	algaCD	algaCE	algaCF	algaCG	algaCH	algaCI	algaCJ	algaCK	algaCL	algaCM	algaCN	algaCO	algaCP	algaCQ	algaCR	algaCS	algaCT	algaCU	algaCV	algaCW	algaCX	algaCY	algaCZ	algaDA	algaDB	algaDC	algaDD	algaDE	algaDF	algaDG	algaDH	algaDI	algaDJ	algaDK	algaDL	algaDM	algaDN	algaDO	algaDP	algaDQ	algaDR	algaDS	algaDT	algaDU	algaDV	algaDW	algaDX	algaDY	algaDZ	algaEA	algaEB	algaEC	algaED	algaEE	algaEF	algaEG	algaEH	algaEI	algaEJ	algaEK	algaEL	algaEM	algaEN	algaEO	algaEP	algaEQ	algaER	algaES	algaET	algaEU	algaEV	algaEW	algaEX	algaEY	algaEZ	algaFA	algaFB	algaFC	algaFD	algaFE	algaFF	algaFG	algaFH	algaFI	algaFJ	algaFK	algaFL	algaFM	algaFN	algaFO	algaFP	algaFQ	algaFR	algaFS	algaFT	algaFU	algaFV	algaFW	algaFX	algaFY	algaFZ	algaGA	algaGB	algaGC	algaGD	algaGE	algaGF	algaGG	algaGH	algaGI	algaGJ	algaGK	algaGL	algaGM	algaGN	algaGO	algaGP	algaGQ	algaGR	algaGS	algaGT	algaGU	algaGV	algaGW	algaGX	algaGY	algaGZ	algaHA	algaHB	algaHC	algaHD	algaHE	algaHF	algaHG	algaHH	algaHI	algaHJ	algaHK	algaHL	algaHM	algaHN	algaHO	algaHP	algaHQ	algaHR	algaHS	algaHT	algaHU	algaHV	algaHW	algaHX	algaHY	algaHZ	algaIA	algaIB	algaIC	algaID	algaIE	algaIF	algaIG	algaIH	algaII	algaIJ	algaIK	algaIL	algaIM	algaIN	algaIO	algaIP	algaIQ	algaIR	algaIS	algaIT	algaIU	algaIV	algaIW	algaIX	algaIY	algaIZ	algaJA	algaJB	algaJC	algaJD	algaJE	algaJF	algaJG	algaJH	algaJI	algaJJ	algaJK	algaJL	algaJM	algaJN	algaJO	algaJP	algaJQ	algaJR	algaJS	algaJT	algaJU	algaJV	algaJW	algaJX	algaJY	algaJZ	algaKA	algaKB	algaKC	algaKD	algaKE	algaKF	algaKG	algaKH	algaKI	algaKJ	algaKL	algaKM	algaKN	algaKO	algaKP	algaKQ	algaKR	algaKS	algaKT	algaKU	algaKV	algaKW	algaKX	algaKY	algaKZ	algaLA	algaLB	algaLC	algaLD	algaLE	algaLF	algaLG	algaLH	algaLI	algaLJ	algaLK	algaLL	algaLM	algaLN	algaLO	algaLP	algaLQ	algaLR	algaLS	algaLT	algaLU	algaLV	algaLW	algaLX	algaLY	algaLZ	algaMA	algaMB	algaMC	algaMD	algaME	algaMF	algaMG	algaMH	algaMI	algaMJ	algaMK	algaML	algaMM	algaMN	algaMO	algaMP	algaMQ	algaMR	algaMS	algaMT	algaMU	algaMV	algaMW	algaMX	algaMY	algaMZ	algaNA	algaNB	algaNC	algaND	algaNE	algaNF	algaNG	algaNH	algaNI	algaNJ	algaNK	algaNL	algaNM	algaNN	algaNO	algaNP	algaNQ	algaNR	algaNS	algaNT	algaNU	algaNV	algaNW	algaNX	algaNY	algaNZ	algaOA	algaOB	algaOC	algaOD	algaOE	algaOF	algaOG	algaOH	algaOI	algaOJ	algaOK	algaOL	algaOM	algaON	algaOO	algaOP	algaOQ	algaOR	algaOS	algaOT	algaOU	algaOV	algaOW	algaOX	algaOY	algaOZ	algaPA	algaPB	algaPC	algaPD	algaPE	algaPF	algaPG	algaPH	algaPI	algaPJ	algaPK	algaPL	algaPM	algaPN	algaPO	algaPP	algaPQ	algaPR	algaPS	algaPT	algaPU	algaPV	algaPW	algaPX	algaPY	algaPZ	algaQA	algaQB	algaQC	algaQD	algaQE	algaQF	algaQG	algaQH	algaQI	algaQJ	algaQK	algaQL	algaQM	algaQN	algaQO	algaQP	algaQQ	algaQR	algaQS	algaQT	algaQU	algaQV	algaQW	algaQX	algaQY	algaQZ	algaRA	algaRB	algaRC	algaRD	algaRE	algaRF	algaRG	algaRH	algaRI	algaRJ	algaRK	algaRL	algaRM	algaRN	algaRO	algaRP	algaRQ	algaRR	algaRS	algaRT	algaRU	algaRV	algaRW	algaRX	algaRY	algaRZ	algaSA	algaSB	algaSC	algaSD	algaSE	algaSF	algaSG	algaSH	algaSI	algaSJ	algaSK	algaSL	algaSM	algaSN	algaSO	algaSP	algaSQ	algaSR	algaSS	algaST	algaSU	algaSV	algaSW	algaSX	algaSY	algaSZ	algaTA	algaTB	algaTC	algaTD	algaTE	algaTF	algaTG	algaTH	algaTI	algaTJ	algaTK	algaTL	algaTM	algaTN	algaTO	algaTP	algaTQ	algaTR	algaTS	algaTT	algaTU	algaTV	algaTW	algaTX	algaTY	algaTZ	algaUA	algaUB	algaUC	algaUD	algaUE	algaUF	algaUG	algaUH	algaUI	algaUJ	algaUK	algaUL	algaUM	algaUN	algaUO	algaUP	algaUQ	algaUR	algaUS	algaUT	algaUU	algaUV	algaUW	algaUX	algaUY	algaUZ	algaVA	algaVB	algaVC	algaVD	algaVE	algaVF	algaVG	algaVH	algaVI	algaVJ	algaVK	algaVL	algaVM	algaVN	algaVO	algaVP	algaVQ	algaVR	algaVS	algaVT	algaVU	algaVV	algaVW	algaVX	algaVY	algaVZ	algaWA	algaWB	algaWC	algaWD	algaWE	algaWF	algaWG	algaWH	algaWI	algaWJ	algaWK	algaWL	algaWM	algaWN	algaWO	algaWP	algaWQ	algaWR	algaWS	algaWT	algaWU	algaWV	algaWW	algaWX	algaWY	algaWZ	algaXA	algaXB	algaXC	algaXD	algaXE	algaXF	algaXG	algaXH	algaXI	algaXJ	algaXK	algaXL	algaXM	algaXN	algaXO	algaXP	algaXQ	algaXR	algaXS	algaXT	algaXU	algaXV	algaXW	algaXX	algaXY	algaXZ	algaYA	algaYB	algaYC	algaYD	algaYE	algaYF	algaYG	algaYH	algaYI	algaYJ	algaYK	algaYL	algaYM	algaYN	algaYO	algaYP	algaYQ	algaYR	algaYS	algaYT	algaYU	algaYV	algaYW	algaYX	algaYY	algaYZ	algaZA	algaZB	algaZC	algaZD	algaZE	algaZF	algaZG	algaZH	algaZI	algaZJ	algaZK	algaZL	algaZM	algaZN	algaZO	algaZP	algaZQ	algaZR	algaZS	algaZT	algaZU	algaZV	algaZW	algaZX	algaZY	algaZZ	NUM FOUND
DIS2-DAID-WYND20_XX	96.3	97.16	-	94.97	-	82.09	100	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9	-	95.49	97.9																																																																																																																																																																								