

Table S1 Number of assembled viral contigs in each sample (length > 5 Kb).

WWTPs (wastewater)	Influent	Primary sedimentation tank effluent	Anoxic pond 1	Anoxic pond 2	Aerobic pond	Secondary sedimentation tank effluent	Tertiary treatment effluent	Disinfection effluent
SZNS	933	/	3461	2431	4036	3976	4859	4452
HBZZ	1494	/	/	1834	3037	1134	/	946
SGP	234	694	/	/	/	5772	819	326

WWTPs (sludge)	Anoxic pond 1	Anoxic pond 2	Aerobic pond	Secondary sedimentation tank effluent	Tertiary treatment effluent
SZNS	529	703	777	496	3073
HBZZ	/	403	490	542	/
SGP	/	/	/	6116	/

Table S2 Number and corresponding virus and pathogen species name.

Tax_ID	Tax_name	Tax_ID	Tax_name
path001	Bacteroides uniformis	path193	Haemophilus haemolyticus
path002	Bacteroides ovatus	path194	Mannheimia haemolytica
path003	Bacteroides fragilis	path195	Campylobacter coli
path004	Achromobacter xylosoxidans	path196	Citrobacter koseri
path005	Ralstonia solanacearum	path197	Streptococcus porcinus
path006	Laribacter hongkongensis	path198	Cronobacter malonaticus
path007	Escherichia coli	path199	Corynebacterium aurimucosum
path008	Pseudomonas putida	path200	Leptotrichia buccalis
path009	Pseudomonas aeruginosa	path201	Leptospira weilii
path010	Bacteroides caccae	path202	Streptococcus sanguinis
path011	Delftia acidovorans	path203	Providencia alcalifaciens
path012	Parabacteroides distasonis	path204	Bartonella quintana
path013	Pseudomonas mendocina	path205	Bartonella vinsonii
path014	Klebsiella pneumoniae	path206	Simkania negevensis
path015	Bordetella hinzii	path207	Ehrlichia chaffeensis
path016	Alistipes onderdonkii	path208	Leptospira mayottensis
path017	Cupriavidus metallidurans	path209	Tatumella ptyseos
path018	Pandoraea pnomenusa	path210	Aggregatibacter segnis
path019	Aeromonas hydrophila	path211	Rickettsia prowazekii
path020	Elizabethkingia anophelis	path212	Actinotignum schaalii
path021	Pseudomonas alcaligenes	path213	Lawsonia intracellularis
path022	Chryseobacterium gleum	path214	Erwinia pyrifoliae
path023	Burkholderia multivorans	path215	Bartonella bacilliformis
path024	Chromobacterium violaceum	path216	Porphyromonas asaccharolytica
path025	Odoribacter splanchnicus	path217	Cryptobacterium curtum
path026	Pseudomonas syringae	path218	Vibrio splendidus
path027	Lachnospira eligens	path219	Waddlia chondrophila
path028	Paraburkholderia xenovorans	path220	Actinomyces oris
path029	Burkholderia glumae	path221	Campylobacter concisus
path030	Eggerthella lenta	path222	Leptospira borgpetersenii
path031	Bordetella holmesii	path223	Lactococcus garvieae
path032	Acinetobacter haemolyticus	path224	Erwinia amylovora
path033	Leishmania major	path225	Shigella dysenteriae
path034	Myroides odoratimimus	path226	Leptospira kmetyi
path035	Capnocytophaga cynodegmi	path227	Finegoldia magna
path036	Prevotella intermedia	path228	Corynebacterium diphtheriae
path037	Pseudomonas oleovorans	path229	Listeria grayi
path038	Plasmodium falciparum	path230	Gemella morbillorum

path039	<i>Thermothielavioides terrestris</i>	path231	<i>Streptococcus mitis</i>
path040	<i>Roseomonas gilardii</i>	path232	<i>Xanthomonas perforans</i>
path041	<i>Prevotella dentalis</i>	path233	<i>Corynebacterium urealyticum</i>
path042	<i>Clostridioides difficile</i>	path234	<i>Rickettsia canadensis</i>
path043	<i>Capnocytophaga sputigena</i>	path235	<i>Mycobacterium leprae</i>
path044	<i>Azotobacter vinelandii</i>	path236	<i>Helicobacter pullorum</i>
path045	<i>Acinetobacter bereziniae</i>	path237	<i>Haemophilus parahaemolyticus</i>
path046	<i>Acinetobacter pittii</i>	path238	<i>Staphylococcus hominis</i>
path047	<i>Acinetobacter lwoffii</i>	path239	<i>Staphylococcus haemolyticus</i>
path048	<i>Prevotella denticola</i>	path240	<i>Bartonella clarridgeiae</i>
path049	<i>Legionella pneumophila</i>	path241	<i>Campylobacter fetus</i>
path050	<i>Leclercia adecarboxylata</i>	path242	<i>Listeria welshimeri</i>
path051	<i>Xanthomonas vesicatoria</i>	path243	<i>Tropheryma whippelii</i>
path052	<i>Xanthomonas citri</i>	path244	<i>Streptococcus oralis</i>
path053	<i>Bordetella parapertussis</i>	path245	<i>Streptococcus dysgalactiae</i>
path054	<i>Streptococcus salivarius</i>	path246	<i>Actinobacillus suis</i>
path055	<i>Tannerella forsythia</i>	path247	<i>Listeria innocua</i>
path056	<i>Burkholderia lata</i>	path248	<i>Campylobacter ureolyticus</i>
path057	<i>Acinetobacter junii</i>	path249	<i>Bartonella elizabethae</i>
path058	<i>Aeromonas caviae</i>	path250	<i>Yersinia kristensenii</i>
path059	<i>Clostridium botulinum</i>	path251	<i>Ehrlichia muris</i>
path060	<i>Burkholderia vietnamiensis</i>	path252	<i>Francisella noatunensis</i>
path061	<i>Acinetobacter radioresistens</i>	path253	<i>Bartonella tribocorum</i>
path062	<i>Mycolicibacterium phlei</i>	path254	<i>Listeria ivanovii</i>
path063	<i>Burkholderia oklahomensis</i>	path255	<i>Staphylococcus arlettae</i>
path064	<i>Acinetobacter calcoaceticus</i>	path256	<i>Gemella haemolysans</i>
path065	<i>Mycolicibacterium chubuense</i>	path257	<i>Leptospira santarosai</i>
path066	<i>Acinetobacter ursingii</i>	path258	<i>Aerococcus urinae</i>
path067	<i>Nocardia cyriacigeorgica</i>	path259	<i>Bartonella henselae</i>
path068	<i>Granulibacter bethesdensis</i>	path260	<i>Candidatus Liberibacter asiaticus</i>
path069	<i>Nocardia brasiliensis</i>	path261	<i>Staphylococcus capitis</i>
path070	<i>Burkholderia pyrrocinia</i>	path262	<i>Streptococcus urinalis</i>
path071	<i>Fusobacterium nucleatum</i>	path263	<i>Bacillus cytotoxicus</i>
path072	<i>Botrytis cinerea</i>	path264	<i>Rickettsia rhipicephali</i>
path073	<i>Nocardia farcinica</i>	path265	<i>Chlamydia pneumoniae</i>
path074	<i>Gordonia bronchialis</i>	path266	<i>Streptococcus sobrinus</i>
path075	<i>Acinetobacter gyllenbergii</i>	path267	<i>Rickettsia typhi</i>
path076	<i>Rothia mucilaginosa</i>	path268	<i>Anaerococcus prevotii</i>
path077	<i>Pseudomonas viridiflava</i>	path269	<i>Streptococcus intermedius</i>
path078	<i>Fusobacterium ulcerans</i>	path270	<i>Bartonella australis</i>
path079	<i>Eikenella corrodens</i>	path271	<i>Campylobacter showae</i>

path080	Burkholderia ambifaria	path272	Helicobacter hepaticus
path081	Neisseria weaveri	path273	Rickettsia felis
path082	Legionella longbeachae	path274	Rickettsia australis
path083	Candida dubliniensis	path275	Mycoplasma hominis
path084	Schaalia odontolytica	path276	Rickettsia rickettsii
path085	Malassezia restricta	path277	Rickettsia akari
path086	Acinetobacter soli	path278	Borrelia miyamotoi
path087	Mycolicibacterium smegmatis	path279	Campylobacter curvus
path088	Paeniclostridium sordellii	path280	Staphylococcus pettenkoferi
path089	Enterococcus casseliflavus	path281	Chlamydia caviae
path090	Haemophilus parainfluenzae	path282	Chlamydia abortus
path091	Klebsiella aerogenes	path283	Borrelia parkeri
path092	Mycolicibacterium fortuitum	path284	Chlamydia felis
path093	Rhodococcus hoagii	path285	Helicobacter suis
path094	Vibrio furnissii	vir001	Kyanoviridae
path095	Clostridium perfringens	vir002	Obolenskivirus
path096	Megasphaera elsdenii	vir003	Peduoviridae
path097	Citrobacter freundii	vir004	Hendrixvirinae
path098	Eremothecium gossypii	vir005	Casjensviridae
path099	Gardnerella vaginalis	vir006	Autographiviridae
path100	Cedecea neteri	vir007	Gracegardnervirinae
path101	Plesiomonas shigelloides	vir008	Weiservirinae
path102	Nocardiopsis dassonvillei	vir009	Sepvirinae
path103	Neisseria subflava	vir010	Mesyanzhinovviridae
path104	Klebsiella oxytoca	vir011	Steigviridae
path105	Bacillus thuringiensis	vir012	Herelleviridae
path106	Mycobacterium kansasii	vir013	Schitoviridae
path107	Nocardia terpenica	vir014	Guernseyvirinae
path108	Nocardia asteroides	vir015	Dolichocephalovirinae
path109	Enterobacter cloacae	vir016	Boydwoodruffvirinae
path110	Corynebacterium xerosis	vir017	Koravirus
path111	Arcanobacterium haemolyticum	vir018	Suoliviridae
path112	Pichia kudriavzevii	vir019	Tybeckvirinae
path113	Yersinia enterocolitica	vir020	Vilmaviridae
path114	Corynebacterium jeikeium	vir021	Triavirus
path115	Mycobacterium colombiense	vir022	Queuovirinae
path116	Pantoea agglomerans	vir023	Deejayvirinae
path117	Pseudomonas luteola	vir024	Straboviridae
path118	Pluralibacter gergoviae	vir025	Demereciviridae
path119	Cardiobacterium hominis	vir026	Casadabanvirus
path120	Kluyveromyces marxianus	vir027	Adenoviridae

path121	<i>Rothia dentocariosa</i>	vir028	Wizardvirus
path122	<i>Vibrio fluvialis</i>	vir029	Vequintavirinae
path123	<i>Enterobacter hormaechei</i>	vir030	Zierdtviridae
path124	<i>Saccharomyces cerevisiae</i>	vir031	Arquatrovirinae
path125	<i>Burkholderia seminalis</i>	vir032	Ounavirinae
path126	<i>Vibrio cholerae</i>	vir033	Ackermannviridae
path127	<i>Klebsiella variicola</i>	vir034	Kostyavirus
path128	<i>Nocardia otitidiscaviarum</i>	vir035	Poxviridae
path129	<i>Morganella morganii</i>	vir036	Zobellviridae
path130	<i>Capnocytophaga gingivalis</i>	vir037	Pakpunavirus
path131	<i>Pasteurella multocida</i>	vir038	Eucampyvirinae
path132	<i>Cutibacterium acnes</i>	vir039	Phycodnaviridae
path133	<i>Enterococcus hirae</i>	vir040	Fernvirus
path134	<i>Grimontia hollisae</i>	vir041	Nclasvirinae
path135	<i>Enterobacter roggenkampii</i>	vir042	Bclasvirinae
path136	<i>Clostridium novyi</i>	vir043	Pclasvirinae
path137	<i>Nocardiopsis alba</i>	vir044	Skunavirus
path138	<i>Vibrio mimicus</i>	vir045	Ceeclamvirinae
path139	<i>Corynebacterium resistens</i>	vir046	Stephanstirmvirinae
path140	<i>Capnocytophaga ochracea</i>	vir047	Bronfenbrennervirinae
path141	<i>Cutibacterium avidum</i>	vir048	Marthavirus
path142	<i>Nocardia arthritidis</i>	vir049	Parvoviridae
path143	<i>Moraxella catarrhalis</i>	vir050	Gladiatorvirus
path144	<i>Providencia rettgeri</i>	vir051	Fromanvirus
path145	<i>Orientia tsutsugamushi</i>	vir052	Asfarviridae
path146	<i>Mycobacterium simiae</i>	vir053	Iridoviridae
path147	<i>Streptococcus suis</i>	vir054	Arenaviridae
path148	<i>Mycobacterium xenopi</i>	vir055	Lipothrixviridae
path149	<i>Leptospira biflexa</i>	vir056	Andromedavirus
path150	<i>Vibrio parahaemolyticus</i>	vir057	Baculoviridae
path151	<i>Hafnia alvei</i>	vir058	Herpesviridae
path152	<i>Mycobacterium haemophilum</i>	vir059	Drexlviridae
path153	<i>Klebsiella michiganensis</i>	vir060	Benedictvirus
path154	<i>Burkholderia dolosa</i>	vir061	Pbunavirus
path155	<i>Streptococcus gallolyticus</i>	vir062	Anelloviridae
path156	<i>Staphylococcus aureus</i>	vir063	Alphaflexiviridae
path157	<i>Acidaminococcus intestini</i>	vir064	Inoviridae
path158	<i>Francisella philomiragia</i>	vir065	Retroviridae
path159	<i>Cronobacter sakazakii</i>	vir066	Rudiviridae
path160	<i>Enterobacter cancerogenus</i>	vir067	Nimaviridae
path161	<i>Clostridium tetani</i>	vir068	Fimoviridae

path162	Aggregatibacter actinomycetemcomitans	vir069	Gclavirinae
path163	Streptococcus lutetiensis	vir070	Azeredovirinae
path164	Aggregatibacter aphrophilus	vir071	Vividuovirus
path165	Escherichia fergusonii	vir072	Geminiviridae
path166	Dickeya chrysanthemi	vir073	Backyardiganvirus
path167	Edwardsiella ictaluri	vir074	Chaseviridae
path168	Neisseria flavescens	vir075	Pleolipoviridae
path169	Campylobacter lari	vir076	Fiersviridae
path170	Parachlamydia acanthamoebae	vir077	Aliusviridae
path171	Aeromonas dhakensis	vir078	Metaviridae
path172	Helicobacter pylori	vir079	Tectiviridae
path173	Mycobacterium intracellulare	vir080	Orlajensenviridae
path174	Cutibacterium granulosum	vir081	Intestiviridae
path175	Fusobacterium necrophorum	vir082	Efquatrovirus
path176	Mycobacterium ulcerans	vir083	Nudiviridae
path177	Providencia stuartii	vir084	Salasmaviridae
path178	Campylobacter jejuni	vir085	Hafunaviridae
path179	Streptobacillus moniliformis	vir086	Spinareoviridae
path180	Acidaminococcus fermentans	vir087	Montyvirus
path181	Hafnia paralvei	vir088	Pahexavirus
path182	Erysipelothrix rhusiopathiae	vir089	Alloherpesviridae
path183	Yokenella regensburgei	vir090	Atkinsviridae
path184	Parvimonas micra	vir091	Closteroviridae
path185	Actinomyces viscosus	vir092	Microviridae
path186	Citrobacter rodentium	vir093	Mymonaviridae
path187	Escherichia albertii	vir094	Papillomaviridae
path188	Campylobacter sputorum	vir095	Phenuiviridae
path189	Leptotrichia trevisanii	vir096	Polyomaviridae
path190	Streptococcus cristatus	vir097	Smacoviridae
path191	Providencia rustigianii	vir098	Tobaniviridae
path192	Rickettsia bellii	vir099	Turbidovirus

Text S1 Pre-treatment and DNA extraction.

Pre-treatment of water samples: The wastewater samples were first pre-filtered (using a 0.22 μm membrane) to remove particulate matter such as unicellular algae, and a filtration pump was used for larger samples. Place the membranes into 50 mL centrifuge tubes (up to 3 membranes per tube), store in the dark at $-80\text{ }^{\circ}\text{C}$, and transport on dry ice. **Pre-treatment of sludge samples:** Take 10~40 mL sludge-wastewater mixture in 20~50 mL sterile centrifuge tube, ensure 5~10 g sediment per tube (if the liquid sludge sample has less sediment, then increase the sampling volume appropriately) centrifuged at 5000 g for 15 min, remove the supernatant, to ensure the activity of the cells, rinsed with PBS buffer, and then repeated three times to put the samples in $-80\text{ }^{\circ}\text{C}$ storage. Genomic DNA was extracted by Guangdong Magigene Biotechnology Co., Ltd. (Guangzhou, China) using the ALFA-SEQ Advanced Soil DNA Kit (mCHIP BioTech Co., LTD, China) according to the manufacturer's instructions. DNA integrity and purity were monitored on 1% agarose gels. DNA concentration and purity were measured using the Qubit 3.0 (Thermo Fisher Scientific, Waltham, USA) and the Nanodrop One (Thermo Fisher Scientific, Waltham, USA) simultaneously.

Text S2 Virus-pathogen network information.

To further investigate the coexistence relationship between pathogens and viruses, a co-occurrence network analysis was conducted on pathogens and viruses detected in three WWTPs (Fig. S7, a-c). The pathogens information was collected from our previous work^[1]. The results revealed that each treatment system displayed significantly distinct structural and complexity characteristics. The co-occurrence network of AAO WWTP comprised 283 nodes and 4612 edges, with a relatively low modularity (0.398). This suggested a high level of connectivity and weak modular separation, indicating potential complex interactions between viruses and pathogens (Fig. S7a). Notably, vir025 (Demerecividae) and vir038 (Eucampyvirinae) were associated with multiple pathogens, implying that they may mediate cross-pathogen gene exchange (Number and corresponding virus and pathogen species name were listed in Table S2). The AAO-MBR WWTP network comprised 218 nodes and 615 edges, with a modularity of 0.818. This network exhibited a more clearly defined interaction structure than that of AAO WWTP, with fewer interactions between viruses and pathogens. Instead, interactions were predominantly internal to pathogens or viruses (Fig. S7b). 290 nodes and 952 edges were established in the MBR WWTP network, with a modularity coefficient of 0.888. The relationships between viruses and pathogens were characterized by high modularity and localized clustering, with limited interactions between modules, similar to those observed in AAO-MBR WWTP (Fig. S7c). The co-occurrence network in the bioreactor sections of AAO WWTP comprised 243 nodes and 1553 edges, with a modularity of 0.571. Notably, vir040 (Fervirus) was associated with multiple pathogens, and vir025 (Demerecividae) was only associated with path242 (*Listeria welshimeri*, a low-infection-risk pathogen), indicating distinct co-occurrence patterns in bioreactor sections that differ from the full-scale view. However, no significant patterns were observed in other WWTPs (Fig. S7, d-e).

Reference

- [1] Zhao YM, Fan L, Gao SH, Huang F, Lei ZL, et al. 2024. Strain-level multidrug-resistant pathogenic bacteria in urban wastewater treatment plants: Transmission, source tracking and evolution. *Water Research* 267 <https://doi.org/10.1016/j.watres.2024.122538>

Text S3 Information of ARGs on viral contigs.

In the comprehensive analysis of viral contigs across all samples, only three contigs (CHNSZ-IFW (k141_2035350), CHNHB-IFW (k141_742160), and SGP-AS (k141_1635139)) were identified as harboring ARGs, including *erm(B)*, *bla*, and *aac(6')-Ie/aph(2'')-Ia*, which are belonging to macrolide, aminoglycoside, and beta-lactam, respectively. The viral contig carrying the *bla* gene has a broad potential host range, including *Citrobacter*, *Escherichia*, *Klebsiella*, and *Salmonella*, suggesting its involvement in complex virus-host interactions within WWTPs and its potential as a vector for ARG dissemination. This result highlighted the dual role of viruses in the WWTP environment: viruses may facilitate the horizontal transfer of ARGs by infecting specific hosts, and they may also directly disseminate ARGs as carriers, thereby expanding the ecological range of resistance gene^[1].

Reference

[1] Wang Q, Wang M, Yang QX, Feng LR, Zhang H, et al. 2025. The role of bacteriophages in facilitating the horizontal transfer of antibiotic resistance genes in municipal wastewater treatment plants. *Water Research* 268 <https://doi.org/10.1016/j.watres.2024.122776>

Fig. S1 Sampling sites arrangement.

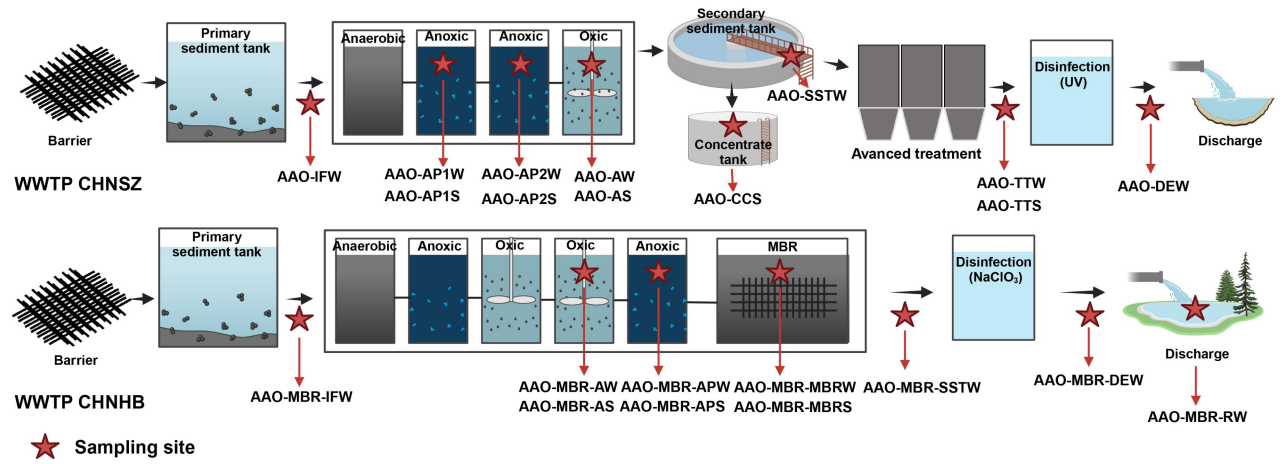


Fig. S2 Density of low-quality to high-quality viral contigs. (a) AAO WWTP; (b) AAO-MBR WWTP; (c) MBR WWTP.

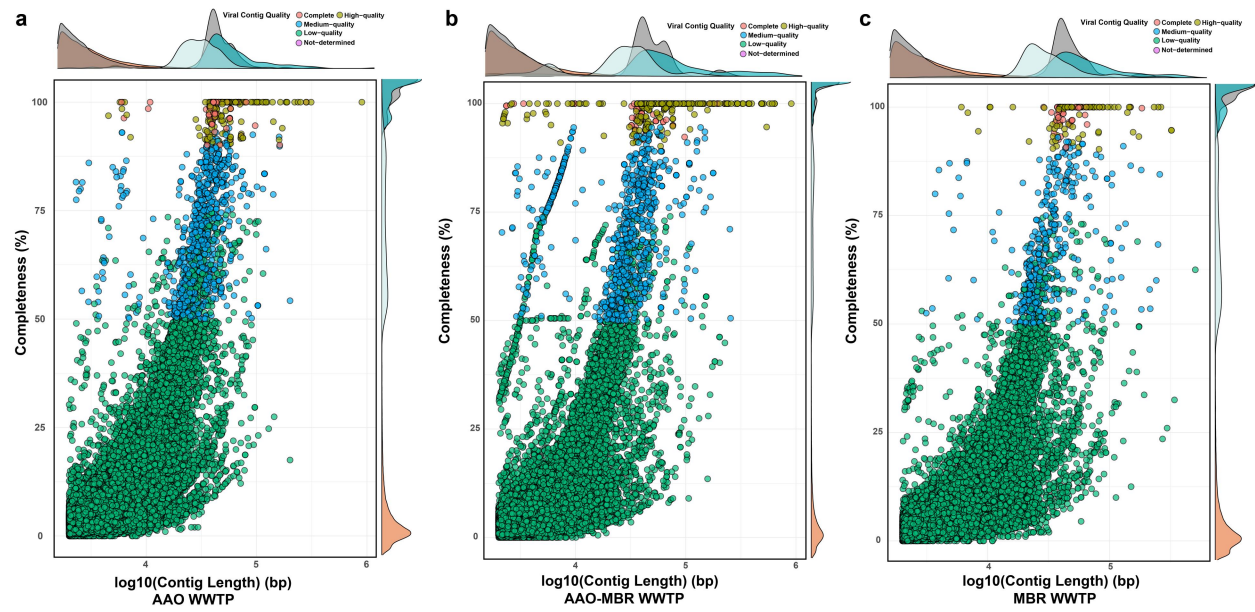


Fig. S3 Linear regression analyses of representative pathogens and viruses with the highest prevalence and abundance. (a)–(j) *E. coli*, $p > 0.05$. (k)–(o) *P. aeruginosa* and *A. caviae*, $p < 0.05$.

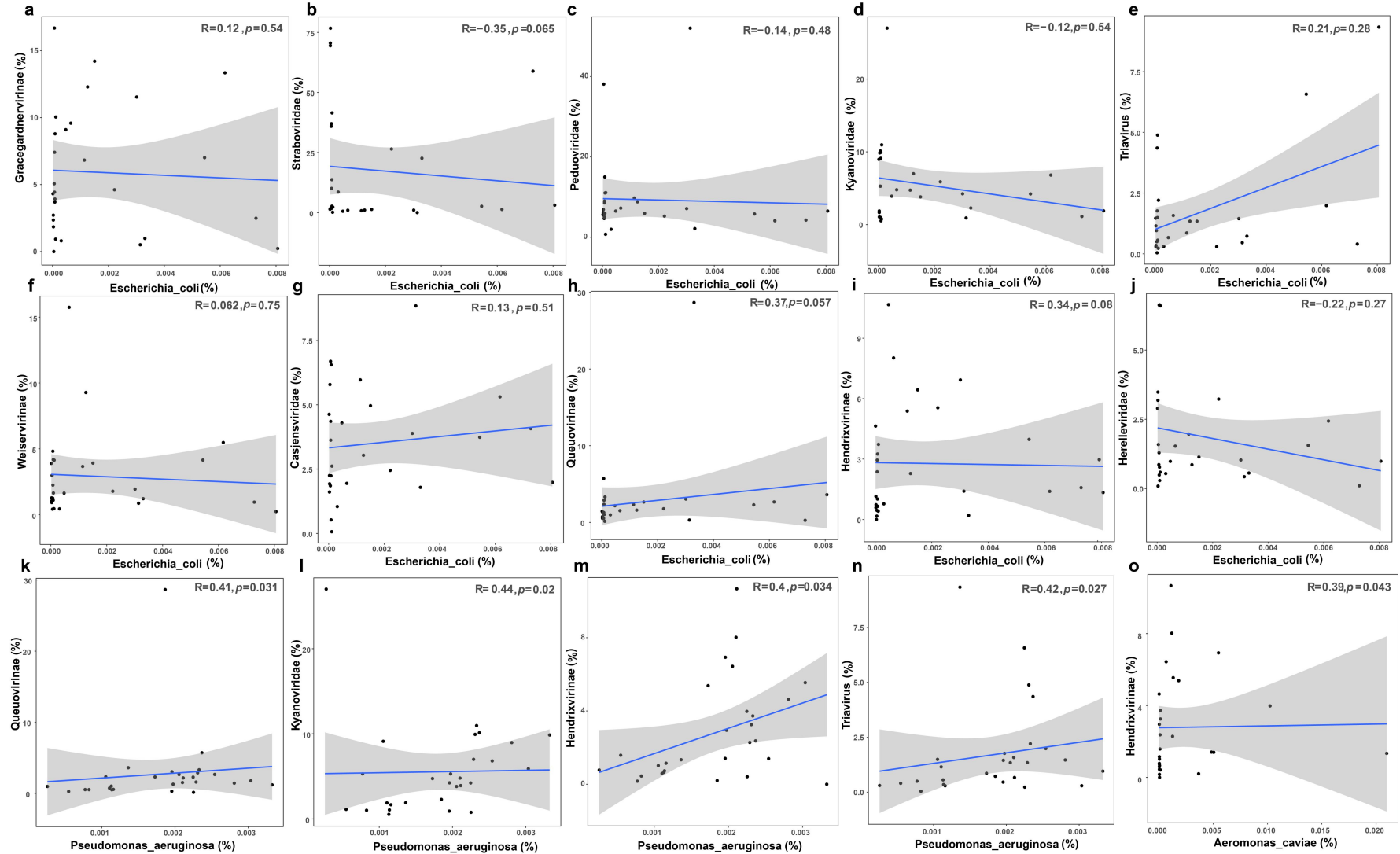


Fig. S5 Predicted number of hosts at each site in three WWTPs.

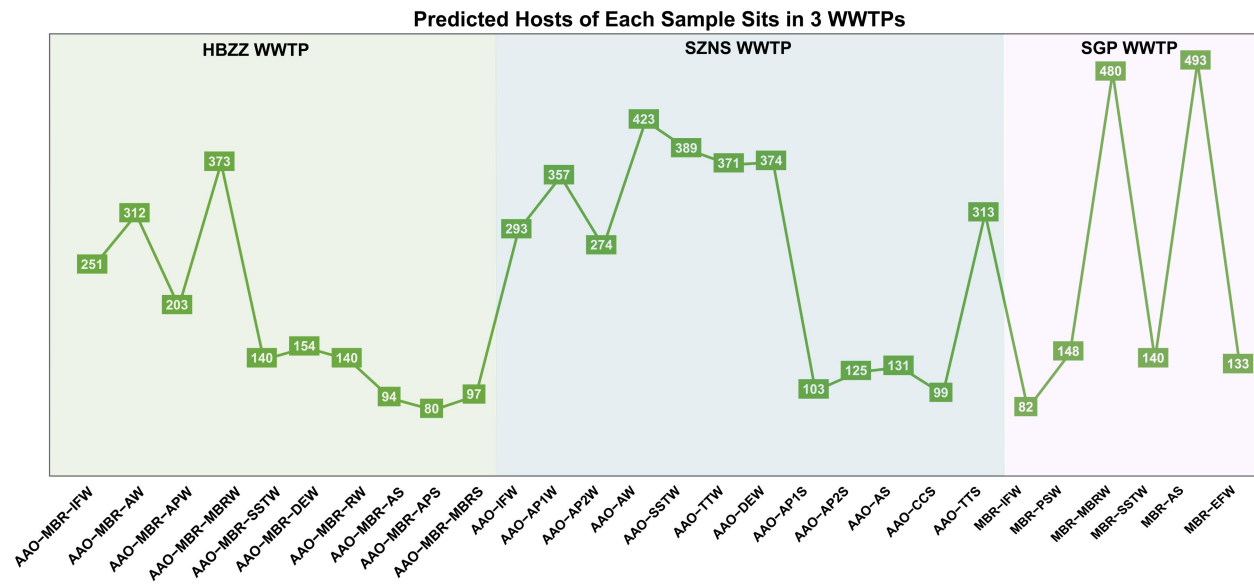


Fig. S6 Phylum-level taxonomy annotation of MAGs in three WWTPs. (a) AAO WWTP; (b) AAO-MBR WWTP; (c) MBR WWTP.

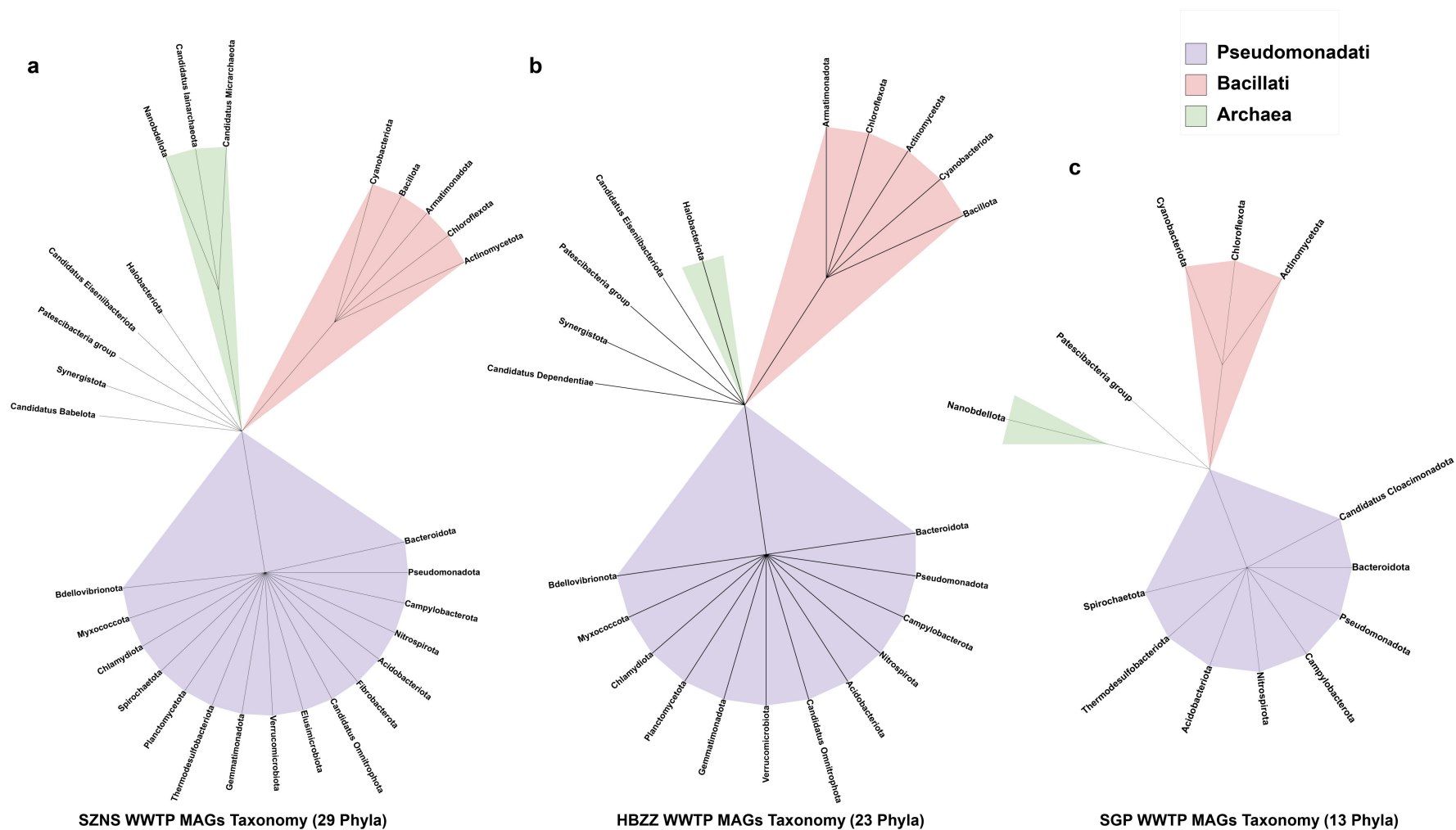


Fig. S7 Co-occurrence network of pathogens and viruses in WWTPs. (a)–(c) Overview of the full-scale AAO, AAO-MBR, and MBR WWTP, respectively. (d), (e) Focusing on bioreactor sections of AAO and AAO-MBR WWTP, respectively. No significant co-occurrence relationship in the bioreactor section of MBR.

