

## Supplementary Materials

### **Removal efficiency of ARGs in different wastewater treatment plants and their potential risks in effluent**

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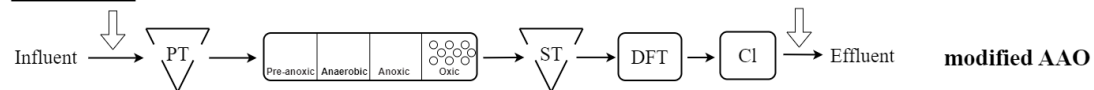
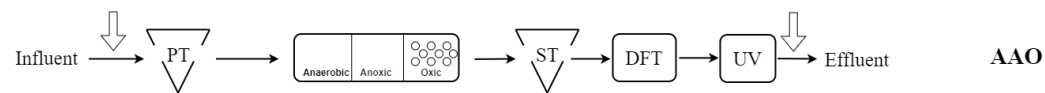
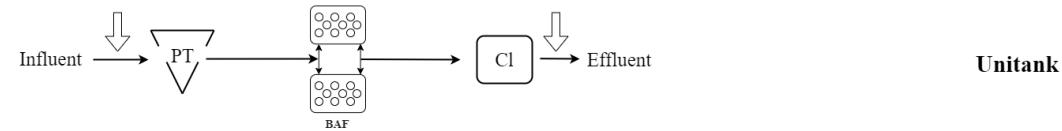
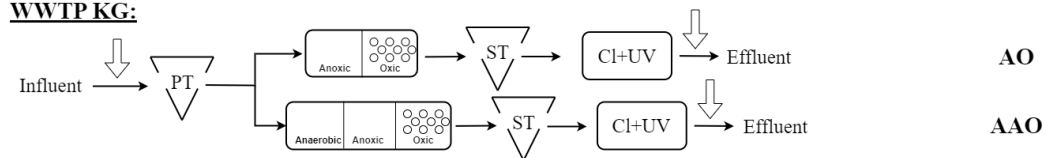
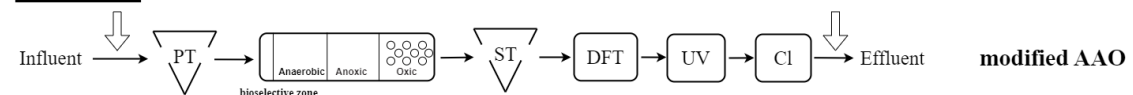
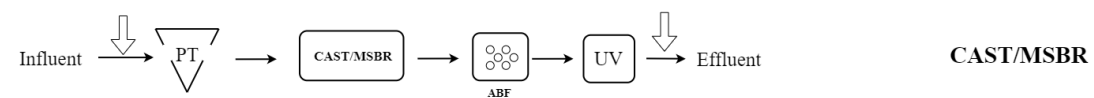
**WWTP BG:****WWTP BJ:****WWTP CB:****WWTP DY:****WWTP KG:****WWTP KX:****WWTP ZJ:**

Figure S1. Process configurations of each WWTP. White arrows indicated the sampling sites of influent or effluent samples. ABF, aeration biological fluidized tank; BAF, biological aeration filter; CAST, cyclic activated sludge technology; Cl, chlorination; DFT, denitrifying filtration tank; MSBR, modified sequencing batch reactor; PT, primary treatment; ST, secondary sedimentation tank; UV, ultraviolet.

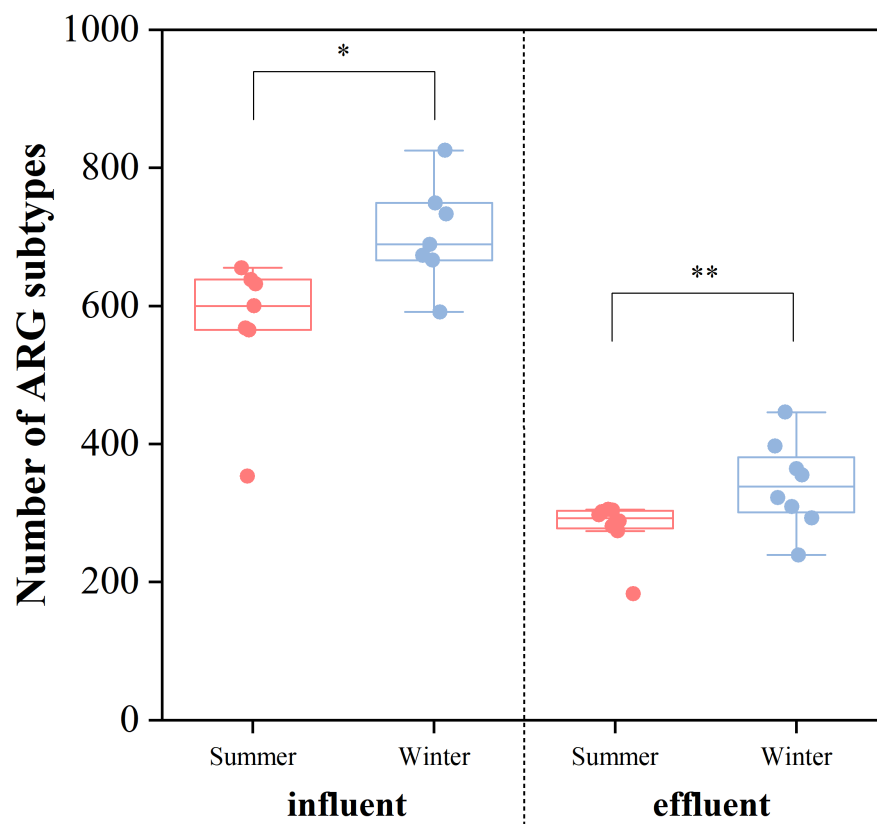


Figure S2. Number of ARG subtypes in influent and effluent samples in different seasons. \*  $p < 0.05$ , \*\*  $p < 0.01$ .

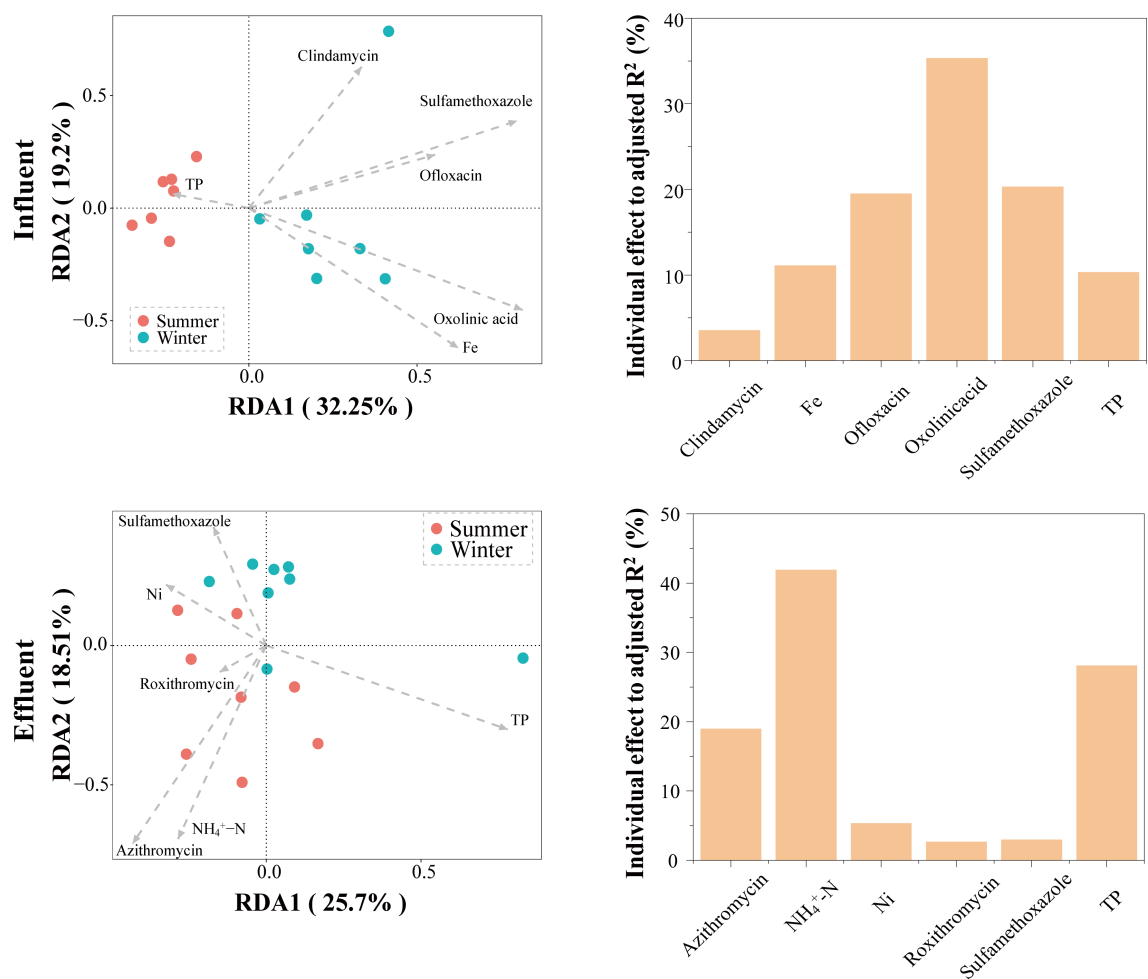


Figure S3. RDA plots showing the relationship between environmental attributes and ARGs in influent and effluent samples, and the individual effect of each environmental attribute on the ARGs.

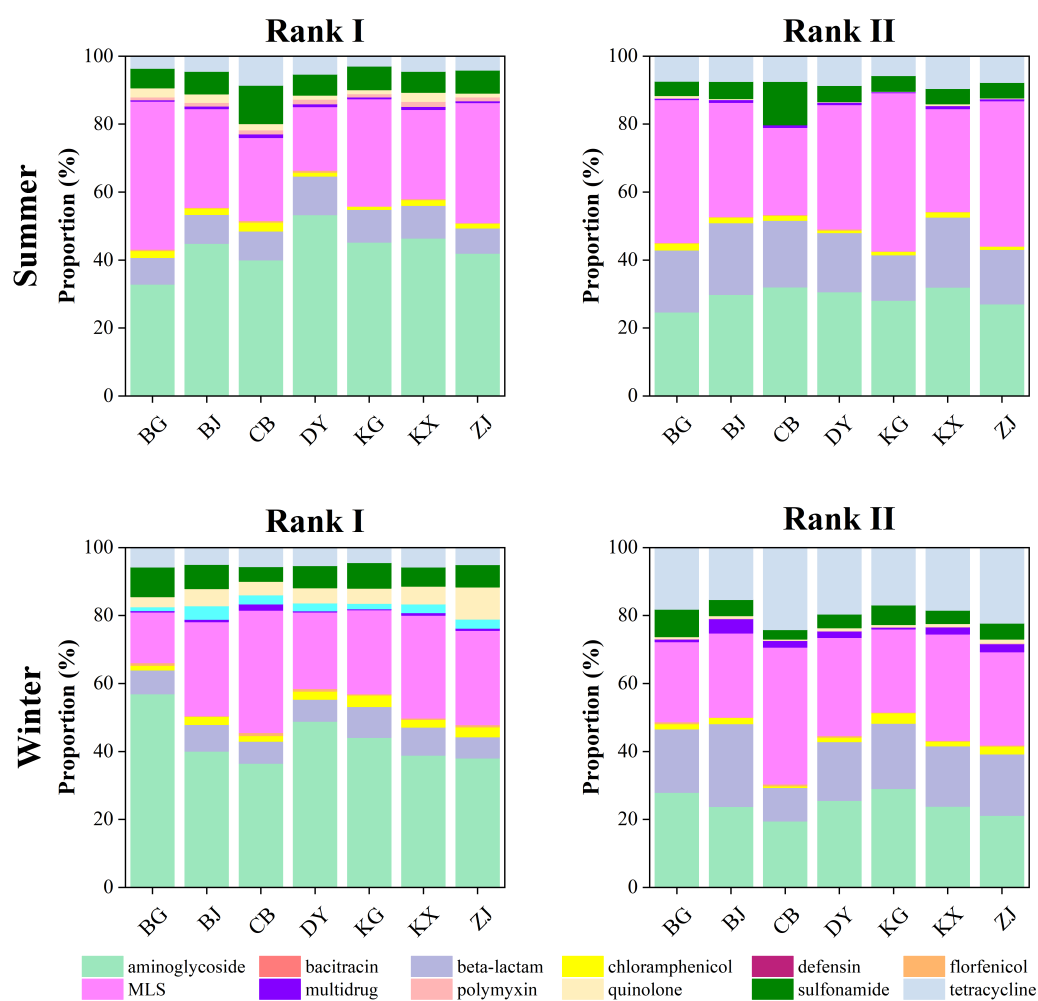


Figure S4. Proportion of different types of ARGs belonging to Rank I and Rank II in the influent samples. MLS, macrolide-lincosamide-streptogramin.

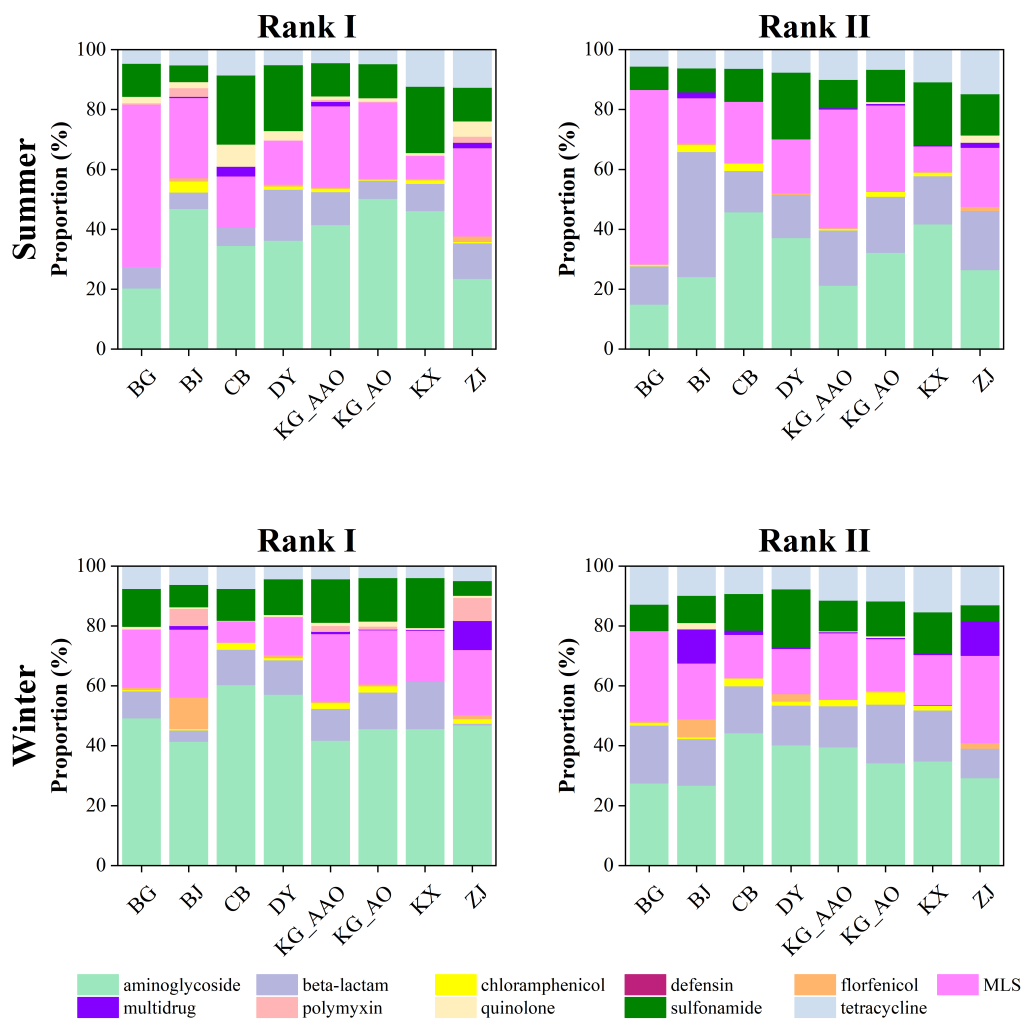


Figure S5. Proportion of different types of ARGs belonging to Rank I and Rank II in the effluent samples. MLS, macrolide-lincosamide-streptogramin.

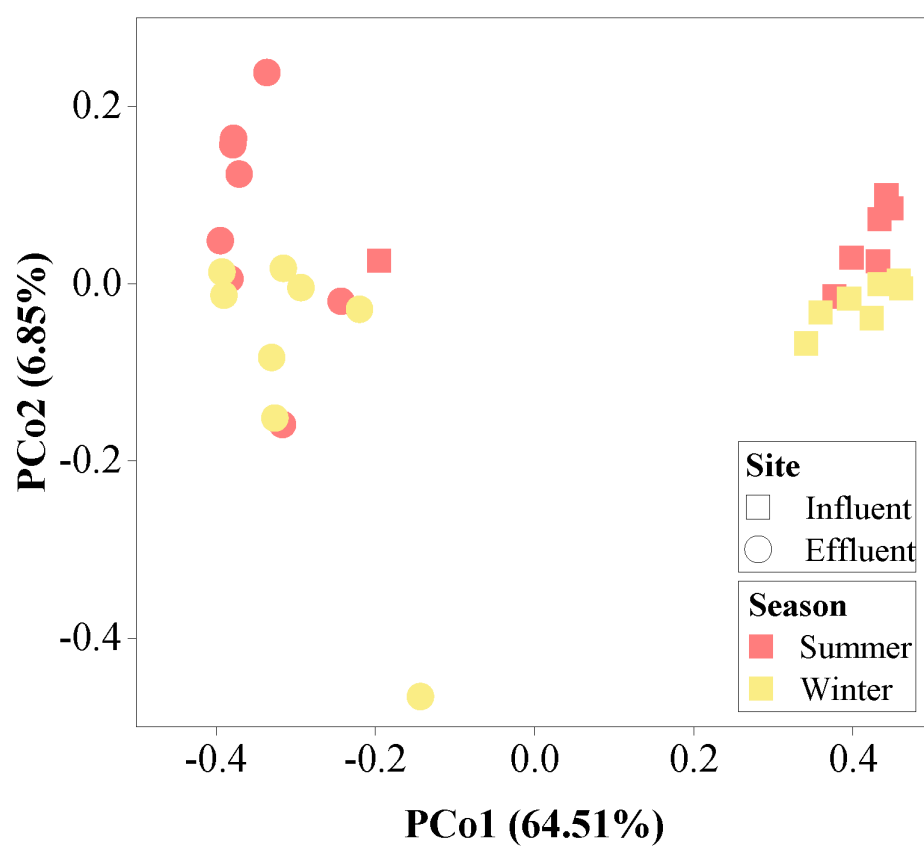


Figure S6. PCoA plot showing the ARGs profiles.

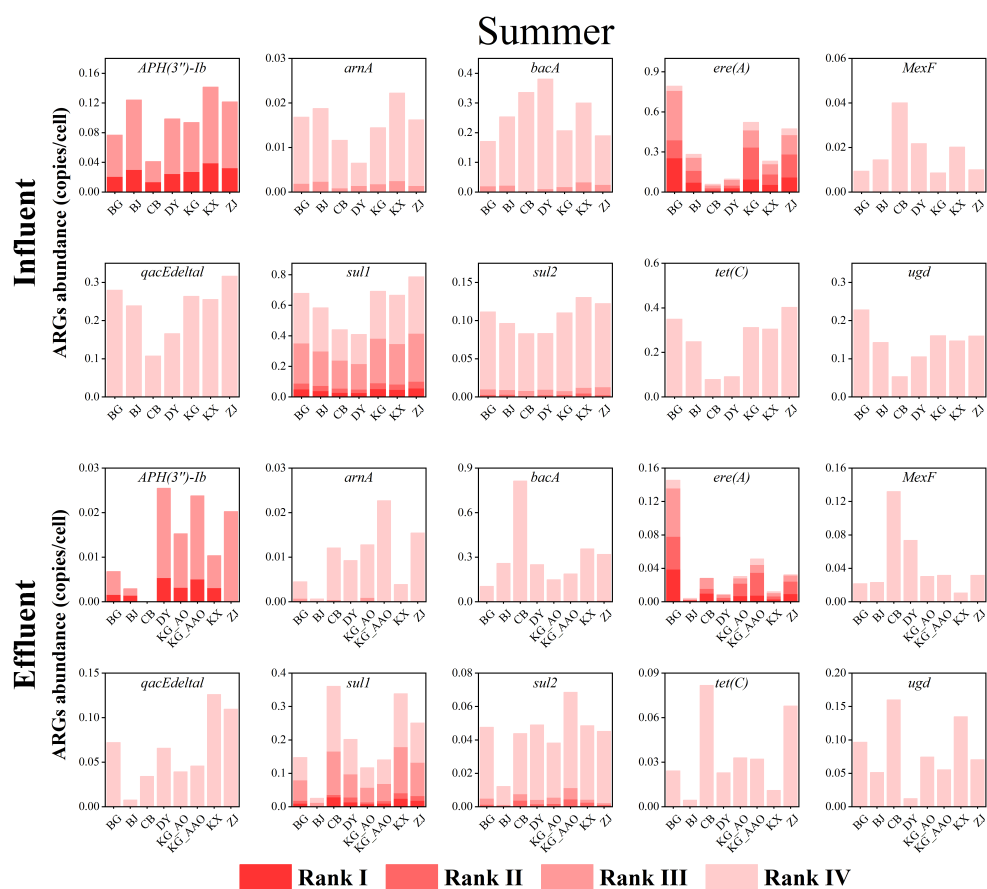


Figure S7. The risky ranks of top 10 ARGs subtypes in influent and effluent in summer.

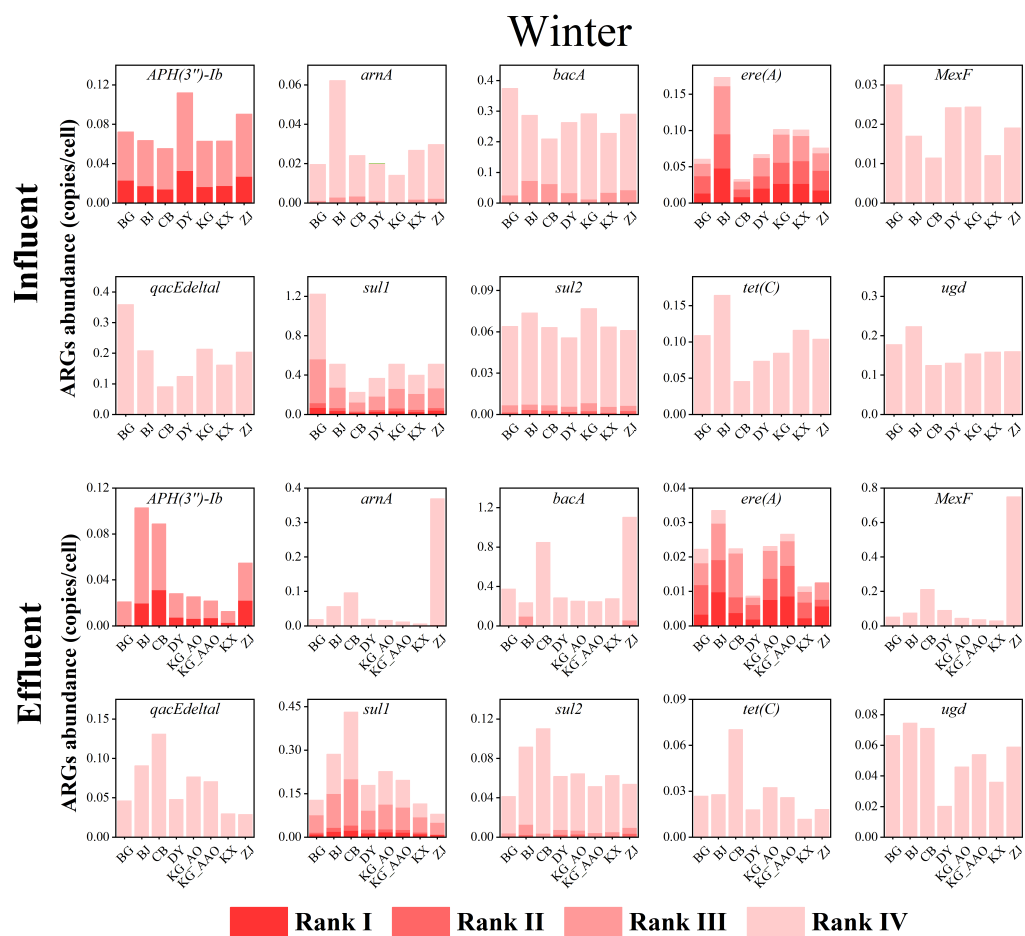


Figure S8. The risky ranks of top 10 ARGs subtypes in influent and effluent in winter.

Table S1. Information for collected influent sample

| BioSample in NCBI | BioProject in NCBI | Country       | Continent     |
|-------------------|--------------------|---------------|---------------|
| SAMEA4527607      | PRJEB23496         | Canada        | North America |
| SAMEA4527609      | PRJEB23496         | Canada        | North America |
| SAMEA4527608      | PRJEB23496         | Canada        | North America |
| SAMEA4527610      | PRJEB23496         | Canada        | North America |
| SAMEA4527625      | PRJEB23496         | Georgia       | North America |
| SAMEA4527669      | PRJEB23496         | USA           | North America |
| SAMEA4527670      | PRJEB23496         | USA           | North America |
| SAMEA4527666      | PRJEB23496         | USA           | North America |
| SAMEA4527672      | PRJEB23496         | USA           | North America |
| SAMEA4527668      | PRJEB23496         | USA           | North America |
| SAMEA4527673      | PRJEB23496         | USA           | North America |
| SAMEA4527674      | PRJEB23496         | USA           | North America |
| SAMEA4527665      | PRJEB23496         | USA           | North America |
| SAMEA4527667      | PRJEB23496         | USA           | North America |
| SAMEA4527671      | PRJEB23496         | USA           | North America |
| SAMEA4527650      | PRJEB23496         | New Zealand   | Oceania       |
| SAMEA4527601      | PRJEB23496         | Australia     | Oceania       |
| SAMEA4527600      | PRJEB23496         | Australia     | Oceania       |
| SAMEA4527606      | PRJEB23496         | Botswana      | Africa        |
| SAMEA4527613      | PRJEB23496         | Côte d'Ivoire | Africa        |
| SAMEA4527623      | PRJEB23496         | Ethiopia      | Africa        |
| SAMEA4527626      | PRJEB23496         | Ghana         | Africa        |
| SAMEA4527637      | PRJEB23496         | Kenya         | Africa        |
| SAMEA4527646      | PRJEB23496         | Nigeria       | Africa        |
| SAMEA4527654      | PRJEB23496         | Senegal       | Africa        |
| SAMEA4527677      | PRJEB23496         | South Africa  | Africa        |
| SAMEA4527664      | PRJEB23496         | Tanzania      | Africa        |
| SAMEA4527662      | PRJEB23496         | Togo          | Africa        |
| SAMEA4527679      | PRJEB23496         | Zambia        | Africa        |
| SAMEA4527678      | PRJEB23496         | Zambia        | Africa        |
| SAMEA4527604      | PRJEB23496         | Brazil        | South America |
| SAMEA4527605      | PRJEB23496         | Brazil        | South America |
| SAMEA4527614      | PRJEB23496         | Colombia      | South America |
| SAMEA4527621      | PRJEB23496         | Ecuador       | South America |
| SAMEA4527620      | PRJEB23496         | Ecuador       | South America |
| SAMEA4527653      | PRJEB23496         | Peru          | South America |
| SAMEA4527599      | PRJEB23496         | Albania       | Europe        |
| SAMEA4527602      | PRJEB23496         | Austria       | Europe        |
| SAMEA4527603      | PRJEB23496         | Bulgaria      | Europe        |
| SAMEA4527628      | PRJEB23496         | Croatia       | Europe        |
| SAMEA4527615      | PRJEB23496         | Czech         | Europe        |
| SAMEA4527619      | PRJEB23496         | Denmark       | Europe        |
| SAMEA4527618      | PRJEB23496         | Denmark       | Europe        |
| SAMEA4527617      | PRJEB23496         | Denmark       | Europe        |
| SAMEA4527624      | PRJEB23496         | Finland       | Europe        |

|                           |              |             |        |
|---------------------------|--------------|-------------|--------|
| SAMEA4527616              | PRJEB23496   | Germany     | Europe |
| SAMEA4527629              | PRJEB23496   | Hungary     | Europe |
| SAMEA4527633              | PRJEB23496   | Iceland     | Europe |
| SAMEA4527631              | PRJEB23496   | Ireland     | Europe |
| SAMEA4527635              | PRJEB23496   | Italy       | Europe |
| SAMEA4527676              | PRJEB23496   | Kosovo      | Europe |
| SAMEA4527641              | PRJEB23496   | Latvia      | Europe |
| SAMEA4527640              | PRJEB23496   | Luxembourg  | Europe |
| SAMEA4527644              | PRJEB23496   | Malta       | Europe |
| SAMEA4527648              | PRJEB23496   | Norway      | Europe |
| SAMEA4527651              | PRJEB23496   | Poland      | Europe |
| SAMEA4527643              | PRJEB23496   | Macedonia   | Europe |
| SAMEA4527642              | PRJEB23496   | Moldova     | Europe |
| SAMEA4527656              | PRJEB23496   | Serbia      | Europe |
| SAMEA4527657              | PRJEB23496   | Slovak      | Europe |
| SAMEA4527658              | PRJEB23496   | Slovenia    | Europe |
| SAMEA4527622              | PRJEB23496   | Spain       | Europe |
| SAMEA4527660              | PRJEB23496   | Sweden      | Europe |
| SAMEA4527659              | PRJEB23496   | Sweden      | Europe |
| SAMEA4939815/SAMEA4939797 | PRJEB28815   | Switzerland | Europe |
| SAMEA4939806/SAMEA4939805 | PRJEB28815   | Switzerland | Europe |
| SAMEA4939818/SAMEA4939817 | PRJEB28815   | Switzerland | Europe |
| SAMEA4939819/SAMEA4939809 | PRJEB28815   | Switzerland | Europe |
| SAMEA4939811/SAMEA4939803 | PRJEB28815   | Switzerland | Europe |
| SAMEA4939802/SAMEA4939801 | PRJEB28815   | Switzerland | Europe |
| SAMEA4939814/SAMEA4939800 | PRJEB28815   | Switzerland | Europe |
| SAMEA4939813/SAMEA4939808 | PRJEB28815   | Switzerland | Europe |
| SAMEA4939810/SAMEA4939804 | PRJEB28815   | Switzerland | Europe |
| SAMEA4939820/SAMEA4939807 | PRJEB28815   | Switzerland | Europe |
| SAMEA4939816/SAMEA4939799 | PRJEB28815   | Switzerland | Europe |
| SAMEA4939812/SAMEA4939798 | PRJEB28815   | Switzerland | Europe |
| SAMEA4527611              | PRJEB23496   | Switzerland | Europe |
| SAMEA4527647              | PRJEB23496   | Netherlands | Europe |
| SAMEA4527638              | PRJEB23496   | Cambodia    | Asia   |
| SAMN10658884              | PRJNA510571  | China       | Asia   |
| SAMEA4527612              | PRJEB23496   | China       | Asia   |
| SAMN37251984              | PRJNA1012295 | China       | Asia   |
| SAMN37251986              | PRJNA1012295 | China       | Asia   |
| SAMN37251980              | PRJNA1012295 | China       | Asia   |
| SAMN37251974              | PRJNA1012295 | China       | Asia   |
| SAMN37251985              | PRJNA1012295 | China       | Asia   |
| SAMN37251979              | PRJNA1012295 | China       | Asia   |
| SAMN37251975              | PRJNA1012295 | China       | Asia   |
| SAMN37251966              | PRJNA1012295 | China       | Asia   |
| SAMN37251987              | PRJNA1012295 | China       | Asia   |
| SAMN37251981              | PRJNA1012295 | China       | Asia   |
| SAMN37251994              | PRJNA1012295 | China       | Asia   |
| SAMN37251998              | PRJNA1012295 | China       | Asia   |

|              |              |            |      |
|--------------|--------------|------------|------|
| SAMN37251983 | PRJNA1012295 | China      | Asia |
| SAMN37251971 | PRJNA1012295 | China      | Asia |
| SAMN37251969 | PRJNA1012295 | China      | Asia |
| SAMN37251988 | PRJNA1012295 | China      | Asia |
| SAMN37251990 | PRJNA1012295 | China      | Asia |
| SAMN37251973 | PRJNA1012295 | China      | Asia |
| SAMN37251968 | PRJNA1012295 | China      | Asia |
| SAMN37251982 | PRJNA1012295 | China      | Asia |
| SAMN37251978 | PRJNA1012295 | China      | Asia |
| SAMN37251989 | PRJNA1012295 | China      | Asia |
| SAMN37251970 | PRJNA1012295 | China      | Asia |
| SAMN37251967 | PRJNA1012295 | China      | Asia |
| SAMN37252000 | PRJNA1012295 | China      | Asia |
| SAMN37251993 | PRJNA1012295 | China      | Asia |
| SAMN37251995 | PRJNA1012295 | China      | Asia |
| SAMN37251972 | PRJNA1012295 | China      | Asia |
| SAMN37251965 | PRJNA1012295 | China      | Asia |
| SAMN37251996 | PRJNA1012295 | China      | Asia |
| SAMN37251992 | PRJNA1012295 | China      | Asia |
| SAMN37251977 | PRJNA1012295 | China      | Asia |
| SAMN37251963 | PRJNA1012295 | China      | Asia |
| SAMN37251991 | PRJNA1012295 | China      | Asia |
| SAMN37251976 | PRJNA1012295 | China      | Asia |
| SAMN37251997 | PRJNA1012295 | China      | Asia |
| SAMN37251964 | PRJNA1012295 | China      | Asia |
| SAMN37251999 | PRJNA1012295 | China      | Asia |
| SAMN37251962 | PRJNA1012295 | China      | Asia |
| SAMEA4527630 | PRJEB23496   | India      | Asia |
| SAMEA4527632 | PRJEB23496   | Iran       | Asia |
| SAMEA4527634 | PRJEB23496   | Israel     | Asia |
| SAMEA4527636 | PRJEB23496   | Kazakhstan | Asia |
| SAMEA4527645 | PRJEB23496   | Malaysia   | Asia |
| SAMEA4527649 | PRJEB23496   | Nepal      | Asia |
| SAMEA4527652 | PRJEB23496   | Pakistan   | Asia |
| SAMEA4527655 | PRJEB23496   | Singapore  | Asia |
| SAMN08707558 | PRJNA438174  | Singapore  | Asia |
| SAMN08707546 | PRJNA438174  | Singapore  | Asia |
| SAMN08707540 | PRJNA438174  | Singapore  | Asia |
| SAMN08707564 | PRJNA438174  | Singapore  | Asia |
| SAMN08707534 | PRJNA438174  | Singapore  | Asia |
| SAMN08707552 | PRJEB23496   | Singapore  | Asia |
| SAMEA4527639 | PRJEB23496   | Sri Lanka  | Asia |
| SAMEA4527663 | PRJEB23496   | Türkiye    | Asia |
| SAMEA4527675 | PRJEB23496   | Vietnam    | Asia |

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Table S2. Information for collected effluent sample

| BioSample in NCBI         | BioProject in NCBI | Country     | Continent     |
|---------------------------|--------------------|-------------|---------------|
| SAMN04262563              | PRJNA300541        | Peru        | South America |
| SAMN04262560              | PRJNA300541        | Peru        | South America |
| SAMN04262552              | PRJNA300541        | Peru        | South America |
| SAMN04262553              | PRJNA300541        | Peru        | South America |
| SAMN04262559              | PRJNA300541        | Peru        | South America |
| SAMN04262562              | PRJNA300541        | Peru        | South America |
| SAMN04262564              | PRJNA300541        | Peru        | South America |
| SAMN07135111              | PRJNA385831        | USA         | North America |
| SAMN07135113              | PRJNA385831        | USA         | North America |
| SAMN07135112              | PRJNA385831        | USA         | North America |
| SAMEA3991470              | PRJEB14051         | Sweden      | Europe        |
| SAMEA3991471              | PRJEB14051         | Sweden      | Europe        |
| SAMEA3991475              | PRJEB14051         | Sweden      | Europe        |
| SAMEA3991474              | PRJEB14051         | Sweden      | Europe        |
| SAMEA5159678/SAMEA5159664 | PRJEB30264         | Switzerland | Europe        |
| SAMEA5159681/SAMEA5159680 | PRJEB30264         | Switzerland | Europe        |
| SAMEA5159672/SAMEA5159666 | PRJEB30264         | Switzerland | Europe        |
| SAMEA5159665/SAMEA5159679 | PRJEB30264         | Switzerland | Europe        |
| SAMEA5159686/SAMEA5159683 | PRJEB30264         | Switzerland | Europe        |
| SAMEA5159673/SAMEA5159667 | PRJEB30264         | Switzerland | Europe        |
| SAMEA5159682/SAMEA5159675 | PRJEB30264         | Switzerland | Europe        |
| SAMEA5159663/SAMEA5159670 | PRJEB30264         | Switzerland | Europe        |
| SAMEA5159685/SAMEA5159677 | PRJEB30264         | Switzerland | Europe        |
| SAMEA5159674/SAMEA5159671 | PRJEB30264         | Switzerland | Europe        |
| SAMEA5159684/SAMEA5159669 | PRJEB30264         | Switzerland | Europe        |
| SAMEA5159662/SAMEA5159676 | PRJEB30264         | Switzerland | Europe        |
| SAMN37252128              | PRJNA1012295       | China       | Asia          |
| SAMN37252138              | PRJNA1012295       | China       | Asia          |
| SAMN37252114              | PRJNA1012295       | China       | Asia          |
| SAMN37252127              | PRJNA1012295       | China       | Asia          |
| SAMN37252131              | PRJNA1012295       | China       | Asia          |
| SAMN37252137              | PRJNA1012295       | China       | Asia          |
| SAMN37252139              | PRJNA1012295       | China       | Asia          |
| SAMN37252133              | PRJNA1012295       | China       | Asia          |
| SAMN37252129              | PRJNA1012295       | China       | Asia          |
| SAMN37252110              | PRJNA1012295       | China       | Asia          |
| SAMN37252122              | PRJNA1012295       | China       | Asia          |
| SAMN37252148              | PRJNA1012295       | China       | Asia          |
| SAMN37252115              | PRJNA1012295       | China       | Asia          |
| SAMN37252120              | PRJNA1012295       | China       | Asia          |
| SAMN37252124              | PRJNA1012295       | China       | Asia          |
| SAMN37252135              | PRJNA1012295       | China       | Asia          |
| SAMN37252126              | PRJNA1012295       | China       | Asia          |
| SAMN37252136              | PRJNA1012295       | China       | Asia          |
| SAMN37252118              | PRJNA1012295       | China       | Asia          |

|              |              |           |      |
|--------------|--------------|-----------|------|
| SAMN37252111 | PRJNA1012295 | China     | Asia |
| SAMN37252140 | PRJNA1012295 | China     | Asia |
| SAMN37252113 | PRJNA1012295 | China     | Asia |
| SAMN37252146 | PRJNA1012295 | China     | Asia |
| SAMN37252130 | PRJNA1012295 | China     | Asia |
| SAMN37252132 | PRJNA1012295 | China     | Asia |
| SAMN37252117 | PRJNA1012295 | China     | Asia |
| SAMN37252134 | PRJNA1012295 | China     | Asia |
| SAMN37252147 | PRJNA1012295 | China     | Asia |
| SAMN37252123 | PRJNA1012295 | China     | Asia |
| SAMN37252121 | PRJNA1012295 | China     | Asia |
| SAMN37252112 | PRJNA1012295 | China     | Asia |
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| SAMN37252142 | PRJNA1012295 | China     | Asia |
| SAMN37252125 | PRJNA1012295 | China     | Asia |
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| SAMN37252119 | PRJNA1012295 | China     | Asia |
| SAMN08707544 | PRJNA438174  | Singapore | Asia |
| SAMN08707568 | PRJNA438174  | Singapore | Asia |
| SAMN08707556 | PRJNA438174  | Singapore | Asia |
| SAMN08707562 | PRJNA438174  | Singapore | Asia |
| SAMN08707561 | PRJNA438174  | Singapore | Asia |
| SAMN08707550 | PRJNA438174  | Singapore | Asia |
| SAMN08707537 | PRJNA438174  | Singapore | Asia |
| SAMN08707555 | PRJNA438174  | Singapore | Asia |
| SAMN08707567 | PRJNA438174  | Singapore | Asia |
| SAMN08707549 | PRJNA438174  | Singapore | Asia |
| SAMN08707543 | PRJNA438174  | Singapore | Asia |

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Table S3. Removal efficiency of ARGs in WWTPs

| Season | WWTP   | Total<br>ARGs | Rank I<br>ARGs | Rank II<br>ARGs | Rank I<br><i>APH(3'')-Ib</i> | Rank I<br><i>ere(A)</i> | Rank I<br><i>sulI</i> |
|--------|--------|---------------|----------------|-----------------|------------------------------|-------------------------|-----------------------|
| Summer | BG     | 93.3%         | 90.1%          | 87.3%           | 92.4%                        | 84.7%                   | 80.4%                 |
|        | BJ     | 88.4%         | 97.6%          | 97.4%           | 95.2%                        | 97.8%                   | 97.8%                 |
|        | CB     | 63.2%         | 50.0%          | 56.6%           | 100.0%                       | 12.3%                   | -2.1%                 |
|        | DY     | 88.2%         | 86.0%          | 86.5%           | 78.3%                        | 93.9%                   | 49.4%                 |
|        | KG_AO  | 90.7%         | 90.7%          | 85.7%           | 88.4%                        | 92.7%                   | 85.5%                 |
|        | KG_AAO | 92.2%         | 86.0%          | 91.8%           | 81.5%                        | 92.1%                   | 81.1%                 |
|        | KX     | 85.8%         | 87.6%          | 89.6%           | 92.0%                        | 94.4%                   | 49.5%                 |
|        | ZJ     | 94.0%         | 81.3%          | 88.9%           | 100.0%                       | 91.3%                   | 68.3%                 |
| Winter | BG     | 94.2%         | 89.8%          | 87.4%           | 95.7%                        | 75.4%                   | 85.2%                 |
|        | BJ     | 87.4%         | 52.0%          | 74.5%           | -13.7%                       | 79.5%                   | 48.1%                 |
|        | CB     | 85.2%         | 50.4%          | 74.3%           | -123.6%                      | 55.0%                   | -21.1%                |
|        | DY     | 87.7%         | 74.3%          | 84.0%           | 77.4%                        | 90.6%                   | 53.2%                 |
|        | KG_AO  | 83.0%         | 73.9%          | 81.9%           | 60.9%                        | 71.6%                   | 48.2%                 |
|        | KG_AAO | 81.1%         | 75.1%          | 79.7%           | 57.8%                        | 67.8%                   | 51.9%                 |
|        | KX     | 89.9%         | 86.9%          | 91.1%           | 84.0%                        | 91.6%                   | 60.9%                 |
|        | ZJ     | 83.5%         | 67.6%          | 86.1%           | 18.0%                        | 67.6%                   | 78.6%                 |
| Total  | BG     | 93.8%         | 90.0%          | 87.3%           | 94.1%                        | 84.3%                   | 83.1%                 |
|        | BJ     | 87.8%         | 77.0%          | 86.1%           | 55.4%                        | 90.5%                   | 73.9%                 |
|        | CB     | 81.4%         | 50.2%          | 69.3%           | -13.4%                       | 30.4%                   | -9.7%                 |
|        | DY     | 87.9%         | 80.1%          | 85.3%           | 77.8%                        | 92.5%                   | 51.4%                 |
|        | KG_AO  | 87.6%         | 84.5%          | 84.2%           | 78.0%                        | 88.1%                   | 69.7%                 |
|        | KG_AAO | 87.7%         | 83.0%          | 87.1%           | 72.6%                        | 86.8%                   | 70.9%                 |
|        | KX     | 87.7%         | 86.4%          | 90.2%           | 89.5%                        | 93.5%                   | 53.6%                 |
|        | ZJ     | 88.1%         | 75.7%          | 87.8%           | 62.7%                        | 88.0%                   | 72.5%                 |

Table S4. Co-occurrence of high-risky ARGs with MGEs in potential pathogens

| contig         | Host                                                                                     | ARGs                                                 | MGEs                                                        |
|----------------|------------------------------------------------------------------------------------------|------------------------------------------------------|-------------------------------------------------------------|
| contig_49805   | <i>Pseudomonas aeruginosa</i>                                                            | <i>sulI</i>                                          | integrase, transposase, recombinase                         |
| contig_631141  | <i>Pseudomonas aeruginosa</i> / <i>Escherichia coli</i> / <i>Acinetobacter baumannii</i> | <i>APH(3'')-Ib</i>                                   | interferase, hypothetical protein, recombinase, transposase |
| contig_450701  | <i>Pseudomonas aeruginosa</i> / <i>Escherichia coli</i>                                  | <i>aadA3</i> , <i>ere(A)</i>                         | transposase                                                 |
| contig_619428  | <i>Pseudomonas aeruginosa</i>                                                            | <i>sulI</i>                                          | intergrase, transposase                                     |
| contig_1458241 | <i>Pseudomonas aeruginosa</i>                                                            | <i>sulI</i>                                          | relaxase                                                    |
| contig_96197   | <i>Salmonella enterica</i> / <i>Escherichia coli</i> / <i>Klebsiella pneumoniae</i>      | <i>APH(6)-Id</i> , <i>APH(3'')-Ib</i>                | recombinase                                                 |
| contig_401414  | <i>Pseudomonas aeruginosa</i>                                                            | <i>sulI</i>                                          | relaxase                                                    |
| contig_413132  | <i>Salmonella enterica</i> / <i>Escherichia coli</i> / <i>Klebsiella pneumoniae</i>      | <i>APH(3'')-Ib</i> , <i>APH(6)-Id</i>                | transposase, recombinase, relaxase                          |
| contig_348836  | <i>Pseudomonas aeruginosa</i>                                                            | <i>qacEdelta1</i> , <i>sulI</i>                      | integrase                                                   |
| contig_170940  | <i>Salmonella enterica</i> / <i>Escherichia coli</i> / <i>Klebsiella pneumoniae</i>      | <i>APH(3'')-Ib</i> , <i>APH(6)-Id</i>                | recombinase                                                 |
| contig_788497  | <i>Salmonella enterica</i> / <i>Escherichia coli</i> / <i>Klebsiella pneumoniae</i>      | <i>APH(3'')-Ib</i> , <i>APH(6)-Id</i>                | recombinase                                                 |
| contig_765414  | <i>Pseudomonas aeruginosa</i> / <i>Escherichia coli</i> / <i>Acinetobacter baumannii</i> | <i>AAC(6')-Ib7</i> , <i>qacEdelta1</i> , <i>sulI</i> | transposase                                                 |
| contig_823633  | <i>Pseudomonas aeruginosa</i> / <i>Escherichia coli</i> / <i>Acinetobacter baumannii</i> | <i>qacEdelta1</i> , <i>sulI</i>                      | TniB family NTP-binding protein                             |
| contig_396098  | <i>Salmonella enterica</i> / <i>Escherichia coli</i> / <i>Klebsiella pneumoniae</i>      | <i>APH(6)-Id</i> , <i>APH(3'')-Ib</i>                | recombinase                                                 |
| contig_666362  | <i>Pseudomonas aeruginosa</i> / <i>Escherichia coli</i> / <i>Klebsiella pneumoniae</i>   | <i>sulI</i>                                          | transposase                                                 |
| contig_555132  | <i>Pseudomonas aeruginosa</i>                                                            | <i>floR</i> , <i>sulI</i>                            | relaxase                                                    |
| contig_1097044 | <i>Salmonella enterica</i> / <i>Escherichia coli</i> / <i>Klebsiella pneumoniae</i>      | <i>APH(3'')-Ib</i> , <i>APH(6)-Id</i>                | transposase, recombinase                                    |
| contig_843868  | <i>Pseudomonas aeruginosa</i> / <i>Klebsiella pneumoniae</i>                             | <i>floR</i> , <i>sulI</i>                            | relaxase, type II toxin-antitoxin system YafQ family toxin  |
| contig_999256  | <i>Salmonella enterica</i> / <i>Escherichia coli</i> / <i>Klebsiella pneumoniae</i>      | <i>APH(6)-Id</i> , <i>APH(3'')-Ib</i>                | recombinase, transposase                                    |
| contig_1259511 | <i>Pseudomonas aeruginosa</i> / <i>Salmonella enterica</i>                               | <i>APH(3'')-Ib</i> , <i>APH(6)-Id</i>                | transposase, recombinase                                    |
| contig_847150  | <i>Pseudomonas aeruginosa</i> / <i>Klebsiella pneumoniae</i>                             | <i>sulI</i> , <i>qacEdelta1</i> , <i>aadA</i>        | transposase                                                 |
| contig_658652  | <i>Pseudomonas aeruginosa</i> / <i>Salmonella enterica</i>                               | <i>APH(3'')-Ib</i>                                   | recombinase                                                 |
| contig_426147  | <i>Pseudomonas aeruginosa</i> / <i>Escherichia coli</i> / <i>Klebsiella pneumoniae</i>   | <i>qacEdelta1</i> , <i>sulI</i>                      | integrase                                                   |
| contig_287841  | <i>Pseudomonas aeruginosa</i> / <i>Salmonella enterica</i>                               | <i>APH(6)-Id</i> , <i>APH(3'')-Ib</i>                | recombinase                                                 |

Table S5. Antibiotic (Beta-lactams, Sulfonamides) concentration

| Season | Type     | Sample | Beta-lactams (ng/L) |           |           | Sulfonamides (ng/L) |               |                  |                    |
|--------|----------|--------|---------------------|-----------|-----------|---------------------|---------------|------------------|--------------------|
|        |          |        | Cefotaxime          | Cefoxitin | Cefazolin | Sulfabenzamide      | Sulfafurazole | Sulfamethoxazole | Sulfamonomethoxine |
| Summer | Influent | BG     | ND                  | ND        | ND        | ND                  | 0.06          | 11.78            | ND                 |
|        |          | BJ     | ND                  | ND        | 3.12      | ND                  | ND            | 2.82             | ND                 |
|        |          | CB     | ND                  | ND        | ND        | ND                  | ND            | 26.82            | ND                 |
|        |          | DY     | ND                  | ND        | 3.48      | ND                  | ND            | 24.02            | ND                 |
|        |          | KG     | ND                  | ND        | ND        | ND                  | ND            | 7.82             | ND                 |
|        |          | KX     | ND                  | ND        | ND        | ND                  | 4.64          | 8.56             | ND                 |
|        |          | ZJ     | ND                  | ND        | 3.32      | ND                  | 0.12          | 21.02            | ND                 |
|        | Effluent | BG     | ND                  | ND        | ND        | ND                  | ND            | 15.79            | 1.65               |
|        |          | BJ     | ND                  | ND        | 1.30      | ND                  | ND            | 2.06             | ND                 |
|        |          | CB     | ND                  | ND        | ND        | 1.27                | ND            | 0.85             | ND                 |
|        |          | DY     | ND                  | ND        | 1.27      | ND                  | ND            | 5.22             | ND                 |
|        |          | KG_AO  | ND                  | ND        | ND        | ND                  | ND            | 6.67             | ND                 |
|        |          | KG_AAO | ND                  | ND        | ND        | ND                  | ND            | 6.93             | ND                 |
|        |          | KX     | ND                  | ND        | ND        | ND                  | ND            | 6.51             | ND                 |
|        |          | ZJ     | ND                  | ND        | 1.19      | ND                  | ND            | 8.74             | ND                 |
| Winter | Influent | BG     | ND                  | ND        | ND        | ND                  | ND            | 24.94            | ND                 |
|        |          | BJ     | ND                  | ND        | ND        | ND                  | ND            | 21.88            | ND                 |
|        |          | CB     | 70.10               | 42.64     | ND        | ND                  | ND            | 70.72            | ND                 |
|        |          | DY     | ND                  | 10.10     | ND        | ND                  | ND            | 20.68            | ND                 |
|        |          | KG     | 9.58                | ND        | ND        | ND                  | ND            | 41.66            | ND                 |
|        |          | KX     | 4.62                | ND        | ND        | ND                  | ND            | 54.68            | ND                 |
|        |          | ZJ     | 8.02                | 17.42     | ND        | ND                  | ND            | 45.76            | ND                 |
|        | Effluent | BG     | ND                  | ND        | ND        | ND                  | ND            | 17.71            | ND                 |
|        |          | BJ     | ND                  | ND        | ND        | ND                  | ND            | 8.38             | ND                 |
|        |          | CB     | 6.81                | ND        | ND        | ND                  | ND            | 4.64             | ND                 |
|        |          | DY     | 8.37                | 1.88      | ND        | ND                  | ND            | 16.49            | ND                 |
|        |          | KG_AO  | ND                  | 6.99      | ND        | ND                  | ND            | 7.34             | ND                 |
|        |          | KG_AAO | ND                  | 14.30     | ND        | ND                  | ND            | 9.95             | ND                 |
|        |          | KX     | ND                  | ND        | ND        | ND                  | ND            | 13.88            | ND                 |
|        |          | ZJ     | ND                  | ND        | ND        | ND                  | ND            | 7.92             | ND                 |

Note: ND, not detected.

Table S6. Antibiotics (Tetracyclines, MLS) concentration

| Season | Type     | Sample | Tetracyclines (ng/L) |              |                 | MLS (ng/L)   |              |                |             |            |               |
|--------|----------|--------|----------------------|--------------|-----------------|--------------|--------------|----------------|-------------|------------|---------------|
|        |          |        | Doxycycline          | Tetracycline | Oxytetracycline | Azithromycin | Erythromycin | Clarithromycin | Clindamycin | Lincomycin | Roxithromycin |
| Summer | Influent | BG     | 5.64                 | ND           | 11.18           | ND           | ND           | 51.68          | 4.50        | ND         | 70.14         |
|        |          | BJ     | 9.10                 | ND           | ND              | 54.12        | 0.86         | 53.52          | 5.80        | ND         | 58.72         |
|        |          | CB     | ND                   | ND           | ND              | 48.96        | 1.26         | 49.38          | 16.64       | 0.28       | 88.78         |
|        |          | DY     | ND                   | ND           | 6.12            | 86.06        | 0.42         | 87.82          | 3.16        | 5.02       | 63.80         |
|        |          | KG     | 7.24                 | ND           | 5.76            | 21.02        | 0.38         | 21.22          | 8.38        | ND         | 63.44         |
|        |          | KX     | 7.56                 | ND           | ND              | 42.70        | 0.66         | 43.34          | 3.22        | ND         | 66.28         |
|        |          | ZJ     | 7.38                 | ND           | 5.98            | 95.64        | 0.92         | 93.64          | 6.00        | 3.20       | 81.66         |
|        | Effluent | BG     | ND                   | ND           | ND              | 7.57         | 1.54         | 8.09           | 13.31       | 1.22       | 18.48         |
|        |          | BJ     | ND                   | ND           | ND              | 4.81         | 0.39         | 4.58           | 2.63        | ND         | 9.78          |
|        |          | CB     | ND                   | ND           | ND              | 30.41        | 2.08         | 29.48          | ND          | ND         | 32.75         |
|        |          | DY     | ND                   | ND           | ND              | 19.32        | 0.79         | 19.71          | ND          | ND         | 15.81         |
|        |          | KG_AO  | ND                   | ND           | 2.49            | 13.48        | 0.42         | 12.59          | 27.05       | 0.64       | 24.08         |
|        |          | KG_AAO | 3.32                 | ND           | ND              | 9.91         | 0.37         | 9.72           | 31.27       | 0.43       | 18.25         |
|        |          | KX     | ND                   | ND           | ND              | 37.00        | 0.95         | 36.42          | 29.20       | 0.10       | 89.87         |
|        |          | ZJ     | ND                   | ND           | ND              | 22.68        | 0.83         | 22.36          | 33.93       | 3.65       | 25.33         |
| Winter | Influent | BG     | 30.94                | ND           | 47.26           | 42.48        | 5.98         | 42.62          | 8.44        | 1.24       | 70.88         |
|        |          | BJ     | 31.62                | ND           | 24.14           | 20.34        | 3.86         | 19.94          | 14.88       | 3.54       | 59.44         |
|        |          | CB     | 33.50                | 161.64       | 54.22           | 66.38        | 3.10         | 66.28          | 21.48       | 1.86       | 153.34        |
|        |          | DY     | 37.92                | ND           | 38.46           | 68.84        | 3.60         | 67.66          | 2.96        | ND         | 111.82        |
|        |          | KG     | 13.64                | ND           | 33.68           | 24.60        | 8.86         | 24.82          | 6.94        | ND         | 178.16        |
|        |          | KX     | 29.30                | ND           | 35.80           | 38.14        | 3.30         | 38.50          | 7.26        | 1.04       | 101.32        |
|        |          | ZJ     | 31.78                | ND           | 31.52           | 59.64        | 3.94         | 59.70          | 6.10        | 21.28      | 83.26         |
|        | Effluent | BG     | 4.99                 | ND           | 19.92           | 10.00        | 5.71         | 9.75           | 55.63       | 0.63       | 54.51         |
|        |          | BJ     | 4.94                 | ND           | 18.53           | 1.55         | 1.13         | 1.71           | 1.54        | ND         | 14.50         |
|        |          | CB     | 4.18                 | ND           | 17.04           | 27.56        | 3.59         | 26.73          | 6.24        | ND         | 133.47        |
|        |          | DY     | 4.39                 | ND           | 36.41           | 14.62        | 2.35         | 14.83          | 7.88        | ND         | 52.06         |
|        |          | KG_AO  | 7.68                 | ND           | 22.27           | 5.62         | 1.38         | 5.37           | 91.51       | 0.89       | 44.56         |
|        |          | KG_AAO | 20.14                | ND           | 12.92           | 7.45         | 1.26         | 7.66           | 6.50        | ND         | 27.69         |
|        |          | KX     | 7.51                 | ND           | 32.66           | 5.13         | 3.44         | 4.89           | 38.80       | ND         | 72.90         |
|        |          | ZJ     | 8.28                 | ND           | 18.04           | 2.04         | 0.96         | 2.26           | 0.72        | ND         | 9.58          |

Note: ND, not detected; MLS, macrolide-lincosamide-streptogramin.

Table S7. Antibiotics (Quinolones, Fluoroquinolones, Chloramphenicols) concentration

| Season | Type     | Sample | Quinolones (ng/L) |                | Fluoroquinolones (ng/L) |              |              |           | Chloramphenicols (ng/L) |                 |            |
|--------|----------|--------|-------------------|----------------|-------------------------|--------------|--------------|-----------|-------------------------|-----------------|------------|
|        |          |        | Oxolinic Acid     | Nalidixic acid | Enrofloxacin            | Moxifloxacin | Sirofloxacin | Ofloxacin | Levofloxacin            | Chloramphenicol | Flofenicol |
| Summer | Influent | BG     | 1.40              | 0.90           | 5.30                    | ND           | 63.60        | 53.32     | 52.68                   | 15.06           | 6.52       |
|        |          | BJ     | 0.58              | 1.14           | 5.06                    | 10.90        | 28.32        | 30.76     | 30.80                   | 6.24            | 2.90       |
|        |          | CB     | 2.88              | 0.92           | 3.56                    | 24.02        | ND           | 53.94     | 53.02                   | 11.12           | 2.30       |
|        |          | DY     | 0.28              | 0.62           | 3.26                    | 11.34        | ND           | 68.52     | 65.68                   | ND              | 3.36       |
|        |          | KG     | 0.28              | 1.04           | 4.00                    | 12.68        | 58.32        | 39.64     | 39.60                   | 5.68            | 3.86       |
|        |          | KX     | 2.10              | 1.82           | 3.68                    | 17.06        | 75.22        | 39.52     | 37.08                   | 7.58            | 2.44       |
|        |          | ZJ     | 2.52              | 2.64           | 4.50                    | 23.34        | 72.86        | 54.60     | 54.00                   | 6.82            | 4.84       |
|        | Effluent | BG     | 0.38              | 0.81           | ND                      | ND           | ND           | 12.47     | 13.12                   | ND              | 1.65       |
|        |          | BJ     | 0.63              | 0.24           | ND                      | ND           | ND           | 8.78      | 8.86                    | ND              | 2.38       |
|        |          | CB     | ND                | 0.45           | ND                      | ND           | ND           | 3.57      | 3.55                    | ND              | 1.54       |
|        |          | DY     | 0.19              | 0.23           | ND                      | 12.29        | ND           | 17.17     | 16.82                   | ND              | 0.93       |
|        |          | KG_AO  | 0.47              | 1.17           | ND                      | ND           | ND           | 11.24     | 11.49                   | ND              | 1.06       |
|        |          | KG_AAO | 0.37              | 1.31           | ND                      | ND           | ND           | 13.23     | 12.67                   | ND              | 1.39       |
|        |          | KX     | 0.31              | 1.03           | ND                      | ND           | ND           | 10.07     | 9.63                    | 1.53            | 1.81       |
|        |          | ZJ     | ND                | 1.13           | 2.35                    | ND           | ND           | 33.56     | 34.05                   | ND              | 0.98       |
| Winter | Influent | BG     | 9.52              | 1.32           | ND                      | 49.60        | ND           | 147.86    | 132.70                  | ND              | ND         |
|        |          | BJ     | 17.96             | 2.36           | ND                      | 11.78        | ND           | 18.18     | 16.34                   | ND              | ND         |
|        |          | CB     | 8.10              | 2.18           | ND                      | 66.20        | ND           | 115.56    | 108.52                  | ND              | ND         |
|        |          | DY     | 20.04             | 1.04           | ND                      | 23.20        | ND           | 71.04     | 65.54                   | ND              | 7.82       |
|        |          | KG     | 17.94             | 5.16           | ND                      | ND           | ND           | 16.54     | 15.72                   | 4.66            | 5.98       |
|        |          | KX     | 21.20             | 2.32           | ND                      | 32.60        | ND           | 65.88     | 61.96                   | 12.18           | ND         |
|        |          | ZJ     | 18.78             | 1.94           | ND                      | 64.40        | ND           | 112.18    | 106.00                  | ND              | ND         |
|        | Effluent | BG     | 10.18             | 0.66           | ND                      | ND           | ND           | 18.57     | 17.45                   | ND              | ND         |
|        |          | BJ     | 9.47              | 0.26           | ND                      | ND           | ND           | 3.84      | 3.90                    | ND              | ND         |
|        |          | CB     | 8.59              | 0.40           | ND                      | ND           | ND           | 9.51      | 9.17                    | ND              | ND         |
|        |          | DY     | 4.97              | 1.58           | ND                      | 12.40        | ND           | 35.80     | 31.70                   | ND              | 3.78       |
|        |          | KG_AO  | 9.18              | 2.93           | ND                      | 5.51         | ND           | 7.90      | 7.52                    | ND              | ND         |
|        |          | KG_AAO | 11.01             | 0.54           | ND                      | 5.95         | ND           | 9.52      | 8.77                    | ND              | ND         |
|        |          | KX     | 23.08             | 1.91           | ND                      | 8.59         | ND           | 15.96     | 14.40                   | ND              | ND         |
|        |          | ZJ     | 7.17              | 1.63           | ND                      | ND           | ND           | 3.54      | 3.38                    | ND              | ND         |

Note: ND, not detected.

Table S8. Basic information for WWTPs

| WWTP | Sample name | Capacity (m <sup>3</sup> /day) | Component                          | Latitude  | Longitude  |
|------|-------------|--------------------------------|------------------------------------|-----------|------------|
| BG   | WWTP BG     | 5,000                          | domestic wastewater                | 32.169 °N | 118.837 °E |
| BJ   | WWTP BJ     | 35,000                         | domestic wastewater                | 31.877 °N | 118.588 °E |
| CB   | WWTP CB     | 300,000                        | domestic wastewater                | 32.106 °N | 118.751 °E |
| DY   | WWTP DY     | 45,000                         | domestic and industrial wastewater | 32.166 °N | 119.041 °E |
| KG   | WWTP KG_AO  | 40,000                         | domestic wastewater                | 31.816 °N | 118.797 °E |
|      | WWTP KG_AAO |                                |                                    |           |            |
| KX   | WWTP KX     | 160,000                        | domestic wastewater                | 31.900 °N | 118.857 °E |
| ZJ   | WWTP ZJ     | 120,000                        | domestic wastewater                | 32.018 °N | 118.642 °E |

Table S9. Physicochemical attributes in each sample

| Season | Type     | Sample | NH <sub>4</sub> <sup>+</sup> -N<br>(mg/L) | TP<br>(mg/L) | TN<br>(mg/L) | pH   | Fe<br>(µg/L) | Mn<br>(µg/L) | Cu<br>(µg/L) | Zn<br>(µg/L) | Ni<br>(µg/L) |
|--------|----------|--------|-------------------------------------------|--------------|--------------|------|--------------|--------------|--------------|--------------|--------------|
| Summer | Influent | BG     | 21.15                                     | 2.41         | 27.50        | 8.12 | 5.53         | 16.72        | ND           | 1.38         | 1.58         |
|        |          | BJ     | 11.26                                     | 1.57         | 16.58        | 7.71 | 23.24        | 5.98         | 0.07         | 6.18         | 1.80         |
|        |          | CB     | 23.79                                     | 4.17         | 33.21        | 8.13 | 4.10         | 7.56         | ND           | 4.59         | 1.50         |
|        |          | DY     | 7.77                                      | 0.69         | 11.21        | 8.19 | 4.18         | 14.70        | ND           | 13.68        | 2.84         |
|        |          | KG     | 10.73                                     | 1.42         | 15.15        | 7.54 | 9.42         | 6.70         | ND           | 3.73         | 4.12         |
|        |          | KX     | 23.9                                      | 1.53         | 30.74        | 7.39 | 41.90        | 201.55       | 2.97         | 17.73        | 2.37         |
|        |          | ZJ     | 21.3                                      | 1.87         | 21.35        | 7.98 | 7.98         | 4.14         | ND           | 7.08         | 1.41         |
|        | Effluent | BG     | 4.02                                      | 0.16         | 8.89         | 7.45 | ND           | 4.64         | ND           | 2.96         | 0.56         |
|        |          | BJ     | 3.15                                      | 0.05         | 3.88         | 7.72 | ND           | 9.34         | ND           | 3.15         | 0.92         |
|        |          | CB     | 2.19                                      | 0.41         | 10.67        | 7.89 | 8.78         | 16.46        | 2.02         | 10.96        | 4.84         |
|        |          | DY     | 0.22                                      | 0.14         | 7.76         | 7.79 | 2.80         | 4.44         | 5.92         | 4.60         | 5.26         |
|        |          | KG_AO  | 3.94                                      | 0.10         | 6.70         | 7.40 | 0.35         | 22.64        | 0.08         | 10.71        | 13.97        |
|        |          | KG_AAO | 4.09                                      | 0.17         | 5.92         | 8.10 | 5.74         | 6.48         | 0.92         | 11.23        | 24.81        |
|        |          | KX     | 3.70                                      | 0.30         | 9.68         | 7.43 | 2.28         | 5.10         | 0.20         | 18.07        | 3.92         |
|        |          | ZJ     | 4.69                                      | 0.10         | 10.55        | 7.79 | 1.38         | 12.51        | 1.76         | 8.31         | 1.42         |
| Winter | Influent | BG     | 20.79                                     | 2.04         | 22.02        | 8.11 | 15.06        | 324.12       | ND           | 16.23        | 1.46         |
|        |          | BJ     | 26.39                                     | 2.00         | 29.47        | 8.11 | 64.88        | 359.67       | ND           | 7.44         | 2.10         |
|        |          | CB     | 19.46                                     | 1.65         | 37.32        | 7.88 | 9.37         | 22.71        | ND           | 8.85         | 1.27         |
|        |          | DY     | 14.03                                     | 0.71         | 18.12        | 8.21 | 39.36        | 104.04       | 1.11         | 19.39        | 2.27         |
|        |          | KG     | 12.29                                     | 1.53         | 30.44        | 7.94 | 27.13        | 59.09        | 0.62         | 19.39        | 8.30         |
|        |          | KX     | 24.54                                     | 1.70         | 25.41        | 6.97 | 77.05        | 117.68       | 1.67         | 30.40        | 5.83         |
|        |          | ZJ     | 27.80                                     | 1.82         | 28.97        | 8.16 | 95.09        | 195.79       | ND           | 13.56        | 1.27         |
|        | Effluent | BG     | 0.22                                      | 0.20         | 9.44         | 7.71 | 2.18         | 6.56         | ND           | 18.17        | 1.31         |
|        |          | BJ     | 0.19                                      | 0.04         | 8.97         | 7.67 | 8.03         | 27.70        | 0.91         | 13.14        | 2.25         |
|        |          | CB     | 0.58                                      | 0.08         | 10.04        | 8.01 | 13.00        | 7.68         | 0.87         | 5.72         | 1.10         |
|        |          | DY     | 0.27                                      | 0.13         | 6.66         | 7.74 | 7.68         | 25.13        | 1.45         | 17.45        | 4.41         |
|        |          | KG_AO  | 0.21                                      | 0.13         | 8.62         | 7.37 | 16.85        | 358.52       | 0.48         | 20.36        | 8.50         |
|        |          | KG_AAO | 0.22                                      | 0.21         | 7.63         | 7.69 | 8.82         | 364.40       | ND           | 17.64        | 7.00         |
|        |          | KX     | 0.20                                      | 0.03         | 9.98         | 7.81 | 8.55         | 60.36        | ND           | 47.54        | 3.16         |
|        |          | ZJ     | 0.35                                      | 0.71         | 6.79         | 7.27 | 270.44       | 44.51        | 9.23         | 39.25        | 3.33         |

Note: ND, not detected.

Table S10 The detection and quantitation limits for antibiotics

| Antibiotics      |                    | Detection limit (ng/L) | Quantitation limit (ng/L) |
|------------------|--------------------|------------------------|---------------------------|
| Beta-lactams     | Cefotaxime         | 0.51                   | 2.05                      |
|                  | Cefoxitin          | 0.59                   | 2.35                      |
|                  | Cefazolin          | 0.83                   | 3.32                      |
| Sulfonamides     | Sulfabenzamide     | 0.21                   | 0.83                      |
|                  | Sulfafurazole      | 0.35                   | 1.41                      |
|                  | Sulfamethoxazole   | 0.34                   | 1.34                      |
|                  | Sulfamonomethoxine | 0.98                   | 3.91                      |
| Tetracyclines    | Doxycycline        | 0.53                   | 2.12                      |
|                  | Tetracycline       | 0.97                   | 3.87                      |
|                  | Oxytetracycline    | 1.27                   | 5.09                      |
| MLS              | Azithromycin       | 0.11                   | 0.45                      |
|                  | Erythromycin       | 1.51                   | 6.03                      |
|                  | Clarithromycin     | 0.13                   | 0.52                      |
|                  | Clindamycin        | 0.45                   | 1.79                      |
|                  | Lincomycin         | 0.55                   | 2.19                      |
|                  | Roxithromycin      | 0.15                   | 0.59                      |
| Quinolones       | Oxolinic acid      | 0.45                   | 1.78                      |
|                  | Nalidixic acid     | 0.21                   | 0.86                      |
| Fluoroquinolones | Enrofloxacin       | 0.05                   | 0.20                      |
|                  | Moxifloxacin       | 0.76                   | 3.06                      |
|                  | Sirofloxacin       | 0.32                   | 1.30                      |
|                  | Ofloxacin          | 0.46                   | 1.85                      |
|                  | Levofloxacin       | 0.67                   | 2.69                      |
| Chloramphenicols | Chloramphenicol    | 0.48                   | 1.93                      |
|                  | Flofenicol         | 0.59                   | 2.34                      |

MLS, macrolide-lincosamide-streptogramin.