Using metagenomic Hi-C data to discover broad host-range plasmids conferring antimicrobial resistance

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Abstract

Horizontal gene transfer (HGT) trough plasmids is one of the main contributors to the rapid increase of antimicrobial resistance (AMR). Studying wastewater from wastewater treatment plants (WWTPs) allows us new insights into HGT as bacteria from different sources come together. Currently the analysis of HGT is limited, as plasmids cannot be linked to their host species with only metagenomic samples, however when combined with Hi-C sequencing data, sequences from the same cell can be linked together.

We developed a method that uses metagenomic Hi-C data to link bacterial genera together with detected plasmid consensus clusters and resistance genes. Using this method, we analysed datasets from two sources: activated sludge put into a reactor with an antibiotic pressure and a WWTP entrance. The activated sludge dataset was sequenced at two timepoints with an increasing antibiotic concentration. This allowed us to compare degrees of antibiotic resistance in different antibiotic pressures as well as detect broad host-range resistant plasmids. We detected an increase in acquired resistance in environments with a higher antibiotic pressure and detected a resistant plasmid in both locations, linked to both pathogenic as well as bacteria found in active sludge.

Introduction

Infectious bacteria have acquired antimicrobial resistance (AMR) genes, which has grown into a global health crisis (World Health Organization, 2012). AMR is estimated to be associated with 4.95 million deaths in 2019 (Murray et al., 2022), partly due to its fast transfer within bacterial communities through horizontal gene transfer (HGT), which allows genes to transfer between bacterial lineages (Baker et al., 2018). The main contributor of the spread of AMR through HGT are plasmids (Dimitriu, 2022). Plasmids are extrachromosomal DNA containing a set of genes that can confer beneficial traits to their bacterial host, including antibiotic resistance genes (ARG). Therefore, it is important to know how AMR can spread via plasmids between bacterial lineages, as knowing which specific plasmids spread AMR can help with the targeting of specific plasmids to control the spread of AMR (Vrancianu et al., 2020). However, currently only limited research has been done on linking plasmids to their bacterial hosts (Stalder et al., 2019) in metagenomic datasets.

Wastewater treatment plants can serve as a treasure-trove of information of AMR, as they function as a hotspot where ARGs accumulate (Guo et al., 2017). The water from wastewater treatment plants comes from different environmental sources like medical, agricultural, and industrial. Which leads to a good data source for research, as wastewater can be used for measuring the amount of AMR coming from different environments, allowing for surveillance for AMR spread. The surveillance of AMR in combination with treatment of wastewater can play an important role in the control of AMR spread (Nguyen et al., 2021).

Currently a lot of research has been done on AMR in metagenomic wastewater datasets (G. Chen et al., 2022; Chu et al., 2018; Garrido-Cardenas et al., 2017; Guo et al., 2017). However, while binning methods do exist for clustering contigs from the same species together for metagenomic data, these methods use read depth and sequence similarity to link contigs together (Kang et al., 2015; Wu et al., 2016). As chromosomal and plasmidic reads differ in read depth and sequence similarity, these binning methods often fail to link plasmid contigs together with chromosomal contigs from the same host species.

By using Hi-C sequencing in combination with metagenomic sequencing, we can add proximity data to our dataset, allowing us to link sequences from the same cell together (Press et al., 2017a). Hi-C sequencing links DNA fragments together not by location on the genome, but by their location physically (Burton et al., 2014). This technique allows us to link plasmids to their host and determine which plasmids are able to replicate in the same host cell (Ivanova et al., 2022).

In this study we developed a method to detect broad host-range resistant plasmids in metagenomic Hi-C datasets originating from WWTPs. We use datasets from two different sources: from activated sludge put in a controlled reactor sequenced at two different levels of kanamycin concentrations and from the entrance of a WWTP. Activated sludge is used in a complex process that uses microbes to remove substrates from wastewater in WTTPs (Orhon, 2015). From these datasets we try to detect broad host-range resistant plasmids and if bacteria used in activated sludge can host resistant plasmids, which could aid the spread of AMR. Additionally, we analyse the detected resistance between the datasets to find if an antibiotic concentration changes the amount of acquired resistance.

Methods

Our method as can be seen in Figure 1 can be divided up into three parts, the Hi-C pipeline, classification, and visualization. The Hi-C pipeline consists of the necessary steps to build metagenomic assembled genomes (MAGs) from shotgun and hi-c sequencing reads. From the MAGs we can further classify the sequences to find genus, plasmid and AMR gene clusters and link them together to visualize them into a graph.

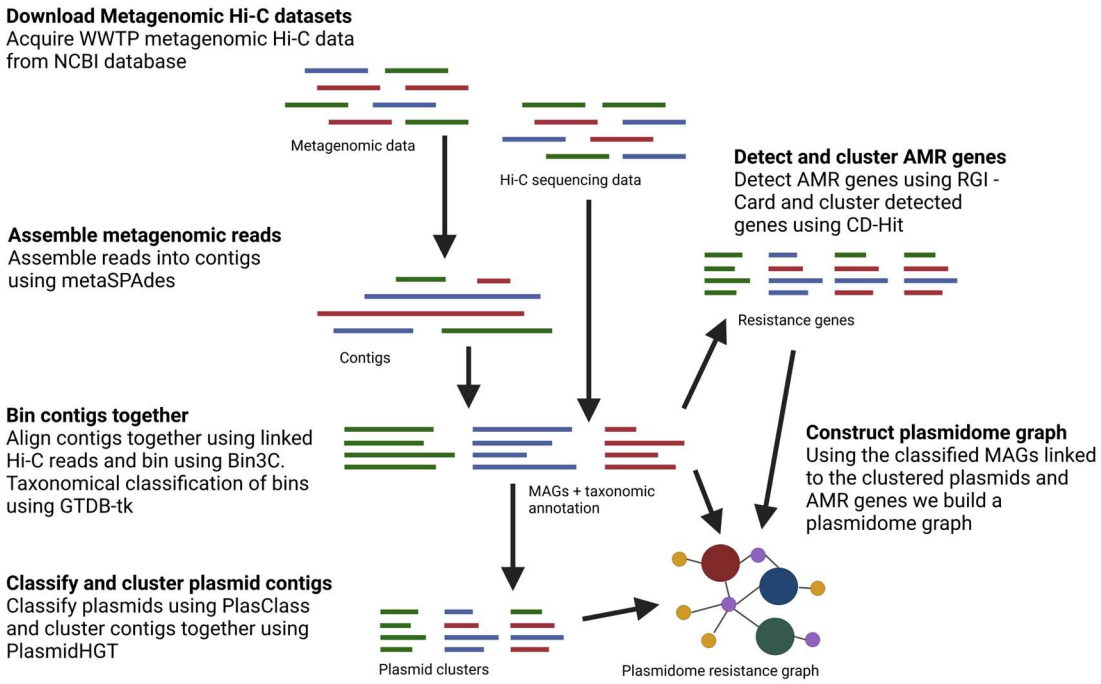

Figure 1: Overview of the methodology. We use two data types for each dataset, metagenomic and Hi-C sequencing data. This allows us to link assembled contigs from the same cell together into MAGs. By detecting and clustering plasmids contigs and resistance genes found in each MAG, we can build a plasmidome resistance graph.

Dataset

Each dataset consists of two parts, metagenomic shotgun sequencing data and spatial Hi-C sequencing data. The first dataset we use is sludge harvested from a wastewater treatment plant in West-Amsterdam put into a controlled reactor and spiked with a plasmid and then observed at different concentrations of kanamycin, an antibiotic solution, at different concentrations (Calderón-Franco et al., 2022). The controlled reactor was sampled at two timepoints with an increasing kanamycin concentration, at 2.5mg/L and at 50mg/L, we refer to these datasets as the low and high concentration datasets respectively. The difference in antibiotic concentrations allows us to study the differences in AMR both intrinsic and acquired. Additionally, we analysed a dataset originating from the Moscow WWTP in Idaho (USA) from the entrance of the WWTP (Stalder et al., 2019). This additional dataset offers us a sample that we can compare the reactor dataset to, as this dataset does not contain activated sludge or an antibiotic concentration.

We controlled the quality of the sequenced reads by using FastQC (Andrews, 2010) to analyse the quality of the reads and used FastP (S. Chen et al., 2018) to trim the reads to improve read quality.

Hi-C pipeline

We use a Hi-C pipeline to construct MAGs from the metagenomic and Hi-C reads. The first step in the pipeline is the assembly of the metagenomic reads into contigs using metaSPAdes (Nurk et al., 2017). Once assembled the contigs are mapped against the Hi-C reads to find links using BWA-MEM (Li, 2013), pairing of the reads are done using SAMBLASTER (Faust & Hall, 2014) and filtering of the reads are done using samtools (Li et al., 2009). Spatial linkage between contigs can be inferred by using the links found using the paired Hi-C data in combination with the contigs. This data is then used in combination with the contigs in order to cluster contigs together into MAGs based on their Hi-C linkage using Bin3C (DeMaere & Darling, 2019).

Classification

From the MAGs we extract three types of clusters: genus, plasmid, and antimicrobial resistance. Genus information is gathered by using a taxonomical analysis on the MAGs using GTDB-tk (Chaumeil et al., 2020). We labelled the MAGs on genus level based on the taxonomical analysis.

Plasmid consensus clusters are built by classifying plasmid contigs from the MAGs and then clustering similar plasmids together. For the classification of plasmid contigs PlasClass (Pellow et al., 2020) was used with threshold of 0.7. The identified plasmid contigs were then clustered on structural and gene similarity using PlasmidHGT (Teixeira et al., 2023) and put into plasmid consensus clusters. Plasmid clusters were then further analysed using MOB-suite (Robertson & Nash, 2018). The AMR genes were found by using Card - RGI (Alcock et al., 2020) to identify AMR genes on the contigs. The identified genes were clustered based on nucleotide similarity using CD-Hit (Fu et al., 2012) to bin genes from different contigs together.

Visualization

As all the clusters from the MAGs can be linked back to the contigs inside of the MAGs the construction of the plasmid resistance graph is straightforward. Every cluster becomes a node in the graph. And an edge is drawn between the nodes if they are linked, for example: an AMR gene on a contig or a plasmid contig in a MAG. In our visual graph we colour the genera based on their phylogenetic order, plasmids purple and resistance genes red.

Results

We analysed three datasets, two of which are from a controlled reactor containing wastewater sludge with increasing antibiotic concentrations and the other from the entrance of a wastewater plant. The wastewater entrance dataset allows us to infer differences between the wastewater at the entrance of a wastewater treatment plant and that of activated sludge. However, since the datasets are sampled from different sources and an antibiotic concentration is added to the controlled reactor dataset no concrete conclusions can be made.

Resistome analysis

How much resistance do we detect in genera?

By analysing the linked AMR genes of genera and their plasmids we can compare the datasets on their detected AMR. In Table 1 we compare the datasets on the percentage of genera that have at least one ARG linked, genera with more than one ARG and resistance against multiple antibiotics, and genera with acquired resistance.

From Table 1 we can make four observations. (1) The low kanamycin dataset has the highest percentage of genera that have at least one resistance type. This could indicate that in the lower concentration dataset the selective pressure is lower, which allows genera to survive with a low number of AMR genes. (2) The high kanamycin dataset has the highest percentage of genera that are multi resistant as well as the most acquired resistance. This indicates that a higher degree of resistance genes as well as acquired resistance plays an important role in the survival of bacteria in an environment with a high antibiotic concentration. (3) The combined dataset averages out most of the values that we find for the low and high concentration datasets, this is to be expected as it combines the low and high concentration datasets. (4) The wastewater dataset has similar values for resistant and multi-resistant genera, however, has a lower value for acquired resistance found. This is not an unexpected result as it has been shown that the transconjugation rate of plasmids is lower in an environment with no antibiotic pressure (Schuurmans et al., 2014).

	Low kanamycin	High kanamycin	Combined	Wastewater
resistant	91.3%	73.1%	78.4%	75.4%
multi resistant	60.9%	69.2%	62.2%	54.4%
acquired resistance	30.4%	38.5%	35.1%	3.5%

Table 1: Comparison of the percentage of genera with resistance between the different datasets. The percentages are calculated by the number of genera with a type of resistance divided by the total number of genera detected.

How much resistance do we detect in plasmids?

From the resistance genes linked to the plasmid contigs we can calculate the amount of resistance on plasmids as can be seen in Table 2. The amount of resistance does not vary much between datasets, with around 3.5% of plasmids containing at least 1 ARG and around 1.5% being multi-resistant. With the high kanamycin dataset containing a little more resistance on plasmids than the low concentration dataset. A clear increase can be seen between the amount of broad host-range plasmids of the low and high kanamycin dataset. The increase of resistant broad host-range plasmids in the higher concentration kanamycin dataset can be explained by a higher selective pressure over a longer period which leads to an increase in HGT (Rensing et al., 2002). In the wastewater dataset we again see many of the values remaining similar between the datasets, except for the relative amount of broad host-range plasmids. The relative amount of broad host-range plasmids can be explained by the increased number of genera and decreased number of plasmids detected in the wastewater dataset. With only

149 plasmids and 114 genera detected in the wastewater dataset, instead of the 696 plasmids and 37 genera in the combined dataset.

	Low kanamycin	High canamycin	Combined	Wastewater
resistant	3.0%	3.3%	3.4%	3.4%
multi resistant	1.3%	1.2%	1.5%	2.0%
broad host-range	3.2%	6.1%	8.6%	13.4%
broad host-range resistant	0.4%	1.0%	1.1%	0.7%

Table 2: Comparison of the percentage of plasmids with resistance between the different datasets. The resistance percentages are calculated by the number of plasmids with a type of resistance divided by the total number of plasmid clusters. Broad host-range is defined as plasmids that are linked to two or more distinct genera.

Broad host-range resistant plasmids

We were able to detect 8 plasmids conferring resistance to multiple genera which can be seen in Table 3, of which 4 have multiple resistance genes. Using MOB-suite we did a further analysis of the MOB-, MPF- and Inc-type of the broad host-range resistant plasmids. Due to limited amounts of contigs or limited number of plasmids known to MOB-suite not all plasmids were able to be classified. Additional information on each resistance gene can be found in the Supplementary table B.

Plasmid	Detected host-range	Plasmid typing	Resistance gene	Gene family	Gene name
p_1*	*Runella, Zoogloea*	MOBP, MPF_T	r_94	TLA beta-lactamase	TLA-2
			r_123	OXA beta-lactamase	OXA-17
			r_131	OXA beta-lactamase	OXA-x**
			r_154	TLA beta-lactamase	TLA-2
			r_170	OXA beta-lactamase	OXA-x**
p_46	*Kaistia, Bosea, Agrobacterium, Aminobacter*	MPF_T	r_1	resistance-nodulation-cell division (RND) antibiotic efflux pump	adeF
p_54	*Bosea, Agrobacterium*	MOBQ, MPF_T	r_123	OXA beta-lactamase	OXA-17
			r_168	AAC(3)	AAC(3)-Ia
p_60	*Aquabacter, Kaistia, Bosea, Agrobacterium*	-	r_1	resistance-nodulation-cell division (RND) antibiotic efflux pump	adeF
p_63	*Ensifer, Gemmobacter, Agrobacterium, UBA1943*	-	r_103	RCP beta-lactamase	RCP-1
p_143*	*Aquabacter, Kaistia, Acidovorax, Zoogloea*	-	r_119	APH(3')	APH(3')-Ib
p_165	*Gemmobacter, Kaistia, Bosea, UBA1943*	-	r_1	resistance-nodulation-cell division (RND) antibiotic efflux pump	adeF
			r_53	major facilitator superfamily (MFS) antibiotic efflux pump	floR
			r_59	major facilitator superfamily (MFS) antibiotic efflux pump	tet(A), tet(C), tet(D)
p_233	*Enterobacter, Kaistia, Zoogloea*	MOB_Q, MPF_F, IncFIB	r_45	major facilitator superfamily (MFS) antibiotic efflux pump	tet(A), tet(C), tet(D)
			r_57	major facilitator superfamily (MFS) antibiotic efflux pump	tet(A), tet(C), tet(D)
			r_59	major facilitator superfamily (MFS) antibiotic efflux pump	tet(A), tet(C), tet(D)
			r_112	APH(6)	APH(6)-Id
			r_120	sulfonamide resistant sul	sul2
			r_125	APH(3'')	APH(3'')-Ib

Table 3: Table with broad host-range resistant plasmids, their linked genera, MOB-suite typing and detected resistance genes with gene family and gene name. *Plasmid confers resistance to kanamycin **Multiple OXA genes classified, for the whole list look at Supplementary Table B.

Furthermore all broad host-range resistant plasmids have been looked up using the PLSDB database (Galata et al., 2019), to see if any plasmids are known and annotated within the database. We used

search strategy Mash screen with the default parameters. From this lookup only two plasmids had search results: p_1 and p_233.

Synteny analysis broad host-range resistant plasmids consensus clusters

To further analyse the contigs that make up the consensus clusters we visualised the contigs using the GenBank annotations generated using Prokka (Seemann, 2014). The generated synteny visualizations can be found in the Supplementary Figures A-1 to A-8. It becomes apparent when analysing the figures that there is a high variation in amount of contigs per consensus cluster as well as the length of each contig. Additionally, some consensus clusters have genera linked with only a few small contigs, like p_1 with *Zoogloea* which only contains 3 smaller contigs. This could lead to some uncertainty whether the plasmid should be linked to a genus. Currently we assume that if a contig is linked to a plasmid consensus cluster the genus is linked to the plasmid, however the certainty could be improved by scoring the links based on the amount and the length of linked contigs.

Beta lactamase plasmids

Plasmid p_1 and p_54, as can be seen in Figure 2 A and B respectively, confer beta-lactamase genes, none of which were found on the reference genome of any of the linked genera. It is known that plasmids can confer beta-lactamase for a long time as it has been found that plasmids have spread beta-lactamase for millions of years (Barlow & Hall, 2002). As such it is not surprising to find two beta-lactamase conferring plasmids within our dataset.

The host-range was reported as *Runella* genus for p_1 and *Rhizobiales* for p_54 this corresponds to what we were able to find in our datasets as well, as p_1 is linked to the *Runella* genus as well as the *Zoogloea* genus which MOB-suite does not find within host-range. The host-range classification for plasmid p_54 does match up with our findings, with both *Agrobracterium* and *Bosea* being genera of the order *Rhizobiales*.

One beta-lactamase resistance gene was found on p_63, however only on one contig contains this gene, additionally this gene was found on the *Gemmobacter* reference genome as well. For this reason, we assume that p_63 likely contains a misclassified contig. This could be a misclassified contig that ended up into a plasmid cluster or gene has been shared with plasmid by host.

Looking up plasmid p_1 using PLSDB resulted in two hits: one from an *Acinetobacter sp.* and the other from a *Corynebacterium xerosis* bacteria. The only linked genera sharing a taxonomic classification lower than domain are *Zoogloea* and *Acinetobacter.* This indicates that plasmid p_1 has a very broad host-range.

Plasmid p_233

Plasmid p_233 was found in both the low concentration reactor dataset as well as the wastewater dataset, with both conferring the same six resistance genes as can be seen in Figure 2C and D. With the datasets being sequenced at different continents, Europe, and North America this indicates that this plasmid is widespread. The host-range of the plasmid is likely at phylum level as it is linked to both pathogens as well as genera used in activated sludge from the *Pseudomonadota* phylum: *Enterobacter*, *Pseudomonas*, *Escherichia*, *Kaistia* and *Zoogloea*. MOB-suite only classifies this plasmid as Enterobacterales order level host-range which is narrower than what we find in our datasets.

Zoogloea and *Agrobacterium* are both aerobic bacteria that are used in activated sludge in WWTP. This is concerning because these plasmids can spread from both aerobic WWTP bacteria as well as pathogens. The resistance found on the plasmid consists of three types: tetracycline, sulphonamide, and streptomycin.

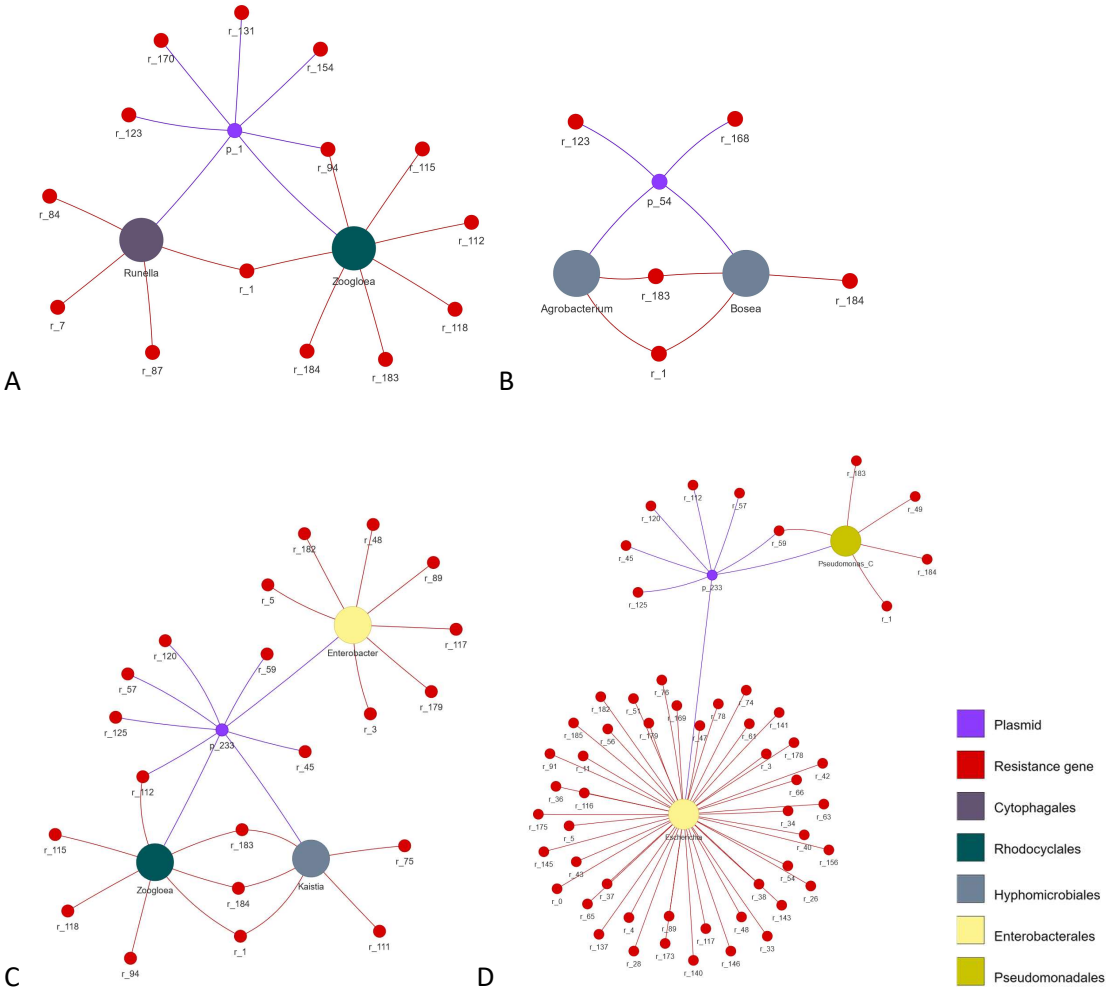

Figure 2: Plasmidome resistance networks of three broad host-range resistant plasmids. (A) Plasmid p_1 confers beta-lactamase genes to the *Runella* and *Zoogloea genera*. (B) Plasmid p_54 confers beta-lactamase resistance to the *Agrobacterium and Bosea genera*. (C) Plasmid p_233 from the reactor dataset confers tetracycline, sulphonamide, and streptomycin resistance to *Enterobacter, Kaistia and Zoogloea*. (D) Plasmid p_233 from the wastewater dataset confers tetracycline, sulphonamide, and streptomycin resistance to *Escherichia* and *Pseudomonas.*

The lookup using PLSDB for plasmid p_233 resulted in 6 hits, of which two were found in *E. coli*, three were found in *K. pneumonia* and one in *Acinetobacter baumanii.* In literature no link has been found so far between this plasmid and *Enterobacter Kaistia* or *Zoogloea*. In one of the studies this plasmid has been shown to confer beta lactamase, sulphonamide and tetracycline resistance (Baron et al., 2020). While we do not detect any beta lactamase genes, we do detect sulphonamide and tetracycline resistance genes, which consistent with their findings. With this plasmid being found in both datasets we sequenced as well as in other studies, we can assume that this plasmid is a common broad host-range resistant plasmid.

Is there a difference in kanamycin resistance in the reactor dataset?

Since kanamycin was added to the reactor of the reactor dataset, we performed an additional analysis of the difference of kanamycin resistance genes as can be seen in Table 4. We detect that broad host-range plasmid p_143 has spread from Zoogloea to *Acidovorax, Aquabacter* and *Kaistia*. What should be noted is that in the high kanamycin dataset we do not detect p_143 in *Zoogloea*, this is most likely due to the misclassification of a contig as either plasmid or chromosome, as intrinsic resistance is detected in *Zoogloea* in the high kanamycin dataset. However, the spread of plasmid p_143 does suggest that HGT has taken place between the sequencing of the low and high kanamycin datasets.

	Low kanamycin	High kanamycin
genera intrinsic	*Hydrogenophaga*	*Runella, Zoogloea*
genera acquired	p_1: *Runella, Zoogloea* p_143: *Zoogloea* p_615: *Zoogloea*	p_1*: Runella* *p_143: Acidovorax, Aquabacter, Kaistia*

Table 4: Comparison genera with kanamycin resistance between the low and high kanamycin concentration datasets. The acquired resistance is shown by the plasmid followed by the genera linked to the plasmid.

Do bacteria used in activated sludge aid the spread of AMR?

The activated sludge process used in WWTPs is a complex system using microbes to remove substrates from the wastewater (Orhon, 2015). By introducing bacteria into an environment by the way of activated sludge it could be possible that some can host broad host-range resistant plasmids and aid the spread of AMR. All eight broad host-range plasmids found in the reactor dataset confer resistance to bacteria that are known to be found in activated sludge. The bacteria found in activated sludge are: *Acidovorax* (Schulze et al., 1999), *Agrobacterium* (McAuliffe et al., 1990), *Aminobacter* (van Bergen et al., 2021), *Ensifer* (Park & Oh, 2020)*, Kaistia* (Lee et al., 2007), *Runella* (Ryu et al., 2006) and *Zoogloea* (Shao et al., 2009). *Kaistia* and *Zoogloea* are linked to plasmid p_233 which is linked to pathogenic bacteria as well. This shows that bacteria used in sludge can host broad host-range resistant plasmids, which could aid the spread of AMR to pathogenic bacteria.

Discussion

To detect and analyse broad host-range plasmids, we developed a method that can link plasmids to host genus by using metagenomic and Hi-C sequencing data. By combining Hi-C sequencing together with metagenomic sequencing, we can link reads from the same cell together, which would not be possible with only metagenomic sequencing data. Our analysis focuses on AMR genes found in both plasmids and bacteria, and using our method we were able to detect multiple broad host-range resistant plasmids. This has allowed us to better analyse HGT of resistant plasmids by using Hi-C data to link plasmid contigs to chromosomal contigs.

We were able to detect 8 broad host-range resistant plasmids using our method, of which one was detected in both the reactor as well as the wastewater dataset. This plasmid, plasmid p_233, has 6 resistance genes that confer resistance for tetracycline, sulphonamide, and streptomycin. The host-range is broad as well, with species from different phylogenetic classes within the *Pseudomonadota* phylum. Within this host-range both pathogenic as well as bacteria used in activated sludge reside. This indicates that bacteria in activated sludge can play a role in the spread of AMR, and this should be examined further. However, in environments with no antibiotic pressure this role might be negligible.

By combining datasets, we were able to get a better picture of plasmid host-ranges, as datasets do not contain the full host-range for each plasmid, combining allows us to link more genera to plasmids that might not have been detected otherwise. Our analysis can help paint a clearer picture of what genera can host resistant plasmids, and how it spreads within bacterial communities. Which could help with selecting bacteria that can be used for activated sludge in WWTPs as well as finding specific plasmids to target to control the spread of AMR.

By analysing the amount of resistance in the two datasets with different kanamycin concentrations we find that in an environment with a higher kanamycin concentration more multi-resistant genera and more acquired resistance is present. This finding is in line with other studies done that found that

found that the rate of transconjugation increases in sublethal antibiotic pressures (Schuurmans et al., 2014).

Hi-C deconvolution has been used before to link plasmid to bacteria in MAGs and AMR analysis was performed on the MAGs. In most studies only AMR analysis has been done on the MAGs and no HGT was analysed (Ivanova et al., 2022; Press et al., 2017b; Stalder et al., 2019). In one other study HGT was studied using Hi-C data for gut microbiome and could identify gene links between MAGs, while this study does differentiate between plasmid an chromosome contigs, it does not cluster plasmids together (Kent et al., 2020). In our method we can link resistance to specific plasmids and use the Hi-C links to analyse possible HGT events.

The main limitation of this method is the reliance on many tools for our pipeline which reduce specificity. Binning methods are limited by the number of Hi-C links, as not all contigs have Hi-C links, which can lead to contigs not being used further into the method. This results in less contigs being used but does offer a higher confidence that contigs are linked together. When classifying the bins using GTDB-tk not all bins are classified which also reduces the amount of usable data, as only classified bins can offer information about host-range. The plasmid clustering can link similar plasmids together, but it is not required for the plasmid contigs to be identical, which might lead to plasmids being linked to more genera than they are. However, the overlap in plasmid-host links between the two datasets from the same source indicates that the method used for plasmid clustering works well. Another limitation outside of our method is the low availability of datasets that have both metagenomic and Hi-C sequencing data. This limits the amount of research we can do using this method. With a greater availability of datasets plasmid host-ranges and the HGT of ARGs can be analysed by combining datasets together into a more complete plasmidome graph. Datasets from other sources like gut microbiome or other clinical settings could also give more insight into resistant plasmids as well as HGT in an environment with an antibiotic pressure.

Conclusion

Pathogenic bacteria acquiring resistance against antibiotics is a growing global health concern, which is driven by the overuse of antibiotics (Amann et al., 2019). To better control the spread of AMR a good understanding of the HGT of AMR is needed (Kessler et al., 2023). However, currently little research has been performed on finding broad host-range resistant plasmids that play a key role in the spread of AMR in bacterial communities. For this study we chose WTTPs as the source for our datasets as WTTPs function as hotspots for AMR as water from different sources gather here.

In this study we offer a method that can use Hi-C links to link plasmids to their host to build a plasmidome network. By detecting AMR genes, we can find instances of intrinsic and acquired resistance. The detection of broad host-range plasmids is performed by clustering plasmids together into plasmid consensus clusters based on their structural and gene similarities. Using the before mentioned method we analysed datasets from two different sources: activated sludge in a controlled reactor with an antibiotic pressure and from the entrance of a WWTP.

When comparing the datasets on the amount of resistance found, we detected a higher degree of acquired resistance in the datasets with a higher antibiotic pressure. This is in line with other studies found on the effects of antibiotic pressure on the transconjugation of plasmids.

We found eight broad host-range resistant plasmids, of which one was detected in datasets from different sources and which host-range consisted of pathogens as well as bacteria used in activated sludge. This indicates that the bacteria used for wastewater treatment could aid the spread of AMR to pathogenic bacteria. However, as the detected HGT of AMR does seem to be linked to the antibiotic

pressure found in the environment the health concerns for using these bacteria in activated sludge can be moderate if antibiotics are removed from wastewater.

By better understanding which plasmids play a role in spreading AMR through bacterial communities we hope that this can help with finding solutions to controlling the spread of AMR by targeting specific broad host-range plasmids. Additionally, this study reiterates the importance of limiting the use of antibiotics as this is one of the driving factors of spreading AMR.

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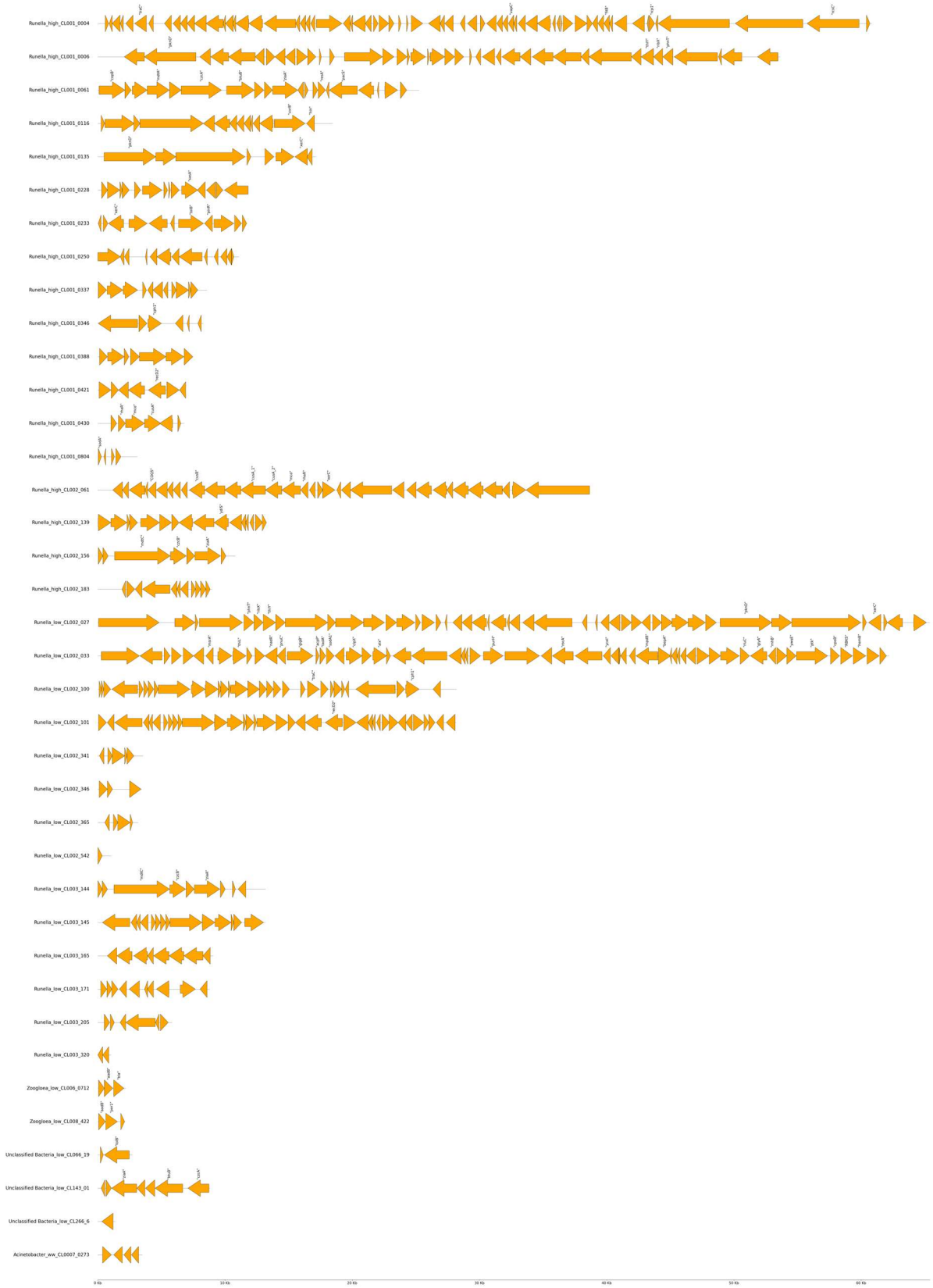
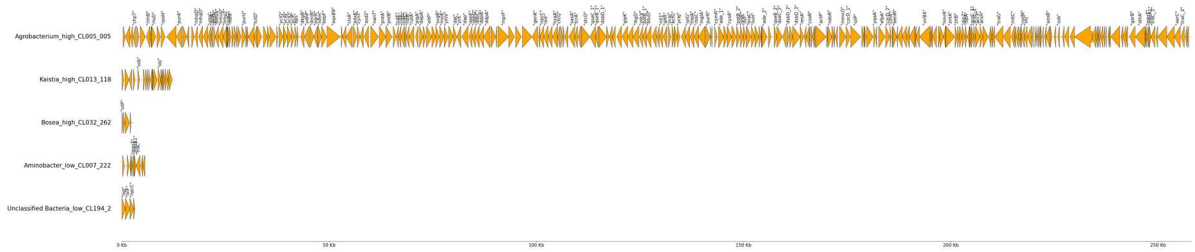
Supplementary

A. Broad host-range resistant plasmids

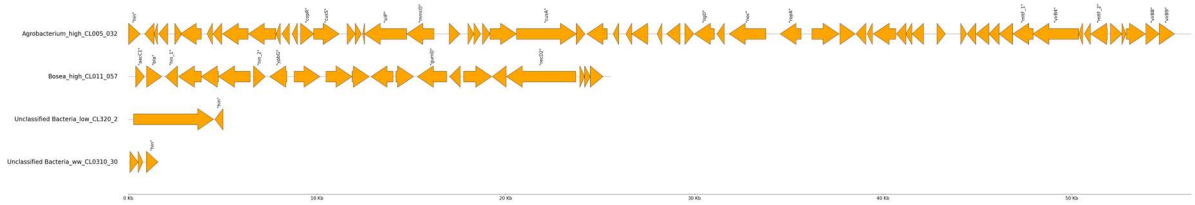
1. p_1



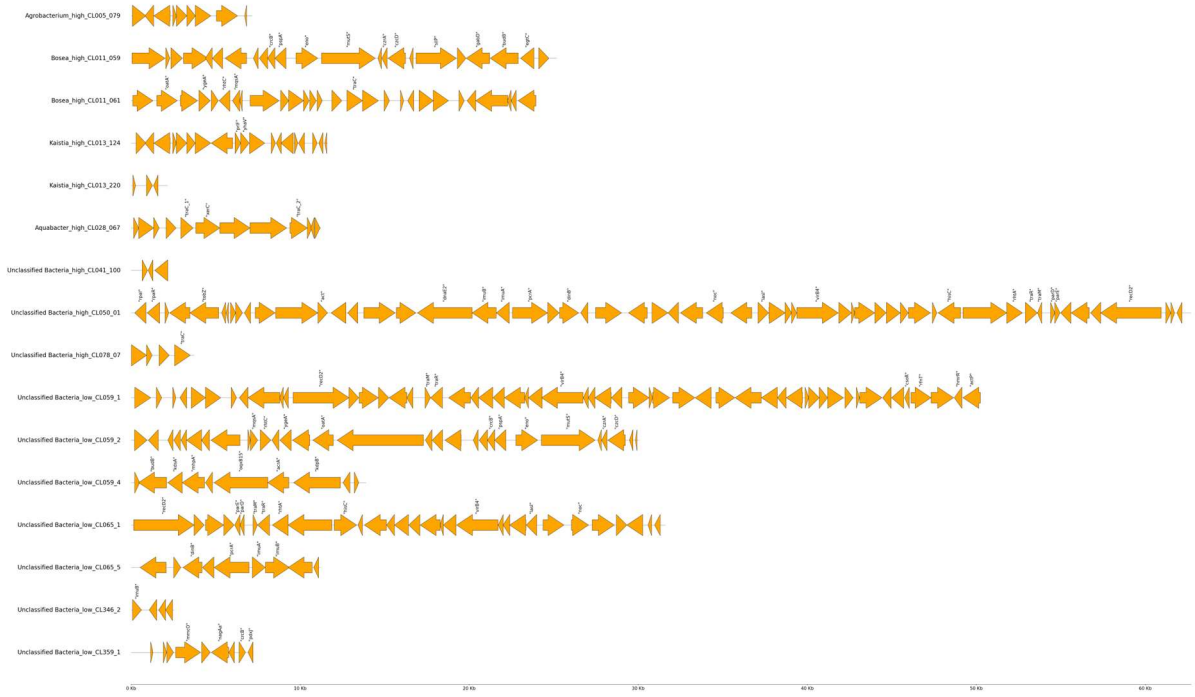
2. p_46



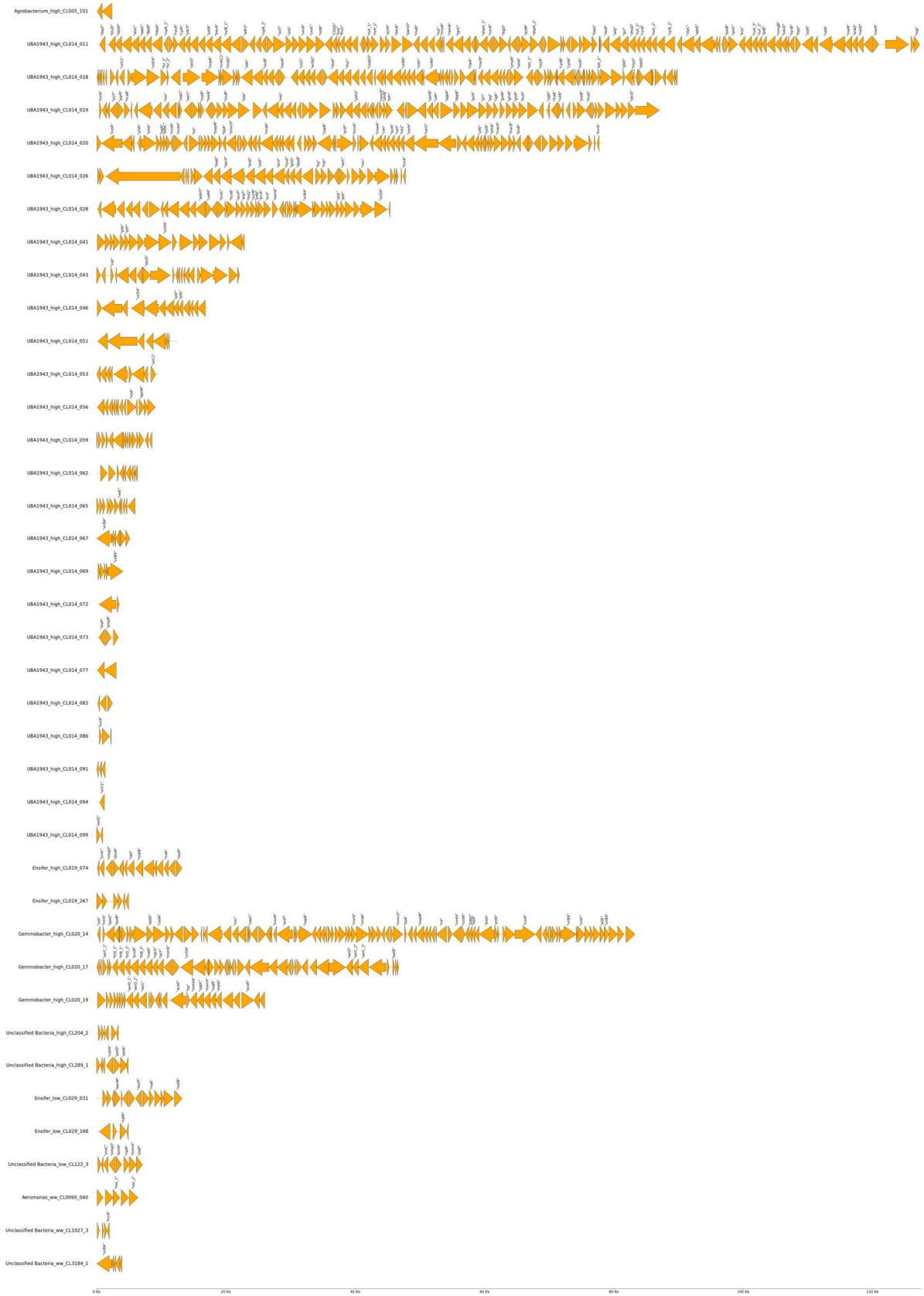
3. p_54



4. p_60



5. p_63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UBA1943_high_CL014_011
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UBA1943_high_CL014_020
UBA1943_high_CL014_026
UBA1943_high_CL014_028
UBA1943_high_CL014_041
UBA1943_high_CL014_043
UBA1943_high_CL014_046
UBA1943_high_CL014_051
UBA1943_high_CL014_053
UBA1943_high_CL014_056
UBA1943_high_CL014_059
UBA1943_high_CL014_062
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UBA1943_high_CL014_077
UBA1943_high_CL014_082
UBA1943_high_CL014_086
UBA1943_high_CL014_091
UBA1943_high_CL014_094
UBA1943_high_CL014_099
Ensifer_high_CL019_074
Ensifer_high_CL019_267
Gemmobacter_high_CL020_14
Gemmobacter_high_CL020_17
Gemmobacter_high_CL020_19
Unclassified Bacteria_high_CL204_2
Unclassified Bacteria_high_CL289_1
Ensifer_low_CL029_031
Ensifer_low_CL029_168
Unclassified Bacteria_low_CL122_3
Aeromonas_ww_CL0060_040
Unclassified Bacteria_ww_CL1027_3
Unclassified Bacteria_ww_CL3184_1
0 Kb
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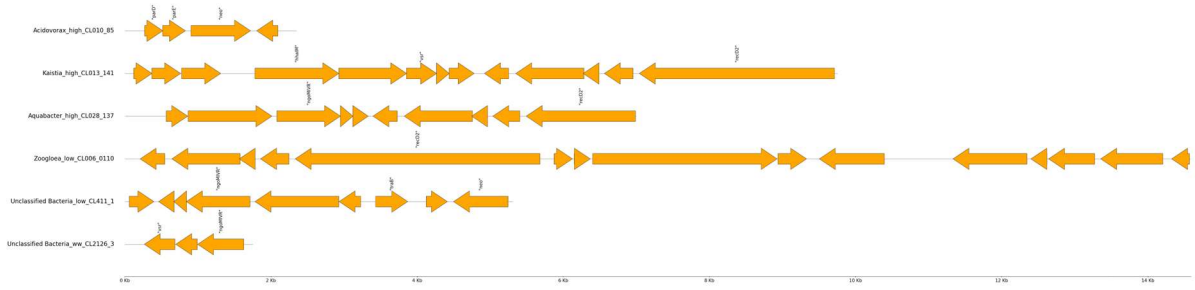
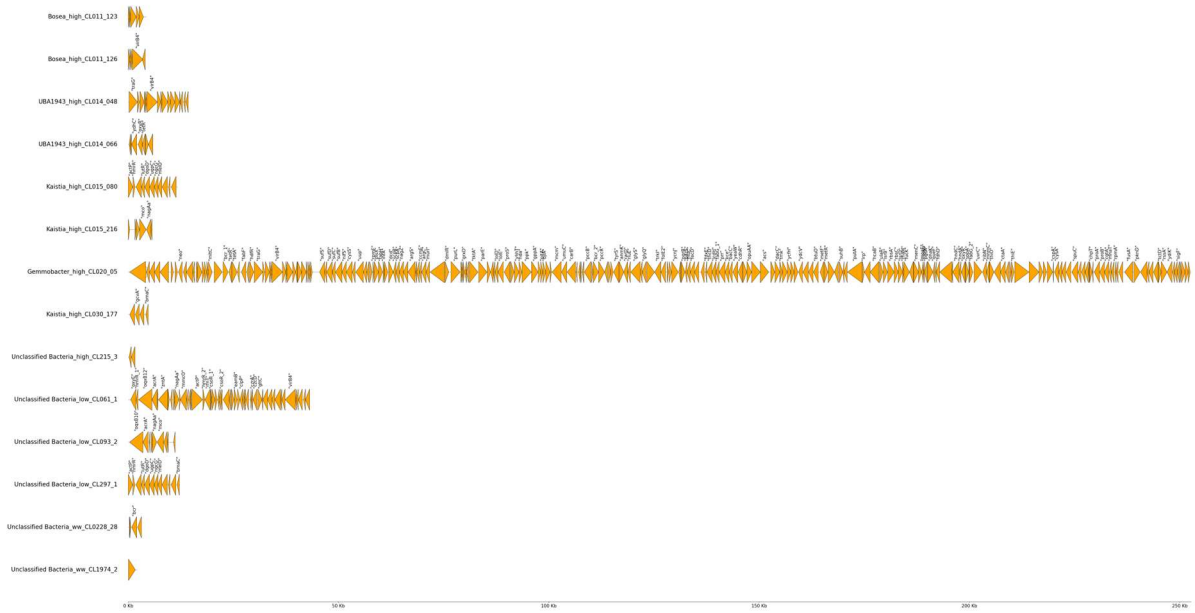
40 Kb
60 Kb
80 Kb
100 Kb
120 Kb

6. p_143


Acidovorax_high_CL010_85
Kaistia_high_CL013_141
Aquabacter_high_CL028_137
Zoogloea_low_CL006_0110
Unclassified Bacteria_low_CL411_1
Unclassified Bacteria_ww_CL2126_3
0 Kb
2 Kb
4 Kb
6 Kb
8 Kb
10 Kb
12 Kb
14 Kb

7. p_165


Bosea_high_CL011_123
Bosea_high_CL011_126
UBA1943_high_CL014_048
UBA1943_high_CL014_066
Kaistia_high_CL015_080
Kaistia_high_CL015_216
Gemmobacter_high_CL029_05
Kaistia_high_CL030_177
Unclassified Bacteria_high_CL215_3
Unclassified Bacteria_low_CL061_1
Unclassified Bacteria_low_CL093_2
Unclassified Bacteria_low_CL297_1
Unclassified Bacteria_ww_CL0228_28
Unclassified Bacteria_ww_CL1974_2
0 Kb
50 Kb
100 Kb
150 Kb
200 Kb
250 Kb

8. p_233

B. Resistance gene annotation

Gene cluster	Gene family	Antibiotic	Gene name
r_0	resistance-nodulation-cell division (RND) antibiotic efflux pump, major facilitator superfamily (MFS) antibiotic efflux pump	cloxacillin, erythromycin, oxacillin, tetracycline, norfloxacin	evgA, emrY, emrK, evgS
r_1	resistance-nodulation-cell division (RND) antibiotic efflux pump	tetracycline	adeF
r_2	resistance-nodulation-cell division (RND) antibiotic efflux pump	cefepime, tetracycline, erythromycin	OprZ, AxyY, AxyX, adeF
r_3	resistance-nodulation-cell division (RND) antibiotic efflux pump	novobiocin	mdtC, mdtB, mdtA
r_4	resistance-nodulation-cell division (RND) antibiotic efflux pump	cloxacillin, erythromycin, oxacillin, norfloxacin	gadW, gadX, mdtE, mdtF
r_5	resistance-nodulation-cell division (RND) antibiotic efflux pump	novobiocin	mdtC, mdtB, mdtA
r_6	ATP-binding cassette (ABC) antibiotic efflux pump		LptD
r_7	glycopeptide resistance gene cluster, vanT	vancomycin	vanT gene in vanG cluster
r_8	Van ligase, vanY, vanT, vanXY, glycopeptide resistance gene cluster	vancomycin, teicoplanin	vanXY gene in vanG cluster, vanC, vanN, vanT gene in vanC cluster, vanXY gene in vanC cluster, vanY gene in vanM cluster, vanXY gene in vanL cluster, vanTr gene in vanL cluster, vanY gene in vanG cluster, vanY gene in vanA cluster, vanXY gene in vanE cluster, vanTm gene in vanL cluster, vanG, vanL, vanXY gene in vanN cluster, vanY gene in vanB cluster, vanT gene in vanN cluster, vanT gene in vanG cluster, vanT gene in vanE cluster, vanY gene in vanF cluster, vanE
r_9	Van ligase, vanY, vanT, vanXY, glycopeptide resistance gene cluster	vancomycin, teicoplanin	vanXY gene in vanG cluster, vanC, vanN, vanT gene in vanC cluster, vanXY gene in vanC cluster, vanY gene in vanM cluster, vanXY gene in vanL cluster, vanTr gene in vanL cluster, vanY gene in vanG cluster, vanY gene in vanA cluster, vanXY gene in vanE cluster, vanTm gene in vanL cluster, vanG, vanL, vanXY gene in vanN cluster, vanY gene in vanB cluster, vanT gene in vanN cluster, vanT gene in vanG cluster, vanT gene in vanE cluster, vanY gene in vanF cluster, vanE
r_10	Van ligase, vanY, vanT, vanXY, glycopeptide resistance gene cluster	vancomycin, teicoplanin	vanXY gene in vanG cluster, vanC, vanN, vanT gene in vanC cluster, vanXY gene in vanC cluster, vanY gene in vanM cluster, vanXY gene in vanL cluster, vanTr gene in vanL cluster, vanY gene in vanG cluster, vanY gene in vanA cluster, vanXY gene in vanE cluster, vanTm gene in vanL cluster, vanG, vanL, vanXY gene in vanN cluster, vanY gene in vanB cluster, vanT gene in vanN cluster, vanT gene in vanG cluster, vanT gene in vanE cluster, vanY gene in vanF cluster, vanE
r_11	major facilitator superfamily (MFS) antibiotic efflux pump	acriflavine, puromycin	mdtP, mdtN, mdtO
r_12	tetracycline-resistant ribosomal protection protein	demeclocycline, doxycycline, minocycline, tetracycline, chlortetracycline, oxytetracycline	tet(36), tet(44), tet(T), tet(Q), tet(32), tet(O), tetB(P), tet(S), tet(W), Streptomyces rimosus otr(A), tet(W/N/W), tet(M)
r_13	tetracycline-resistant ribosomal protection protein	demeclocycline, doxycycline, minocycline, tetracycline, chlortetracycline, oxytetracycline	tet(36), tet(44), tet(T), tet(Q), tet(32), tet(O/W/32/O), tet(O), tet(O/W), tet(S),

			tet(W), tet(O/W/O), tet(W/N/W), tet(W/32/O), tet(M)
r_14	tetracycline-resistant ribosomal protection protein	demeclocycline, doxycycline, minocycline, tetracycline, chlortetracycline, oxytetracycline	tet(36), tet(44), tet(T), tet(Q), tet(32), tet(O), tetB(P), tet(S), tet(W), Streptomyces rimosus otr(A), tet(W/N/W), tet(M)
r_15	tetracycline-resistant ribosomal protection protein	demeclocycline, doxycycline, minocycline, tetracycline, chlortetracycline, oxytetracycline	tet(36), tet(44), tet(T), tet(Q), tet(32), tet(O/W/32/O), tet(O), tet(O/W), tet(S), tet(W), tet(O/W/O), tet(W/N/W), tet(W/32/O), tet(M)
r_16	tetracycline-resistant ribosomal protection protein	demeclocycline, doxycycline, minocycline, tetracycline, chlortetracycline, oxytetracycline	tet(36), tet(44), tet(T), tet(Q), tet(32), tet(O/W/32/O), tet(O), tet(O/W), tet(S), tet(W), tet(O/W/O), tet(W/N/W), tet(W/32/O), tet(M)
r_17	tetracycline-resistant ribosomal protection protein	demeclocycline, doxycycline, minocycline, tetracycline, chlortetracycline, oxytetracycline	tet(36), tet(44), tet(T), tet(Q), tet(32), tet(O/W/32/O), tet(O), tet(O/W), tet(S), tet(W), tet(O/W/O), tet(W/N/W), tet(W/32/O), tet(M)
r_18	tetracycline-resistant ribosomal protection protein	demeclocycline, doxycycline, minocycline, tetracycline, chlortetracycline, oxytetracycline	tet(36), tet(44), tet(T), tet(Q), tet(32), tet(O/W/32/O), tet(O), tet(O/W), tet(S), tet(W), tet(O/W/O), tet(W/N/W), tet(W/32/O), tet(M)
r_19	tetracycline-resistant ribosomal protection protein	demeclocycline, doxycycline, minocycline, tetracycline, chlortetracycline, oxytetracycline	tet(36), tet(44), tet(T), tet(Q), tet(32), tet(O/W/32/O), tet(O), tet(O/W), tet(S), tet(W), tet(O/W/O), tet(W/N/W), tet(W/32/O), tet(M)
r_20	tetracycline-resistant ribosomal protection protein	demeclocycline, doxycycline, minocycline, tetracycline, chlortetracycline, oxytetracycline	tet(36), tet(44), tet(T), tet(Q), tet(32), tet(O/W/32/O), tet(O), tet(O/W), tet(S), tet(W), tet(O/W/O), tet(W/N/W), tet(W/32/O), tet(M)
r_21	tetracycline-resistant ribosomal protection protein	demeclocycline, doxycycline, minocycline, tetracycline, chlortetracycline, oxytetracycline	tet(36), tet(44), tet(T), tet(Q), tet(32), tet(O/W/32/O), tet(O), tet(O/W), tet(S), tet(W), tet(O/W/O), tet(W/N/W), tet(W/32/O), tet(M)
r_22	tetracycline-resistant ribosomal protection protein	demeclocycline, doxycycline, minocycline, tetracycline, chlortetracycline, oxytetracycline	tet(36), tet(44), tet(T), tet(Q), tet(32), tet(O/W/32/O), tet(O), tet(O/W), tet(S), tet(W), tet(O/W/O), tet(W/N/W), tet(W/32/O), tet(M)
r_23	tetracycline-resistant ribosomal protection protein	demeclocycline, doxycycline, minocycline, tetracycline, chlortetracycline, oxytetracycline	tet(36), tet(44), tet(T), tet(Q), tet(32), tet(O/W/32/O), tet(O), tet(O/W), tet(S), tet(W), tet(O/W/O), tet(W/N/W), tet(W/32/O), tet(M)
r_24	tetracycline-resistant ribosomal protection protein	demeclocycline, doxycycline, minocycline, tetracycline, chlortetracycline, oxytetracycline	tet(36), tet(44), tet(T), tet(Q), tet(32), tet(O/W/32/O), tet(O), tet(O/W), tet(S), tet(W), tet(O/W/O), tet(W/N/W), tet(W/32/O), tet(M)
r_25	tetracycline-resistant ribosomal protection protein	demeclocycline, doxycycline, minocycline, tetracycline, chlortetracycline, oxytetracycline	tet(36), tet(44), tet(T), tet(Q), tet(32), tet(O/W/32/O), tet(O), tet(O/W), tetB(P), tet(S), tet(O/M/O), tet(W), tet(O/W/O), tet(W/N/W), Streptomyces rimosus otr(A), tet(O/32/O), tet(W/32/O), tet(M)
r_26	Penicillin-binding protein mutations conferring resistance to beta-lactam antibiotics	cefdinir, ampicillin, cefaclor, ceftriaxone, cefotaxime, cefditoren	Haemophilus influenzae PBP3 conferring resistance to beta-lactam antibiotics
r_27	ATP-binding cassette (ABC) antibiotic efflux pump	ciprofloxacin, norfloxacin	patB
r_28	ATP-binding cassette (ABC) antibiotic efflux pump	metronidazole	msbA
r_29	intrinsic colistin resistant phosphoethanolamine transferase	colistin B, colistin A	ICR-Mo, ICR-Mc
r_30	pmr phosphoethanolamine transferase	colistin B, polymyxin B, colistin A	eptB
r_31	ATP-binding cassette (ABC) antibiotic efflux pump	ciprofloxacin, norfloxacin	patA, patB

r_32	intrinsic colistin resistant phosphoethanolamine transferase	colistin B, colistin A	ICR-Mo, ICR-Mc
r_33	ATP-binding cassette (ABC) antibiotic efflux pump	microcin J25	YojI
r_34	pmr phosphoethanolamine transferase	polymyxin B	eptA
r_35	Miscellaneous ABC-F subfamily ATP-binding cassette ribosomal protection proteins	linezolid, doxycycline, chloramphenicol, tetracycline, florfenicol	poxtA
r_36	resistance-nodulation-cell division (RND) antibiotic efflux pump, major facilitator superfamily (MFS) antibiotic efflux pump	cloxacillin, erythromycin, oxacillin, tetracycline, norfloxacin	evgA, emrY, emrK, evgS
r_37	major facilitator superfamily (MFS) antibiotic efflux pump	nalidixic acid	emrR, emrB, emrA
r_38	ATP-binding cassette (ABC) antibiotic efflux pump, resistance-nodulation-cell division (RND) antibiotic efflux pump, major facilitator superfamily (MFS) antibiotic efflux pump	nalidixic acid, polymyxin B, tetracycline, polymyxin B3, triclosan, tigecycline, polymyxin B1, novobiocin, erythromycin, ampicillin, azithromycin, chloramphenicol, rifampin, polymyxin B4, polymyxin B2, piperacillin, streptomycin, ertapenem, cloxacillin, cefalotin, ticarcillin, oxacillin, ciprofloxacin, norfloxacin, spectinomycin, imipenem, gentamicin C, tobramycin, ceftazidime, gentamicin	TolC
r_39	macrolide phosphotransferase (MPH), msr-type ABC-F protein	erythromycin	msrE, mphE
r_40	major facilitator superfamily (MFS) antibiotic efflux pump	acriflavine, puromycin	mdtP, mdtN, mdtO
r_41	resistance-nodulation-cell division (RND) antibiotic efflux pump	cefepime, tetracycline, erythromycin	OprZ, AxyY, AxyX, adeF
r_42	resistance-nodulation-cell division (RND) antibiotic efflux pump	novobiocin	cpxA
r_43	antibiotic-resistant GlpT	fosfomycin	Escherichia coli GlpT with mutation conferring resistance to fosfomycin
r_44	major facilitator superfamily (MFS) antibiotic efflux pump	fosfomycin	Acinetobacter baumannii AbaF
r_45	major facilitator superfamily (MFS) antibiotic efflux pump	tetracycline	tet(A), tet(D), tet(C)
r_46	major facilitator superfamily (MFS) antibiotic efflux pump	chloramphenicol	cmlA5
r_47	resistance-nodulation-cell division (RND) antibiotic efflux pump	novobiocin	mdtC, mdtB, mdtA
r_48	major facilitator superfamily (MFS) antibiotic efflux pump	tetracycline, benzalkonium chloride	Escherichia coli mdfA
r_49	major facilitator superfamily (MFS) antibiotic efflux pump	tetracycline	tet(31), tet(D)
r_50	elfamycin resistant EF-Tu	pulvomycin	Escherichia coli EF-Tu mutants conferring resistance to Pulvomycin
r_51	major facilitator superfamily (MFS) antibiotic efflux pump	enoxacin, fosfomycin, norfloxacin	mdtH, mdtG
r_52	major facilitator superfamily (MFS) antibiotic efflux pump	tetracycline	tet(33), tet(D)
r_53	major facilitator superfamily (MFS) antibiotic efflux pump	florfenicol, chloramphenicol	floR
r_54	major facilitator superfamily (MFS) antibiotic efflux pump	enoxacin, fosfomycin, norfloxacin	mdtH, mdtG
r_55	major facilitator superfamily (MFS) antibiotic efflux pump		Mef(En2)

r_56	resistance-nodulation-cell division (RND) antibiotic efflux pump	ampicillin, cefalotin, chloramphenicol, rifampin, tetracycline, ceftazidime, ciprofloxacin, triclosan, tigecycline	Escherichia coli AcrAB-TolC with AcrR mutation conferring resistance to ciprofloxacin, tetracycline, and ceftazidime, Escherichia coli acrA
r_57	major facilitator superfamily (MFS) antibiotic efflux pump	tetracycline	tet(A), tet(D), tet(C)
r_58	major facilitator superfamily (MFS) antibiotic efflux pump	tetracycline	tet(39)
r_59	major facilitator superfamily (MFS) antibiotic efflux pump	tetracycline	tet(A), tet(D), tet(C)
r_60	resistance-nodulation-cell division (RND) antibiotic efflux pump	cefepime, tetracycline, erythromycin	OprZ, AxyY, AxyX, adeF
r_61	major facilitator superfamily (MFS) antibiotic efflux pump	nalidixic acid	emrR, emrB, emrA
r_62	LHK beta-lactamase, ampC-type beta-lactamase		LHK-2, LHK-6, LHK-1, LHK-5, LHK-3, Laribacter hongkongensis ampC beta-lactamase, LHK-4
r_63	pmr phosphoethanolamine transferase	polymyxin B	ugd
r_64	tetracycline inactivation enzyme	demeclocycline, doxycycline, minocycline, tetracycline, chlortetracycline, oxytetracycline, tigecycline	tet(X), tet(X4)
r_65	resistance-nodulation-cell division (RND) antibiotic efflux pump	cloxacillin, erythromycin, oxacillin, norfloxacin	gadW, gadX, mdtE, mdtF
r_66	resistance-nodulation-cell division (RND) antibiotic efflux pump	ampicillin, cefalotin, chloramphenicol, rifampin, tetracycline, ciprofloxacin, triclosan, tigecycline	AcrS, AcrE
r_67	TRU beta-lactamase, MOX beta-lactamase	cefalotin	TRU-1, MOX-9
r_68	TRU beta-lactamase, MOX beta-lactamase	cefalotin	MOX-13, MOX-3, MOX-11, MOX-2, MOX-10, TRU-1, MOX-7, MOX-8, MOX-4, MOX-12
r_69	TRU beta-lactamase, MOX beta-lactamase	cefalotin	MOX-13, MOX-3, MOX-11, MOX-2, MOX-10, TRU-1, MOX-7, MOX-8, MOX-4, MOX-12
r_70	glycopeptide resistance gene cluster, vanW	vancomycin, teicoplanin	vanW gene in vanG cluster, vanW gene in vanI cluster, vanW gene in vanB cluster
r_71	Van ligase, vanY, vanT, vanXY, glycopeptide resistance gene cluster	vancomycin, teicoplanin	vanXY gene in vanG cluster, vanC, vanN, vanT gene in vanC cluster, vanXY gene in vanC cluster, vanY gene in vanM cluster, vanXY gene in vanL cluster, vanTr gene in vanL cluster, vanY gene in vanG cluster, vanY gene in vanA cluster, vanXY gene in vanE cluster, vanTm gene in vanL cluster, vanG, vanL, vanXY gene in vanN cluster, vanY gene in vanB cluster, vanT gene in vanN cluster, vanT gene in vanG cluster, vanT gene in vanE cluster, vanY gene in vanF cluster, vanE
r_72	Erm 23S ribosomal RNA methyltransferase, tetracycline inactivation enzyme	dalfopristin, pristinamycin IB, tetracycline, oxytetracycline, clindamycin, ostreogrycin B3, vernamycin C, erythromycin, tylosin, azithromycin, virginiamycin M1, madumycin II, telithromycin, dirithromycin, griseoviridin, pristinamycin IC, patricin A, minocycline, pristinamycin IA, clarithromycin, roxithromycin, quinupristin, spiramycin, lincomycin, oleandomycin, virginiamycin S2, patricin B	tet(X1), ErmF
r_73	Van ligase, vanY, vanT, vanXY, glycopeptide resistance gene cluster	vancomycin, teicoplanin	vanXY gene in vanG cluster, vanC, vanN, vanT gene in vanC cluster, vanXY gene in vanC cluster, vanY gene in vanM cluster, vanXY gene in vanL cluster, vanTr gene in vanL cluster, vanY gene in vanG cluster, vanY gene in vanA cluster, vanXY gene in vanE cluster, vanTm gene in vanL cluster, vanG, vanL, vanXY gene in vanN cluster, vanY gene in vanB cluster, vanT gene in vanN cluster, vanT

			gene in vanG cluster, vanT gene in vanE cluster, vanY gene in vanF cluster, vanE
r_74	resistance-nodulation-cell division (RND) antibiotic efflux pump, major facilitator superfamily (MFS) antibiotic efflux pump	cloxacillin, erythromycin, oxacillin, tetracycline, norfloxacin	evgA, emrY, emrK, evgS
r_75	glycopeptide resistance gene cluster, vanH	vancomycin, teicoplanin	vanH gene in vanF cluster, vanH gene in vanO cluster, vanH gene in vanD cluster
r_76	Van ligase, vanY, vanT, vanXY, glycopeptide resistance gene cluster	vancomycin, teicoplanin	vanXY gene in vanE cluster, vanXY gene in vanG cluster, vanTm gene in vanL cluster, vanC, vanN, vanG, vanT gene in vanC cluster, vanL, vanXY gene in vanC cluster, vanY gene in vanM cluster, vanXY gene in vanL cluster, vanXY gene in vanN cluster, vanY gene in vanB cluster, vanT gene in vanN cluster, vanT gene in vanG cluster, vanT gene in vanE cluster, vanTr gene in vanL cluster, vanY gene in vanF cluster, vanE, vanY gene in vanG cluster, vanY gene in vanA cluster
r_77	Van ligase, vanY, vanT, vanXY, glycopeptide resistance gene cluster	vancomycin, teicoplanin	vanXY gene in vanG cluster, vanC, vanN, vanT gene in vanC cluster, vanXY gene in vanC cluster, vanY gene in vanM cluster, vanXY gene in vanL cluster, vanTr gene in vanL cluster, vanY gene in vanG cluster, vanY gene in vanA cluster, vanXY gene in vanE cluster, vanTm gene in vanL cluster, vanG, vanL, vanXY gene in vanN cluster, vanY gene in vanB cluster, vanT gene in vanN cluster, vanT gene in vanG cluster, vanT gene in vanE cluster, vanY gene in vanF cluster, vanE
r_78	major facilitator superfamily (MFS) antibiotic efflux pump	acriflavine, puromycin	mdtP, mdtN, mdtO
r_79	Van ligase, vanY, vanT, vanXY, glycopeptide resistance gene cluster	vancomycin, teicoplanin	vanXY gene in vanG cluster, vanC, vanN, vanT gene in vanC cluster, vanXY gene in vanC cluster, vanY gene in vanM cluster, vanXY gene in vanL cluster, vanTr gene in vanL cluster, vanY gene in vanG cluster, vanY gene in vanA cluster, vanXY gene in vanE cluster, vanTm gene in vanL cluster, vanG, vanL, vanXY gene in vanN cluster, vanY gene in vanB cluster, vanT gene in vanN cluster, vanT gene in vanG cluster, vanT gene in vanE cluster, vanY gene in vanF cluster, vanE
r_80	Van ligase, vanY, vanT, vanXY, glycopeptide resistance gene cluster	vancomycin, teicoplanin	vanXY gene in vanG cluster, vanC, vanN, vanT gene in vanC cluster, vanXY gene in vanC cluster, vanY gene in vanM cluster, vanXY gene in vanL cluster, vanTr gene in vanL cluster, vanY gene in vanG cluster, vanY gene in vanA cluster, vanXY gene in vanE cluster, vanTm gene in vanL cluster, vanG, vanL, vanXY gene in vanN cluster, vanY gene in vanB cluster, vanT gene in vanN cluster, vanT gene in vanG cluster, vanT gene in vanE cluster, vanY gene in vanF cluster, vanE
r_81	CfxA beta-lactamase		CfxA6
r_82	Van ligase, vanY, vanT, vanXY, glycopeptide resistance gene cluster	vancomycin, teicoplanin	vanXY gene in vanG cluster, vanC, vanN, vanT gene in vanC cluster, vanXY gene in vanC cluster, vanY gene in vanM cluster, vanXY gene in vanL cluster, vanTr gene in vanL cluster, vanY gene in vanG cluster, vanY gene in vanA cluster, vanXY gene in vanE cluster, vanTm gene in vanL cluster, vanG, vanL, vanXY gene in vanN cluster, vanY gene in vanB cluster, vanT gene in vanN cluster, vanT gene in vanG cluster, vanT gene in vanE cluster, vanY gene in vanF cluster, vanE

r_83	glycopeptide resistance gene cluster, vanH	vancomycin, teicoplanin	vanH gene in vanF cluster, vanH gene in vanB cluster, vanH gene in vanA cluster, vanH gene in vanM cluster, vanH gene in vanO cluster, vanH gene in vanD cluster
r_84	glycopeptide resistance gene cluster, vanH	vancomycin, teicoplanin	vanH gene in vanA cluster, vanH gene in vanB cluster
r_85	ANT(3'')	spectinomycin, streptomycin	aadA21, ANT(3'')-IIa, aadA25, aadA23, aadA, aadA13, aadA22, aadA24, aadA8
r_86	glycopeptide resistance gene cluster, vanH	vancomycin, teicoplanin	vanH gene in vanF cluster, vanH gene in vanO cluster, vanH gene in vanD cluster
r_87	glycopeptide resistance gene cluster, vanH	vancomycin, teicoplanin	vanH gene in vanA cluster, vanH gene in vanB cluster
r_88	glycopeptide resistance gene cluster, vanH	vancomycin, teicoplanin	vanH gene in vanF cluster, vanH gene in vanO cluster, vanH gene in vanD cluster
r_89	pmr phosphoethanolamine transferase	polymyxin B	PmrF
r_90	CfxA beta-lactamase		CfxA2
r_91	Penicillin-binding protein mutations conferring resistance to beta-lactam antibiotics, major facilitator superfamily (MFS) antibiotic efflux pump	cefdinir, ampicillin, cefaclor, puromycin, ceftriaxone, cefotaxime, acriflavine, cefditoren	leuO, Haemophilus influenzae PBP3 conferring resistance to beta-lactam antibiotics
r_92	glycopeptide resistance gene cluster, vanH	vancomycin, teicoplanin	vanH gene in vanF cluster, vanH gene in vanB cluster, vanH gene in vanM cluster, vanH gene in vanA cluster, vanH gene in vanO cluster, vanH gene in vanD cluster, vanH gene in vanP cluster
r_93	BRO Beta-lactamase		BRO-1
r_94	TLA beta-lactamase		TLA-2
r_95	vanY, glycopeptide resistance gene cluster	vancomycin, teicoplanin	vanY gene in vanB cluster, vanY gene in vanM cluster, vanY gene in vanF cluster, vanY gene in vanG cluster, vanY gene in vanA cluster
r_96	CepA beta-lactamase		CepA-29, CepA-49, cepA, CepA-44
r_97	macrolide phosphotransferase (MPH)	tylosin, erythromycin, azithromycin, clarithromycin, roxithromycin	mphF
r_98	resistance-nodulation-cell division (RND) antibiotic efflux pump, CblA beta-lactamase	tetracycline, cephaloridine	CblA-1, adeF
r_99	macrolide phosphotransferase (MPH), msr-type ABC-F protein	erythromycin	msrE, mphE
r_100	macrolide phosphotransferase (MPH)	erythromycin, clarithromycin, azithromycin	mphG
r_101	vanY, glycopeptide resistance gene cluster	vancomycin, teicoplanin	vanY gene in vanB cluster, vanY gene in vanM cluster, vanY gene in vanF cluster, vanY gene in vanG cluster, vanY gene in vanA cluster
r_102	PAU beta-lactamase		PAU-1
r_103	RCP beta-lactamase		RCP-1
r_104	GES beta-lactamase, trimethoprim resistant dihydrofolate reductase dfr	trimethoprim	DfrB9, GES-1
r_105	AAC(3)	6'-N-ethylnetilmicin, 2'-N-ethylnetilmicin, tobramycin, gentamicin, sisomicin, dibekacin, netilmicin	AAC(3)-IIb, AAC(3)-IIc, AAC(3)-IIg
r_106	OXA beta-lactamase	oxacillin, cloxacillin, cefalotin	OXA-296, OXA-666
r_107	glycopeptide resistance gene cluster, vanW	vancomycin, teicoplanin	vanW gene in vanG cluster, vanW gene in vanI cluster, vanW gene in vanB cluster
r_108	ANT(3'')	spectinomycin, streptomycin	aadA11, aadA6, aadA16, aadA6/aadA10

r_109	ANT(3'')	spectinomycin, streptomycin	aadA11, aadA6, aadA16, aadA6/aadA10
r_110	sulfonamide resistant sul	sulfamethoxazole, sulfasalazine, sulfamethizole, sulfadiazine, sulfadimidine, mafenide, sulfacetamide, sulfadoxine, sulfisoxazole	sul1
r_111	vanY, glycopeptide resistance gene cluster	vancomycin, teicoplanin	vanY gene in vanB cluster, vanY gene in vanM cluster, vanY gene in vanF cluster, vanY gene in vanG cluster, vanY gene in vanA cluster
r_112	APH(6)	streptomycin	APH(6)-Id
r_113	OXA beta-lactamase	oxacillin, cloxacillin, cefalotin	OXA-299
r_114	glycopeptide resistance gene cluster, vanW	vancomycin, teicoplanin	vanW gene in vanG cluster, vanW gene in vanI cluster, vanW gene in vanB cluster
r_115	OXA beta-lactamase	oxacillin, cloxacillin, cefalotin	OXA-21
r_116	resistance-nodulation-cell division (RND) antibiotic efflux pump	cloxacillin, erythromycin, oxacillin, norfloxacin	gadW, gadX, mdtE, mdtF
r_117	undecaprenyl pyrophosphate related proteins	bacitracin, bacitracin B, bacitracin F, bacitracin A	bacA
r_118	APH(3')	ribostamycin, kanamycin A, gentamicin B, lividomycin, neomycin, gentamicin, paromomycin	APH(3')-Ia
r_119	APH(3')	ribostamycin, kanamycin A, gentamicin B, lividomycin, neomycin, gentamicin, paromomycin	APH(3')-Ib
r_120	sulfonamide resistant sul	sulfamethoxazole, sulfasalazine, sulfamethizole, sulfadiazine, sulfadimidine, mafenide, sulfacetamide, sulfadoxine, sulfisoxazole	sul2
r_121	AAC(3)	6'-N-ethylnetilmicin, 2'-N-ethylnetilmicin, tobramycin, gentamicin, sisomicin, dibekacin, netilmicin	AAC(3)-IIb, AAC(3)-IIc, AAC(3)-IIg
r_122	AAC(3)	6'-N-ethylnetilmicin, 2'-N-ethylnetilmicin, tobramycin, gentamicin, sisomicin, dibekacin, netilmicin	AAC(3)-IIb, AAC(3)-IIc, AAC(3)-IIg
r_123	AAC(3), OXA beta-lactamase	cloxacillin, cefalotin, astromicin, oxacillin, gentamicin, sisomicin	AAC(3)-Ia, OXA-17
r_124	vanY, glycopeptide resistance gene cluster	vancomycin, teicoplanin	vanY gene in vanB cluster, vanY gene in vanM cluster, vanY gene in vanF cluster, vanY gene in vanG cluster, vanY gene in vanA cluster
r_125	APH(3'')	streptomycin	APH(3'')-Ib
r_126	Erm 23S ribosomal RNA methyltransferase, tetracycline inactivation enzyme	dalfopristin, pristinamycin IB, tetracycline, oxytetracycline, clindamycin, ostreogrycin B3, vernamycin C, erythromycin, tylosin, azithromycin, virginiamycin M1, madumycin II, telithromycin, dirithromycin, griseoviridin, pristinamycin IC, patricin A, minocycline, pristinamycin IA, clarithromycin, roxithromycin, quinupristin, spiramycin, lincomycin, oleandomycin, virginiamycin S2, patricin B	tet(X1), ErmF
r_127	ANT(3'')	spectinomycin, streptomycin	aadA21, ANT(3'')-IIa, aadA25, aadA23, aadA, aadA13, aadA22, aadA24, aadA8
r_128	OXA beta-lactamase	oxacillin, cloxacillin, cefalotin	OXA-427, OXA-917
r_129	OXA beta-lactamase	oxacillin, cloxacillin, cefalotin	OXA-780, OXA-504
r_130	ANT(3'')	spectinomycin, streptomycin	aadA27
r_131	ANT(2''), AAC(6'), OXA beta-lactamase	amikacin, cloxacillin, 2'-N-ethylnetilmicin, cefalotin, 5-episisomicin, oxacillin,	OXA-10, OXA-520, OXA-147, OXA-233, OXA-16, OXA-7, OXA-935, OXA-56, OXA-656, OXA-17, OXA-827, OXA-928, OXA-13, OXA-256,

		sisomicin, kanamycin A, tobramycin, gentamicin, dibekacin, netilmicin	OXA-824, AAC(6')-Il, OXA-823, OXA-240, OXA-246, OXA-28, OXA-183, OXA-736, OXA-454, OXA-795, OXA-142, OXA-74, OXA-35, OXA-368, OXA-794, OXA-836, OXA-677, OXA-655, OXA-251, OXA-19, ANT(2'')-Ia, OXA-932, OXA-145, OXA-11, OXA-14, OXA-663, OXA-101, OXA-676
r_132	AAC(2')	kasugamycin	AAC(2')-IIa
r_133	NPS beta-lactamase		NPS-1
r_134	Van ligase, vanY, vanT, vanXY, glycopeptide resistance gene cluster	vancomycin, teicoplanin	vanXY gene in vanG cluster, vanC, vanN, vanT gene in vanC cluster, vanXY gene in vanC cluster, vanY gene in vanM cluster, vanXY gene in vanL cluster, vanTr gene in vanL cluster, vanY gene in vanG cluster, vanY gene in vanA cluster, vanXY gene in vanE cluster, vanTm gene in vanL cluster, vanG, vanL, vanXY gene in vanN cluster, vanY gene in vanB cluster, vanT gene in vanN cluster, vanT gene in vanG cluster, vanT gene in vanE cluster, vanY gene in vanF cluster, vanE
r_135	JOHN beta-lactamase		JOHN-1
r_136	Erm 23S ribosomal RNA methyltransferase	erythromycin, azithromycin, quinupristin, spiramycin, lincomycin, clindamycin	Erm(47)
r_137	resistance-nodulation-cell division (RND) antibiotic efflux pump	cloxacillin, erythromycin, oxacillin, norfloxacin	gadW, gadX, mdtE, mdtF
r_138	vanY, glycopeptide resistance gene cluster	vancomycin, teicoplanin	vanY gene in vanB cluster, vanY gene in vanM cluster, vanY gene in vanF cluster, vanY gene in vanG cluster, vanY gene in vanA cluster
r_139	AAC(6'), AAC(6')-Ib-cr, major facilitator superfamily (MFS) antibiotic efflux pump, trimethoprim resistant dihydrofolate reductase dfr, sulfonamide resistant sul	amikacin, sulfamethizole, sulfadimidine, 2'-N-ethylnetilmicin, 5-episisomicin, sisomicin, kanamycin A, trimethoprim, sulfamethoxazole, sulfisoxazole, dibekacin, sulfadiazine, mafenide, ciprofloxacin, sulfacetamide, sulfasalazine, tobramycin, gentamicin, sulfadoxine, netilmicin	qacEdelta1, AAC(6')-Ib-cr8, sul1, AAC(6')-Ib-cr9, dfrB5, AAC(6')-Ib-cr6, AAC(6')-Ib, AAC(6')-Ib8, AAC(6')-Ib-cr3, AAC(6')-Ib-Suzhou, AAC(6')-Ib11, AAC(6')-IIa, AAC(6')-Ib-cr5, AAC(6')-Ib-cr7, DfrB9, AAC(6')-Ib-cr1, AAC(6')-Ib-Hangzhou, AAC(6')-32, AAC(6')-Ib', AAC(6')-Ib9, AAC(6')-Ib-cr4, AAC(6')-IIc, dfrB7, AAC(6')-Ib3, AAC(6')-Ib10, AAC(6')-Ib4
r_140	kdpDE	kanamycin A	kdpE
r_141	resistance-nodulation-cell division (RND) antibiotic efflux pump	ampicillin, cefalotin, chloramphenicol, rifampin, tetracycline, ciprofloxacin, triclosan, tigecycline	AcrS, AcrE
r_142	quinolone resistance protein (qnr)	nalidixic acid, sparfloxacin, ciprofloxacin, gatifloxacin, moxifloxacin, levofloxacin, norfloxacin	QnrS2
r_143	resistance-nodulation-cell division (RND) antibiotic efflux pump	ampicillin, cefalotin, chloramphenicol, rifampin, tetracycline, ceftazidime, ciprofloxacin, triclosan, tigecycline	Escherichia coli AcrAB-TolC with AcrR mutation conferring resistance to ciprofloxacin, tetracycline, and ceftazidime, Escherichia coli acrA
r_144	chloramphenicol acetyltransferase (CAT), trimethoprim resistant dihydrofolate reductase dfr	chloramphenicol, azidamfenicol, trimethoprim, thiamphenicol	dfrA12, catB3
r_145	resistance-nodulation-cell division (RND) antibiotic efflux pump	cloxacillin, erythromycin, oxacillin, norfloxacin	CRP
r_146	resistance-nodulation-cell division (RND) antibiotic efflux pump, major facilitator superfamily (MFS) antibiotic efflux pump	cloxacillin, erythromycin, oxacillin, tetracycline, norfloxacin	evgA, emrY, emrK, evgS
r_147	AAC(6'), AAC(6')-Ib-cr, major facilitator superfamily (MFS) antibiotic efflux pump, trimethoprim resistant dihydrofolate reductase dfr, sulfonamide resistant sul	amikacin, sulfamethizole, sulfadimidine, 2'-N-ethylnetilmicin, 5-episisomicin, sisomicin, kanamycin A, trimethoprim, sulfamethoxazole, sulfisoxazole, dibekacin, sulfadiazine, mafenide, ciprofloxacin, sulfacetamide, sulfasalazine,	qacEdelta1, AAC(6')-Ib-cr8, sul1, AAC(6')-Ib-cr9, dfrB5, AAC(6')-Ib-cr6, AAC(6')-Ib, AAC(6')-Ib8, AAC(6')-Ib-cr3, AAC(6')-Ib-Suzhou, AAC(6')-Ib11, AAC(6')-IIa, AAC(6')-Ib-cr5, AAC(6')-Ib-cr7, DfrB9, AAC(6')-Ib-cr1, AAC(6')-Ib-Hangzhou, AAC(6')-32, AAC(6')-Ib', AAC(6')-

		tobramycin, gentamicin, sulfadoxine, netilmicin	Ib9, AAC(6')-Ib-cr4, AAC(6')-IIc, dfrB7, AAC(6')-Ib3, AAC(6')-Ib10, AAC(6')-Ib4
r_148	Van ligase, vanY, vanT, vanXY, glycopeptide resistance gene cluster	vancomycin, teicoplanin	vanXY gene in vanG cluster, vanC, vanN, vanT gene in vanC cluster, vanXY gene in vanC cluster, vanY gene in vanM cluster, vanXY gene in vanL cluster, vanTr gene in vanL cluster, vanY gene in vanG cluster, vanY gene in vanA cluster, vanXY gene in vanE cluster, vanTm gene in vanL cluster, vanG, vanL, vanXY gene in vanN cluster, vanY gene in vanB cluster, vanT gene in vanN cluster, vanT gene in vanG cluster, vanT gene in vanE cluster, vanY gene in vanF cluster, vanE
r_149	Van ligase, vanY, vanT, vanXY, glycopeptide resistance gene cluster	vancomycin, teicoplanin	vanXY gene in vanG cluster, vanC, vanN, vanT gene in vanC cluster, vanXY gene in vanC cluster, vanY gene in vanM cluster, vanXY gene in vanL cluster, vanTr gene in vanL cluster, vanY gene in vanG cluster, vanY gene in vanA cluster, vanXY gene in vanE cluster, vanTm gene in vanL cluster, vanG, vanL, vanXY gene in vanN cluster, vanY gene in vanB cluster, vanT gene in vanN cluster, vanT gene in vanG cluster, vanT gene in vanE cluster, vanY gene in vanF cluster, vanE
r_150	Van ligase, vanY, vanT, vanXY, glycopeptide resistance gene cluster	vancomycin, teicoplanin	vanXY gene in vanG cluster, vanC, vanN, vanT gene in vanC cluster, vanXY gene in vanC cluster, vanY gene in vanM cluster, vanXY gene in vanL cluster, vanTr gene in vanL cluster, vanY gene in vanG cluster, vanY gene in vanA cluster, vanXY gene in vanE cluster, vanTm gene in vanL cluster, vanG, vanL, vanXY gene in vanN cluster, vanY gene in vanB cluster, vanT gene in vanN cluster, vanT gene in vanG cluster, vanT gene in vanE cluster, vanY gene in vanF cluster, vanE
r_151	Van ligase, vanY, vanT, vanXY, glycopeptide resistance gene cluster	vancomycin, teicoplanin	vanXY gene in vanG cluster, vanC, vanN, vanT gene in vanC cluster, vanXY gene in vanC cluster, vanY gene in vanM cluster, vanXY gene in vanL cluster, vanTr gene in vanL cluster, vanY gene in vanG cluster, vanY gene in vanA cluster, vanXY gene in vanE cluster, vanTm gene in vanL cluster, vanG, vanL, vanXY gene in vanN cluster, vanY gene in vanB cluster, vanT gene in vanN cluster, vanT gene in vanG cluster, vanT gene in vanE cluster, vanY gene in vanF cluster, vanE
r_152	AAC(6'), AAC(6')-Ib-cr, major facilitator superfamily (MFS) antibiotic efflux pump, trimethoprim resistant dihydrofolate reductase dfr, sulfonamide resistant sul	amikacin, sulfamethizole, sulfadimidine, 2'-N-ethylnetilmicin, 5-episisomicin, sisomicin, kanamycin A, trimethoprim, sulfamethoxazole, sulfisoxazole, dibekacin, sulfadiazine, mafenide, ciprofloxacin, sulfacetamide, sulfasalazine, tobramycin, gentamicin, sulfadoxine, netilmicin	qacEdelta1, AAC(6')-Ib-cr8, sul1, AAC(6')-Ib-cr9, dfrB5, AAC(6')-Ib-cr6, AAC(6')-Ib, AAC(6')-Ib8, AAC(6')-Ib-cr3, AAC(6')-Ib-Suzhou, AAC(6')-Ib11, AAC(6')-IIa, AAC(6')-Ib-cr5, AAC(6')-Ib-cr7, DfrB9, AAC(6')-Ib-cr1, AAC(6')-Ib-Hangzhou, AAC(6')-32, AAC(6')-Ib', AAC(6')-Ib9, AAC(6')-Ib-cr4, AAC(6')-IIc, dfrB7, AAC(6')-Ib3, AAC(6')-Ib10, AAC(6')-Ib4
r_153	AAC(6'), AAC(6')-Ib-cr, major facilitator superfamily (MFS) antibiotic efflux pump, trimethoprim resistant dihydrofolate reductase dfr, sulfonamide resistant sul	amikacin, sulfamethizole, sulfadimidine, 2'-N-ethylnetilmicin, 5-episisomicin, sisomicin, kanamycin A, trimethoprim, sulfamethoxazole, sulfisoxazole, dibekacin, sulfadiazine, mafenide, ciprofloxacin, sulfacetamide, sulfasalazine, tobramycin, gentamicin, sulfadoxine, netilmicin	qacEdelta1, AAC(6')-Ib-cr8, sul1, AAC(6')-Ib-cr9, dfrB5, AAC(6')-Ib-cr6, AAC(6')-Ib, AAC(6')-Ib8, AAC(6')-Ib-cr3, AAC(6')-Ib-Suzhou, AAC(6')-Ib11, AAC(6')-IIa, AAC(6')-Ib-cr5, AAC(6')-Ib-cr7, DfrB9, AAC(6')-Ib-cr1, AAC(6')-Ib-Hangzhou, AAC(6')-32, AAC(6')-Ib', AAC(6')-Ib9, AAC(6')-Ib-cr4, AAC(6')-IIc, dfrB7, AAC(6')-Ib3, AAC(6')-Ib10, AAC(6')-Ib4
r_154	ANT(2''), TLA beta-lactamase	kanamycin A, tobramycin, gentamicin, sisomicin, dibekacin	TLA-2, ANT(2'')-Ia
r_155	nitroimidazole reductase	metronidazole	nimA, nimJ, nimD, nimC, nimH, nimI, nimK, nimB, nimG, nimE, nimF

r_156	major facilitator superfamily (MFS) antibiotic efflux pump	nalidixic acid	emrR, emrB, emrA
r_157	nitroimidazole reductase	metronidazole	nimA, nimJ, nimD, nimC, nimH, nimI, nimK, nimB, nimG, nimE, nimF
r_158	nitroimidazole reductase	metronidazole	nimA, nimJ, nimD, nimC, nimH, nimI, nimK, nimB, nimG, nimE, nimF
r_159	nitroimidazole reductase	metronidazole	nimA, nimJ, nimD, nimC, nimH, nimI, nimK, nimB, nimG, nimE, nimF
r_160	chloramphenicol acetyltransferase (CAT), trimethoprim resistant dihydrofolate reductase dfr	chloramphenicol, azidamfenicol, trimethoprim, thiamphenicol	dfrA12, catB3
r_161	nitroimidazole reductase	metronidazole	nimA, nimJ, nimD, nimC, nimH, nimI, nimK, nimB, nimG, nimE, nimF
r_162	nitroimidazole reductase	metronidazole	nimA, nimJ, nimD, nimC, nimH, nimI, nimK, nimB, nimG, nimE, nimF
r_163	nitroimidazole reductase	metronidazole	nimA, nimJ, nimD, nimC, nimH, nimI, nimK, nimB, nimG, nimE, nimF
r_164	nitroimidazole reductase	metronidazole	nimA, nimJ, nimD, nimC, nimH, nimI, nimK, nimB, nimG, nimE, nimF
r_165	nitroimidazole reductase	metronidazole	nimA, nimJ, nimD, nimC, nimH, nimI, nimK, nimB, nimG, nimE, nimF
r_166	trimethoprim resistant dihydrofolate reductase dfr	trimethoprim	dfrA3
r_167	ATP-binding cassette (ABC) antibiotic efflux pump, resistance-nodulation-cell division (RND) antibiotic efflux pump, major facilitator superfamily (MFS) antibiotic efflux pump	cefalotin, tetracycline, ciprofloxacin, triclosan, tigecycline, norfloxacin, ampicillin, chloramphenicol, rifampin, acriflavine	Pseudomonas aeruginosa soxR
r_168	AAC(3)	astromicin, gentamicin, sisomicin	AAC(3)-Ia
r_169	General Bacterial Porin with reduced permeability to beta-lactams, major facilitator superfamily (MFS) antibiotic efflux pump, ATP-binding cassette (ABC) antibiotic efflux pump, resistance-nodulation-cell division (RND) antibiotic efflux pump	nalidixic acid, cefalotin, tetracycline, ciprofloxacin, triclosan, tigecycline, norfloxacin, ampicillin, chloramphenicol, rifampin, enrofloxacin	Escherichia coli soxS with mutation conferring antibiotic resistance, Escherichia coli soxR with mutation conferring antibiotic resistance
r_170	ANT(2''), AAC(6'), OXA beta-lactamase	amikacin, cloxacillin, 2'-N-ethylnetilmicin, cefalotin, 5-episisomicin, oxacillin, sisomicin, kanamycin A, tobramycin, gentamicin, dibekacin, netilmicin	OXA-10, OXA-520, OXA-147, OXA-233, OXA-16, OXA-7, OXA-935, OXA-56, OXA-656, OXA-17, OXA-827, OXA-928, OXA-13, OXA-256, OXA-824, AAC(6')-Il, OXA-823, OXA-240, OXA-246, OXA-28, OXA-183, OXA-736, OXA-454, OXA-795, OXA-142, OXA-74, OXA-35, OXA-368, OXA-794, OXA-836, OXA-677, OXA-655, OXA-251, OXA-19, ANT(2'')-Ia, OXA-932, OXA-145, OXA-11, OXA-14, OXA-663, OXA-101, OXA-676
r_171	nitroimidazole reductase	metronidazole	nimA, nimJ, nimD, nimC, nimH, nimI, nimK, nimB, nimG, nimE, nimF
r_172	nitroimidazole reductase	metronidazole	nimA, nimJ, nimD, nimC, nimH, nimI, nimK, nimB, nimG, nimE, nimF
r_173	resistance-nodulation-cell division (RND) antibiotic efflux pump, General Bacterial Porin with reduced permeability to beta-lactams	ampicillin, cefalotin, chloramphenicol, rifampin, tetracycline, ciprofloxacin, triclosan, tigecycline	Escherichia coli AcrAB-TolC with MarR mutations conferring resistance to ciprofloxacin and tetracycline, marA
r_174	fosfomycin thiol transferase	fosfomycin	FosA8
r_175	resistance-nodulation-cell division (RND) antibiotic efflux pump, major facilitator superfamily (MFS) antibiotic efflux pump	cloxacillin, erythromycin, tetracycline, oxacillin, ciprofloxacin, norfloxacin	H-NS
r_176	fosfomycin thiol transferase	fosfomycin	FosH, FosI

r_177	fosfomycin thiol transferase	fosfomycin	FosH, FosI
r_178	resistance-nodulation-cell division (RND) antibiotic efflux pump, General Bacterial Porin with reduced permeability to beta-lactams	ampicillin, cefalotin, chloramphenicol, rifampin, tetracycline, ciprofloxacin, triclosan, tigecycline	Escherichia coli AcrAB-TolC with MarR mutations conferring resistance to ciprofloxacin and tetracycline, marA
r_179	small multidrug resistance (SMR) antibiotic efflux pump	cefepime, colistin B, tetracycline, ceftriaxone, triclosan, benzalkonium chloride, erythromycin, chlorhexidine, colistin A, rifampin, streptomycin	Klebsiella pneumoniae KpnE, Klebsiella pneumoniae KpnF
r_180	AAC(6'), AAC(6')-Ib-cr, major facilitator superfamily (MFS) antibiotic efflux pump, trimethoprim resistant dihydrofolate reductase dfr, sulfonamide resistant sul	amikacin, sulfamethizole, sulfadimidine, 2'-N-ethylnetilmicin, 5-episisomicin, sisomicin, kanamycin A, trimethoprim, sulfamethoxazole, sulfisoxazole, dibekacin, sulfadiazine, mafenide, ciprofloxacin, sulfacetamide, sulfasalazine, tobramycin, gentamicin, sulfadoxine, netilmicin	qacEdelta1, AAC(6')-Ib-cr8, sul1, AAC(6')-Ib-cr9, dfrB5, AAC(6')-Ib-cr6, AAC(6')-Ib, AAC(6')-Ib8, AAC(6')-Ib-cr3, AAC(6')-Ib-Suzhou, AAC(6')-Ib11, AAC(6')-IIa, AAC(6')-Ib-cr5, AAC(6')-Ib-cr7, DfrB9, AAC(6')-Ib-cr1, AAC(6')-Ib-Hangzhou, AAC(6')-32, AAC(6')-Ib', AAC(6')-Ib9, AAC(6')-Ib-cr4, AAC(6')-IIc, dfrB7, AAC(6')-Ib3, AAC(6')-Ib10, AAC(6')-Ib4
r_181	small multidrug resistance (SMR) antibiotic efflux pump	benzalkonium chloride	qacG, qacL
r_182	small multidrug resistance (SMR) antibiotic efflux pump	cefepime, colistin B, tetracycline, ceftriaxone, triclosan, benzalkonium chloride, erythromycin, chlorhexidine, colistin A, rifampin, streptomycin	Klebsiella pneumoniae KpnE, Klebsiella pneumoniae KpnF
r_183	small multidrug resistance (SMR) antibiotic efflux pump	benzalkonium chloride	qacJ, qacG
r_184	small multidrug resistance (SMR) antibiotic efflux pump	benzalkonium chloride	qacJ, qacG
r_185	General Bacterial Porin with reduced permeability to beta-lactams, major facilitator superfamily (MFS) antibiotic efflux pump, ATP-binding cassette (ABC) antibiotic efflux pump, resistance-nodulation-cell division (RND) antibiotic efflux pump	nalidixic acid, cefalotin, tetracycline, ciprofloxacin, triclosan, tigecycline, norfloxacin, ampicillin, chloramphenicol, rifampin, enrofloxacin	Escherichia coli soxS with mutation conferring antibiotic resistance, Escherichia coli soxR with mutation conferring antibiotic resistance
r_186	AAC(6'), AAC(6')-Ib-cr, major facilitator superfamily (MFS) antibiotic efflux pump, trimethoprim resistant dihydrofolate reductase dfr, sulfonamide resistant sul	amikacin, sulfamethizole, sulfadimidine, 2'-N-ethylnetilmicin, 5-episisomicin, sisomicin, kanamycin A, trimethoprim, sulfamethoxazole, sulfisoxazole, dibekacin, sulfadiazine, mafenide, ciprofloxacin, sulfacetamide, sulfasalazine, tobramycin, gentamicin, sulfadoxine, netilmicin	qacEdelta1, AAC(6')-Ib-cr8, sul1, AAC(6')-Ib-cr9, dfrB5, AAC(6')-Ib-cr6, AAC(6')-Ib, AAC(6')-Ib8, AAC(6')-Ib-cr3, AAC(6')-Ib-Suzhou, AAC(6')-Ib11, AAC(6')-IIa, AAC(6')-Ib-cr5, AAC(6')-Ib-cr7, DfrB9, AAC(6')-Ib-cr1, AAC(6')-Ib-Hangzhou, AAC(6')-32, AAC(6')-Ib', AAC(6')-Ib9, AAC(6')-Ib-cr4, AAC(6')-IIc, dfrB7, AAC(6')-Ib3, AAC(6')-Ib10, AAC(6')-Ib4
r_187	GES beta-lactamase, trimethoprim resistant dihydrofolate reductase dfr	trimethoprim	DfrB9, GES-1
r_188	glycopeptide resistance gene cluster, vanU	vancomycin	vanU gene in vanG cluster