

Comparative transcriptomics of multidrug-resistant *Acinetobacter baumannii* in response to antibiotic treatments

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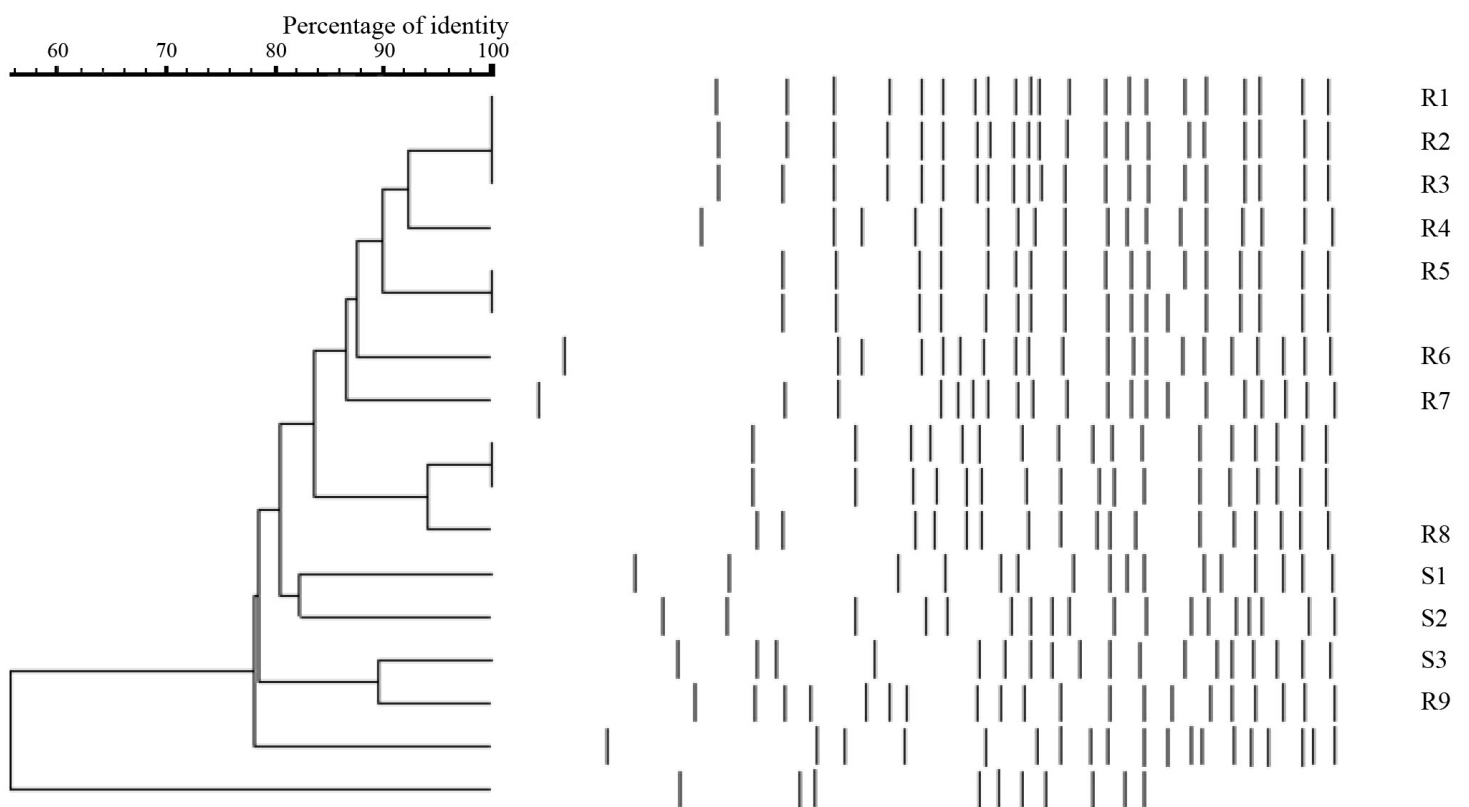


Figure S1. Pulsed-field gel electrophoresis (PFGE) patterns of the *A. baumannii* strains used in this study. PFGE patterns were clustered using Unweighted Pair Group Method with Arithmetic Mean (UPGMA). Different lineages were determined by the similarity cutoff of 80%, with 1.5% position tolerance and 1% band optimization.

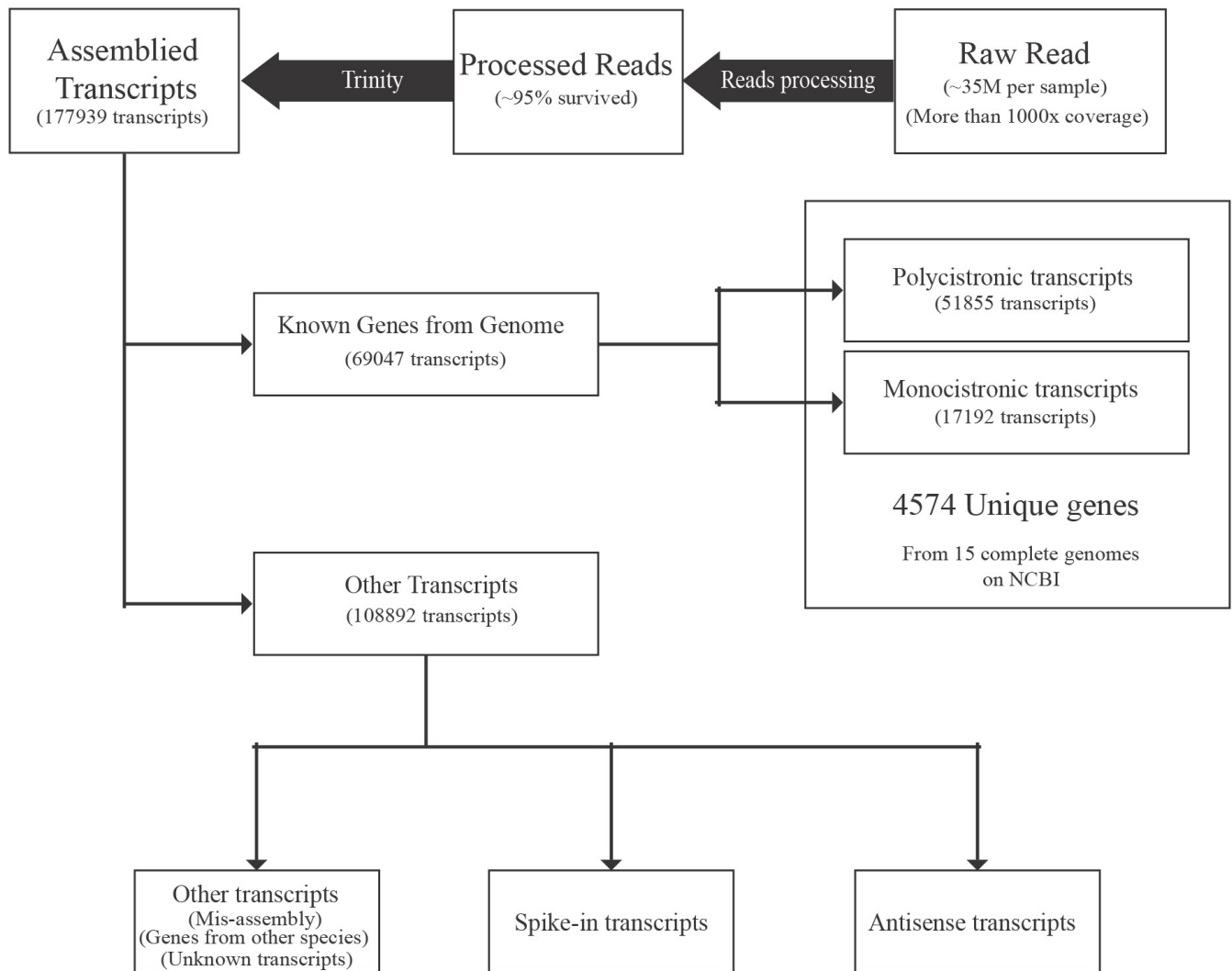


Figure S2. Bioinformatics analysis flow chart.

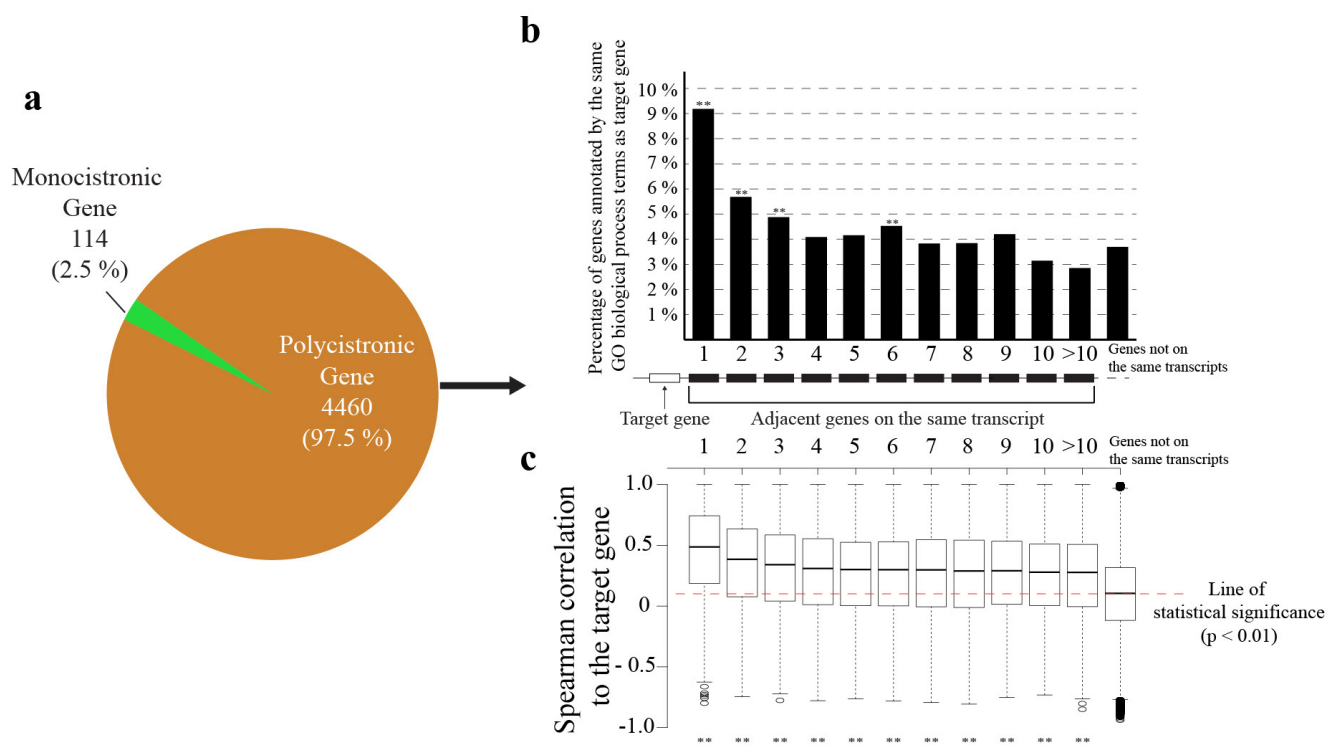


Figure S3. *De novo* assembly could efficiently construct polycistronic transcripts. (a) Distribution of genes being either monocistronic or polycistronic. (b) Proportion of adjacent genes which can be annotated by the same GO (biological process) as the target gene on the same polycistronic transcripts. (c) Boxplots of spearman correlations of adjacent genes with the target gene on the same polycistronic transcripts.

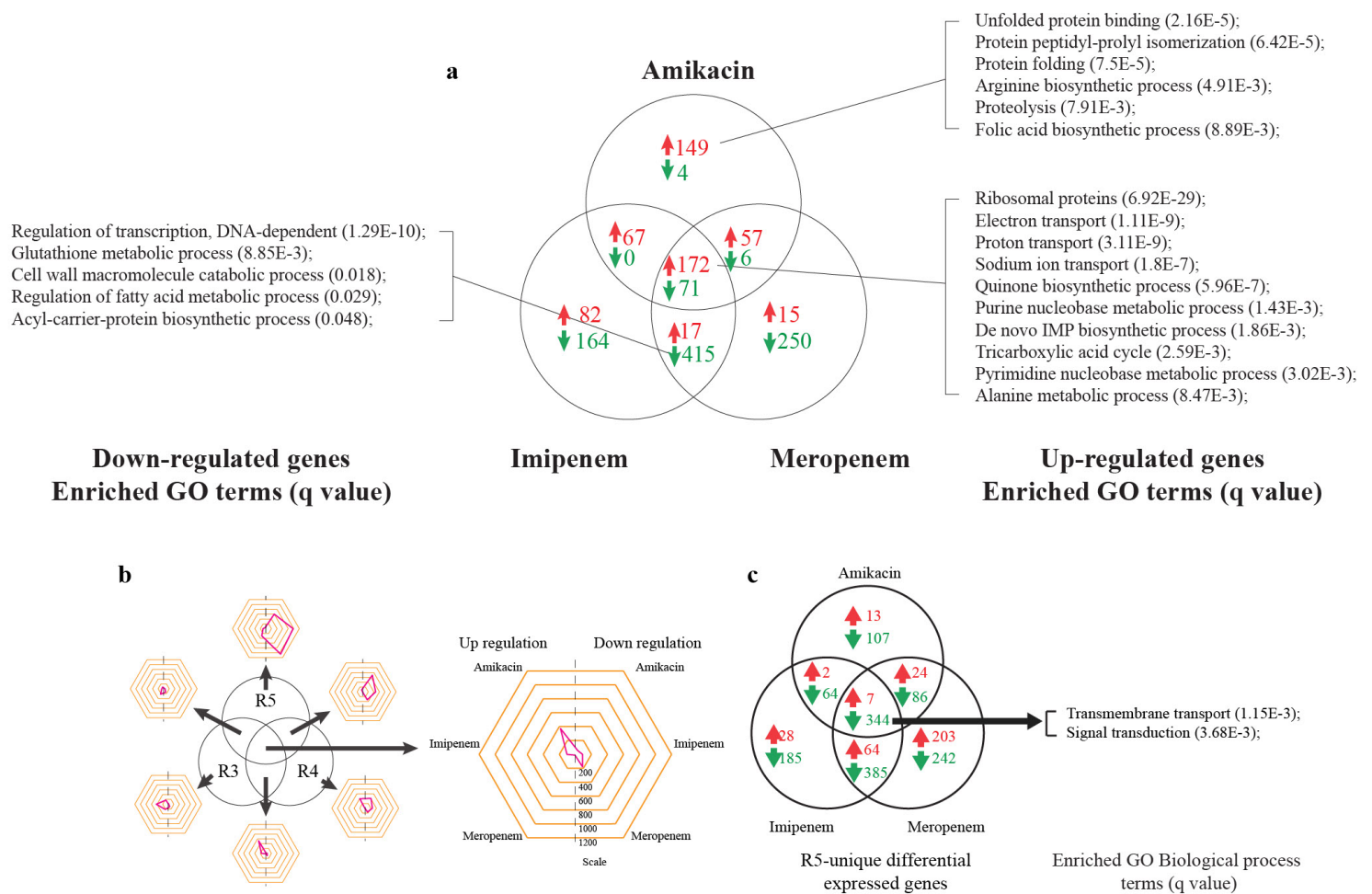
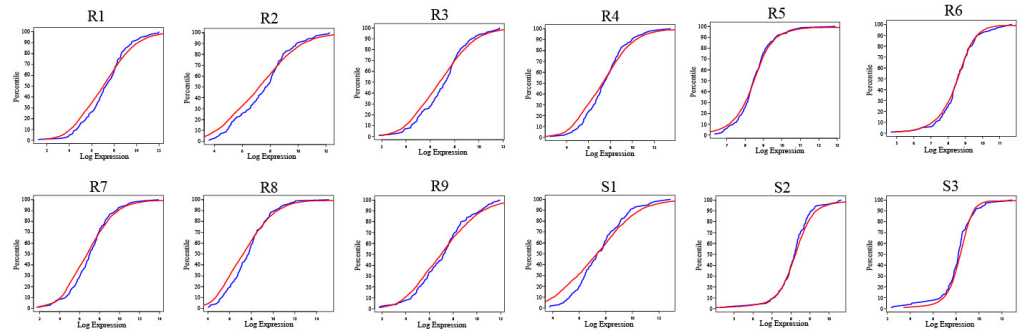
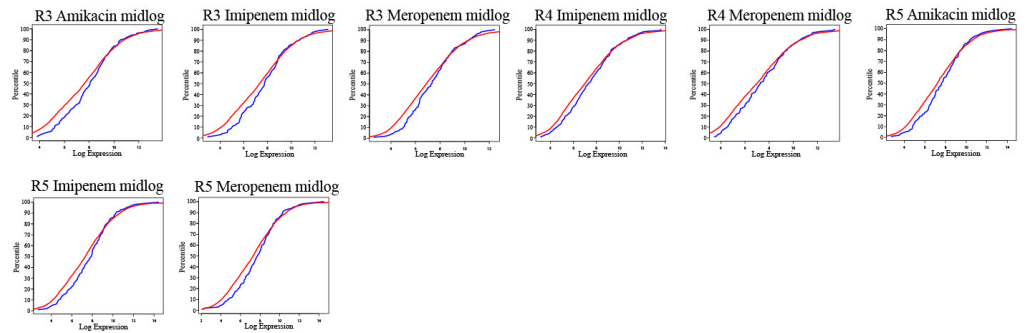


Figure S4. The three multidrug resistant strains showed differential responses under different antibiotic treatments. (a) Venn diagram and gene enrichment analysis of differentially-expressed genes under the three antibiotic treatments revealed both common and differential responses. (b) Venn diagram of differentially-expressed genes under antibiotic treatments in each strain. Scales and dimensions of all radar plots are the same as the middle example, which represents the common differentially-expressed genes among the three strains. (c) Venn diagram and gene enrichment analysis of differentially-expressed genes in R5 under the three antibiotic treatments.

Antibiotic free
Midlog phase



Antibiotic-treated
Midlog phase



Antibiotic-treated
Stationary phase

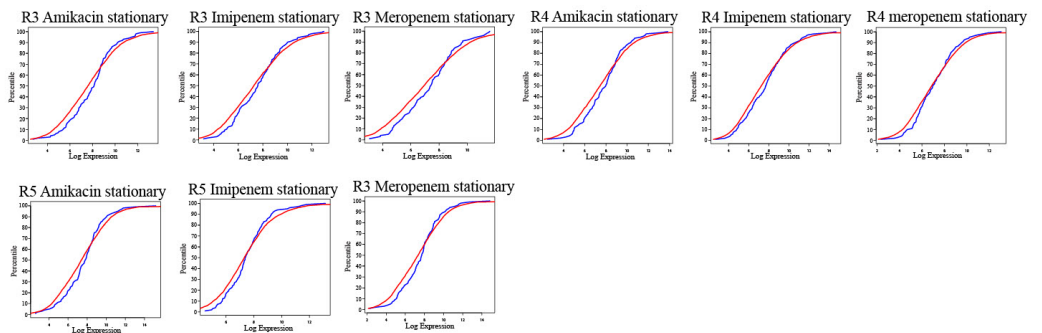


Figure S5. Comparison between transposon-associated and non-transposon-associated genes based on percentile of expression values across individual sample pairs. Blue line represents transposon-associated genes; red line represents non-transposon-associated genes.

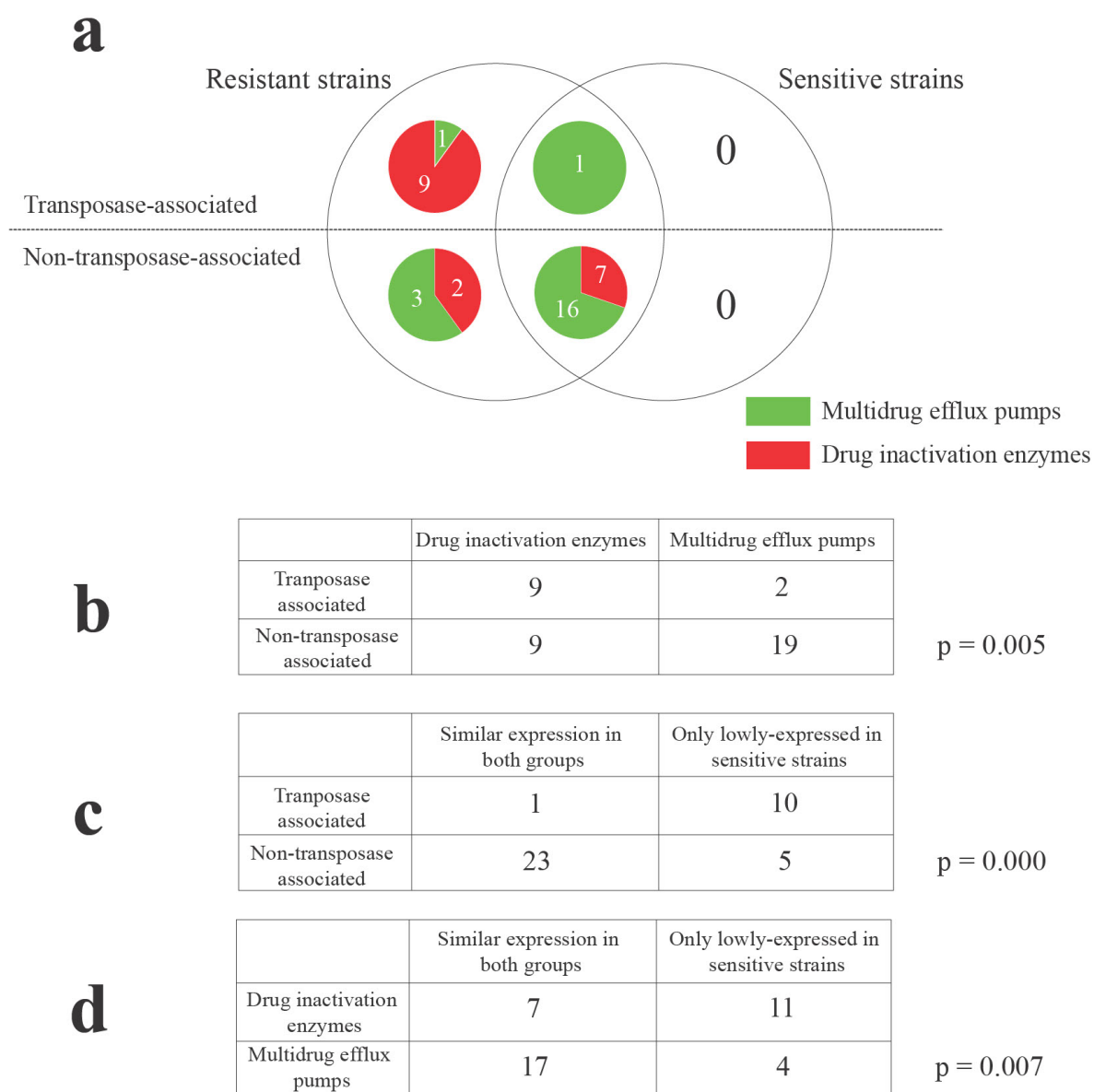


Figure S6. Transposon-associated resistant genes were more likely to be specific to multidrug resistant strains and co-transcribed with drug-inactivation enzymes. (a) Venn diagram of resistant genes. (b-d) Contingency table analysis of resistant genes' properties between: transposon-association vs. functions (b), transposon-association vs. expression patterns (c) and functions vs. expression patterns (d) p-values were calculated by Pearson's chi-square test.

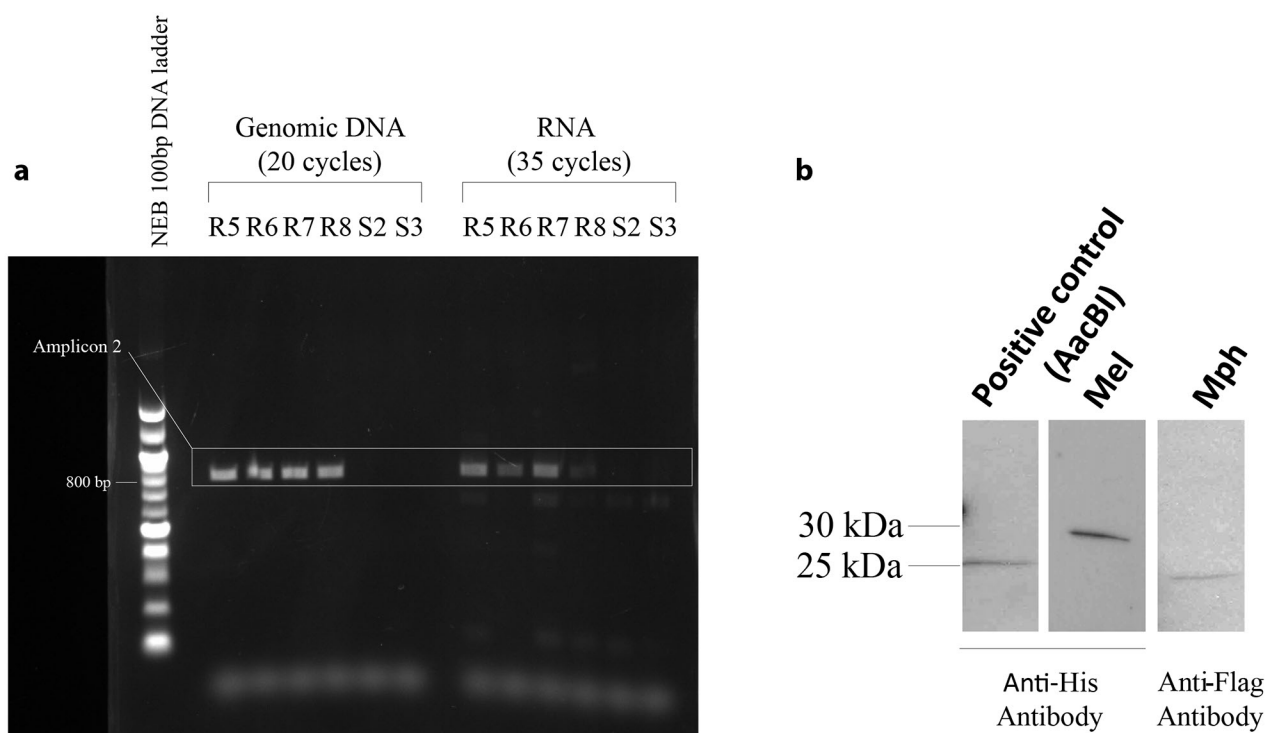


Figure S7. Expression validation of two antibiotic resistance genes in RNA and protein levels. (a) Weak co-transcription between *tnpD* and *mel* can be detected by qPCR after 35 cycles. (b) Western blotting confirmed the expression of Mel and Mph in plasmid-transformed cells.

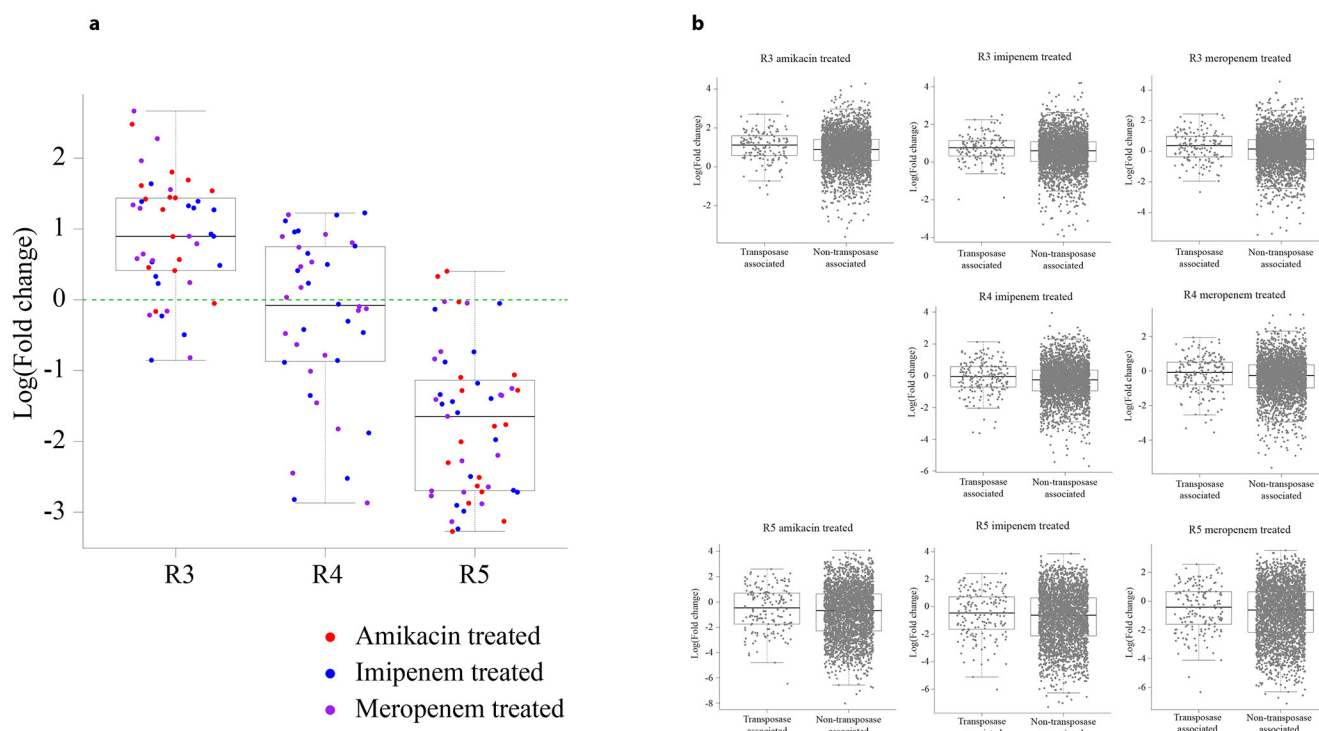


Figure S8. Expression patterns of transposable elements and transposon-associated genes. (a) Expressions patterns of transposable elements were strain-specific under antibiotic treatment. Each point represents a log(fold change) value of a transposable element gene under a certain antibiotic treatment. (b) Transposon-associated genes generally have higher fold changes under antibiotic treatment. Each point represents a log(fold change) value of a gene under a certain antibiotic treatment.