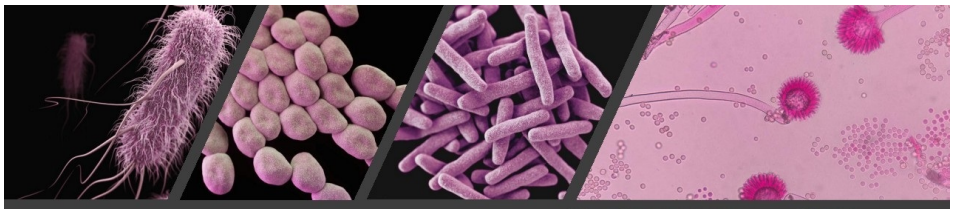

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Chromosomal ampR Regulates Plasmid-Mediated Antibiotic Resistance and Gene Duplication Amplification in Enterobacter cloacae

Thursday 11 September 2025 14:45 (15 minutes)

Background and Objectives: Gene duplication and amplification (GDA) is crucial for bacterial adaptation to antibiotic pressure [1]. In Enterobacter cloacae strain IMT49658-1, ceftazidime induces GDA of a genomic region containing blaDHA and adjacent ampR(DHA) [2]. The strain also carries plasmid-borne blaTEM and blaCTX, and chromosomal blaACT with ampR(ACT). Given the regulatory role of ampR in β-lactamase expression, we aimed to investigate whether ampR(ACT) influences GDA and the expression of plasmid-borne resistance genes, thereby modulating antibiotic susceptibility and bacterial fitness.

Methods: ampR(ACT) was deleted via λ Red recombineering, confirmed by PCR and sequencing. Antibiotic susceptibility was tested by agar disc diffusion assay. GDA copy number was quantified by qPCR of genomic. Meanwhile, Expression of blaDHA and ampR(DHA) was measured by RT-qPCR. ScanLag was used to assess colony appearance and growth times.

Results: Deleting ampR(ACT) unexpectedly increased ceftazidime resistance, despite reduced GDA. RT-qPCR showed blaDHA upregulation and ampR(DHA) downregulation, suggesting altered regulation. ScanLag revealed delayed growth, indicating a fitness cost.

Conclusions: These findings indicate that ampR(ACT) functions as a key regulatory element, influencing both chromosomal and plasmid-borne resistance genes. This study provides new insights into the genetic and regulatory mechanisms shaping antibiotic resistance evolution in E. cloacae.

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