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Investigating conjugative AMR plasmid maintenance using CRISPR-Cas-based plasmid curing

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Bacterial resistance against antibiotics has become commonplace in medical, veterinary, and environmental settings. This development is particularly driven by conjugative plasmids carrying antibiotics resistance genes, which spread easily in environments of high bacterial density. Understanding the dynamics of spread of AMR genes among commercially raised animals is a crucial component of preventing spread to humans. We therefore sought to investigate the contribution of non-AMR genes on the maintenance of conjugative plasmids by curing environmental E. coli isolates of their conjugative AMR plasmids and comparing the resulting plasmid-cured variants to the parent strains. Here, we develop and refine a CRISPR-Cas9-based approach to curing conjugative AMR plasmids carrying ESBL or pAmpC genes using a recombinant plasmid, pCBL (Curing Beta-Lactamases). This modular system can address common challenges of curing conjugative plasmids, such as toxin-antitoxin systems and broad resistances. We employ pCBL to produce plasmid-cured variants of ESBL/pAmpC-carrying E. coli strains isolated from broiler chickens and their environment and characterise these strains Current results showing differences in growth and biofilm formation in some strains will be presented. We aim to provide a fast and reliable method of curing AMR plasmids to assess the physiological impact of plasmid loss in a broad range of isolates and to investigate non-resistance advantages of conjugative AMR plasmids.

Keywords

Plasmid Curing, ESBL, E. coli

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Professional Status of the Speaker

Postdoc

Junior Scientist Status

No, I am not a Junior Scientist.

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