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***mcr-1* Confers Cross-Resistance to the Cationic Host Antimicrobial Lysozyme**

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mcr-1 has become an increasing concern due to the fear of rapid transferable resistance to our last resort antibiotics¹. While *mcr-1* confers resistance to the polymyxin antibiotic colistin, it remains unclear whether this gene also confers resistance to other antimicrobials. Here we report that *mcr-1* confers cross-resistance to the cationic host antimicrobial, lysozyme.

mcr-1 positive *Escherichia coli* isolates, CDF-1 and IHD86_4 (patient isolates from Switzerland² and Cambodia³, respectively), were cured of their *mcr-1* carrying plasmid by serial passage in Lysogeny Broth (LB). Curing of the *mcr-1* gene was confirmed using colony PCR comparing parental *mcr-1* positive strains vs. *mcr-1* cured strains (Fig. 1A). Colony PCR using *E. coli* specific *uspA* primers was performed as a positive control (Fig. 1B). When tested by broth microdilution in 25% LB, both cured strains exhibited a four and two-fold increase in susceptibility to colistin and polymyxin B, respectively.

The *mcr-1* encoded phosphoethanolamine transferase adds a positively charged phosphoethanolamine moiety to the lipid A portion of the bacterial outer membrane component lipopolysaccharide¹. This reduces the overall net negative surface charge of the bacteria, likely leading to repulsion of the cationic antibiotic, colistin. The host's innate immune system employs multiple positively charged antimicrobials to combat bacterial infection. Therefore, we sought to determine if *mcr-1* was capable of providing cross-resistance to the cationic host protein lysozyme. We measured survival rates between *mcr-1* positive isolates and cured strains in the presence of lysozyme in 25% LB. Strains lacking

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Author Contributions

Experiments were conducted by EXS. Data collection and analysis was performed by EXS. Data interpretation was conducted by EXS, DAH, and DSW. The manuscript was prepared by EXS, DAH and DSW. Study was planned and directed by DSW.

Declaration of Interests

The authors declare no competing financial interests.

mcr-1 were killed in the presence of lysozyme while the parental strains were able to grow (Fig. 1C). Specifically, *mcr-1* negative strains were 5 to 20-fold more susceptible to multiple concentrations of lysozyme when comparing percent survival.

To our knowledge, this is the first report of *mcr-1* conferring cross-resistance to a host antimicrobial. Resistance to the host's innate immune defenses through *mcr-1* could drive plasmid maintenance in strains carrying *mcr-1*, *mcr-2*, or other transferable resistance plasmids leading to propagation of colistin resistance.^{4,5} Therefore, mammals may currently or in the future serve as reservoirs for bacteria harboring transmissible colistin resistance regardless of polymyxin exposure. More studies are needed to determine the broader effect of mobilized colistin resistance and resistance to cationic antimicrobials within the context of the host.

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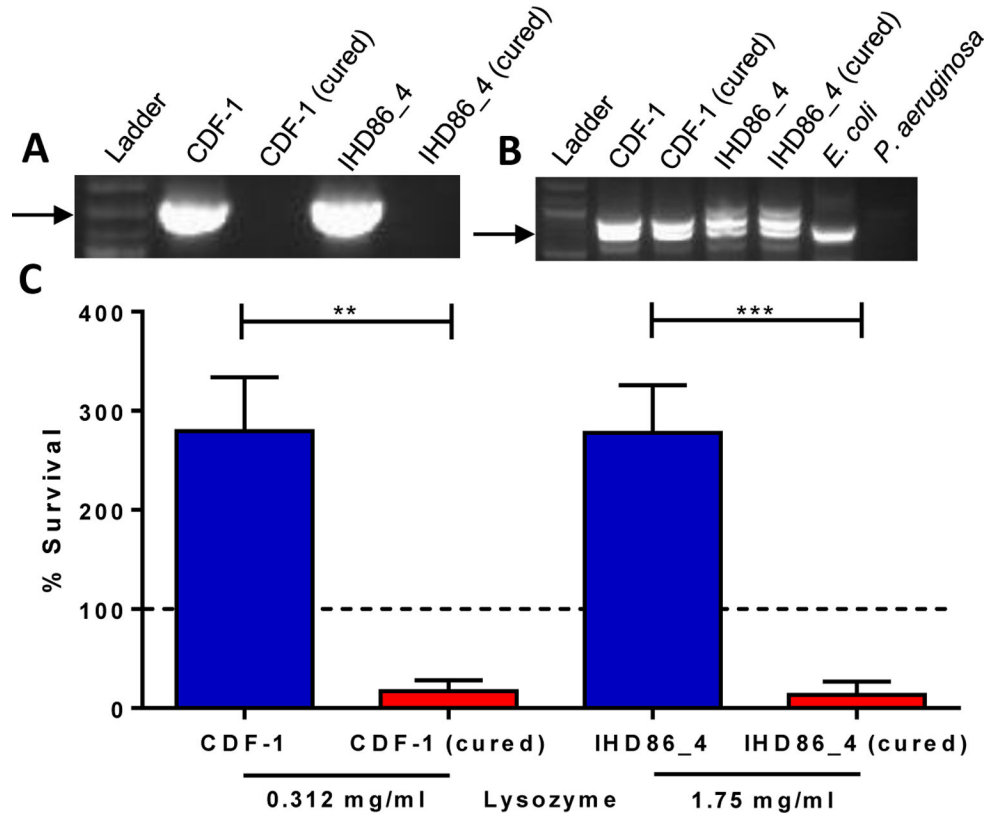


Figure 1. *mcr-1* mediates resistance to lysozyme.

A, B, Colony PCR using (A) *mcr-1* specific or (B) *uspA* specific primers on *mcr-1* positive isolates CDF-1 and IHD86_4 and their corresponding cured, *mcr-1* negative derivatives. A molecular weight ladder was included in each figure, and *E. coli* strain NCM3722 was used as a positive control and *Pseudomonas aeruginosa* strain PAO1 was used as a negative control in Figure 1B. **C,** Percent survival of *mcr-1* positive isolates and their corresponding, cured *mcr-1* negative derivatives, calculated by dividing the surviving CFUs after three-hour incubation with lysozyme by the initial inoculum (represented by dashed line, 1×10^6 CFU/ml). Data shown are representative of three biological replicates. Error bars represent mean \pm standard deviation, ** $P=0-0012$, *** $P=0-0008$.