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Comparative Analysis of Outer Membrane Vesicles from Cationic Adapted Escherichia Coli Isolates Reveals Unique Vesicle Membrane Morphologies and Different Antimicrobial Susceptibilities When Supplemented to Unadapted E. coli.

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Abstract (266 words): Cationic antimicrobials (CA) such as the therapeutic antibiotic colistin (COL) and antiseptics cetrimide (CET) and chlorhexidine (CHX) all exert their mechanism of action by disrupting bacterial membranes, leading to cell content leakage and death. Resistance to CAs is rapidly increasing and of the many antimicrobial resistance mechanisms that may contribute, the role of outer membrane vesicle (OMV) formation is least understood. Here, we gradually adapted *E. coli* BW215113 K-12 to COL, CET, and CHX by prolonged exposure *in vitro* to determine how each CA impacts OMV formation. We assessed OMVs isolated from each CA adapted strain by nanoparticle tracking analysis (NTA) and cryo-transmission electron microscopy (cryo-TEM), and compared OMV proteomes using liquid chromatography-mass spectroscopy. The results of NTA analysis showed that all three CA-adapted strains had increased OMV formation as compared to unadapted BW25113 (WT), where CA-adapted OMVs were significantly larger. Cryo-TEM analyses revealed that each strain's OMVs had distinctive morphological alterations, where CET-OMVs looked similar to unadapted WT OMVs but were encapsulated and aggregated, CHX-OMVs were multilamellar and COL-OMVs were large (4-12X) and tubular as compared to WT OMVs. Proteomic analysis highlighted significant increases in EptC abundance in COL-OMVs and decreased abundance of MlaA in CHX-OMVs. Antimicrobial susceptibility testing of *E. coli* BW25113 K-12 supplemented with purified CA-adapted OMVs demonstrated that CET-OMVs did not alter CET susceptibility, CHX-OMVs enhanced BW25113 tolerance to CHX exposure by 2-fold, and COL-OMV supplementation to BW25113 increased COL susceptibility by 2-fold compared to unadapted WT OMVs. Hence, CA adaptation by *E. coli* has significant ramifications on OMV production and morphology, and CA-adapted OMV exposure has significant consequences for bacterial antimicrobial susceptibility.