Comparative analysis of outer membrane vesicles from cationic adapted *Escherichia coli* isolates reveals unique vesicle membrane morphologies and different antimicrobial susceptibilities when supplemented to un-adapted *E. coli*

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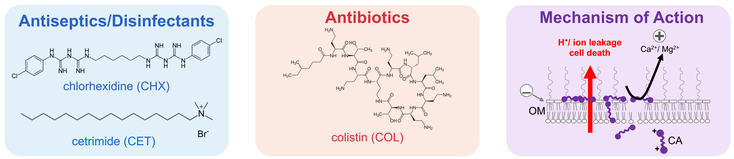
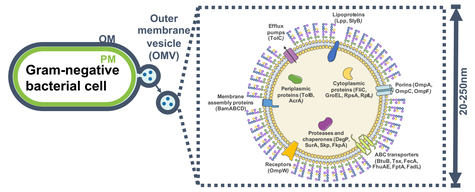
1 INTRODUCTION

Cationic antimicrobials (CAs) such as antiseptics chlorhexidine (CHX) and cetrimide (CET) and the antibiotic colistin (COL) act by disrupting the bacterial outer membrane (OM) and displacing divalent cations, leading to cell content leakage, pore formation, and death[1].

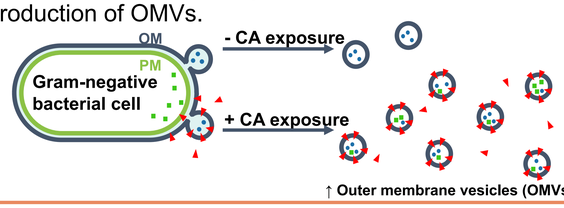


Outer membrane vesicles are small structures produced constitutively by Gram-negative bacteria. They can transport DNA/RNA or virulence factors, participate in host immune invasion or defense, and aid the cell in resisting antimicrobials[2,3].

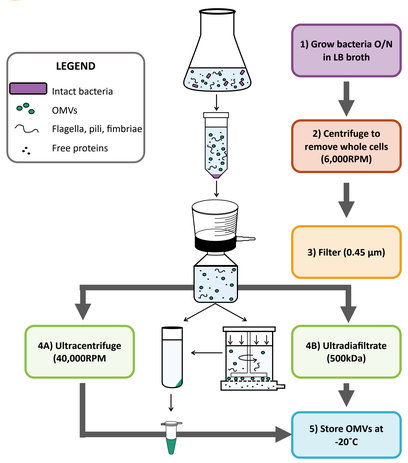


2 STUDY AIM

This study's aim was to identify changes in OMV production and composition after CA exposure, and to test the hypothesis that CA-tolerant strains have increased production of OMVs.



3 METHODS



- *E. coli* BW25113 K-12 was gradually adapted to COL, CET, and CHX by prolonged exposure *in vitro*[4].
- OMVs were isolated from CA-adapted strains (CETR, CHXR, COLR) using an ultradiafiltration and ultracentrifugation protocol[5].
- OMV size and morphology were analyzed using nanoparticle tracking analysis (NTA) and cryo-transmission electron microscopy (cryo-TEM).
- OMV proteomes were compared using liquid chromatography-mass spectroscopy (LC-MS/MS).
- Antimicrobial susceptibility against CAs was assessed with supplementation of OMVs purified from WT or CA-adapted strains.

4 RESULTS

- All CA-adapted strains produced significantly more/larger OMVs than WT (WT = 85.4 nm ± 20.9, CETR = 169 nm ± 42.3, CHXR = 124nm ± 30.2, COLR = 222nm ± 81.8).
- COLR-OMVs were the most polydisperse, exhibiting a wide size range (48-580nm in diameter) of OMVs.
- Cryo-TEM confirmed NTA size estimates and showed that each strain had unique vesicle morphologies.

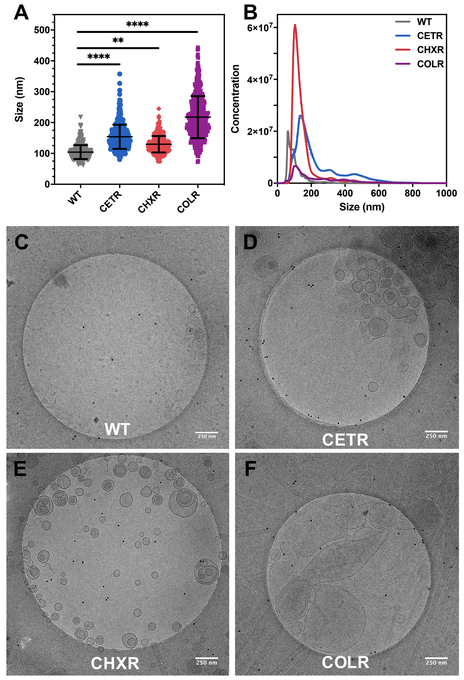
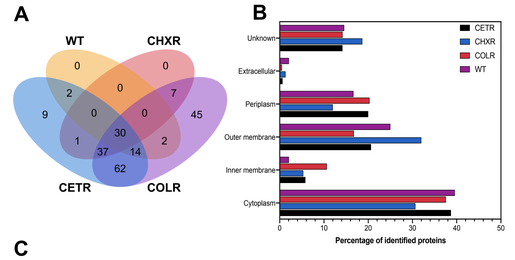


Figure 1. Size and morphology of CA-adapted OMVs as assessed by NanoSight NTA and cryo-TEM. A) Diameter of vesicles as measured by NTA (nm). B) Size distribution and concentration of vesicles by NTA. C-F) Representative cryo-TEM images of C) WT D) CETR E) CHXR and F) COLR OMVs at 14,500X magnification.



C

Enrichment Score	GO Term (Biological Process)	Number of Proteins
0.00	transcription, DNA-templated	
0.04	negative regulation of cellular metabolic process	
0.17	polysaccharide biosynthetic process	
0.31	protein complex assembly/biogenesis	
0.42	aromatic compound catabolic process	
0.51	ion homeostasis	
0.78	posttranscriptional regulation of gene expression	
0.81	monosaccharide metabolic process	
0.84	cell wall biogenesis	
0.93	peptidoglycan metabolic process	
2.38	purine nucleotide metabolic process	
2.48	translation	
2.71	cellular protein localization	
2.75	glycolytic process	
3.16	pyridine nucleotide metabolic process	
3.41	ATP metabolic process	
5.48	Gram-negative-bacterium-type cell outer membrane assembly	

Figure 2. Proteins detected in CA-adapted OMVs as identified by LC-MS/MS. A) Venn diagram of the number of unique and overlapping proteins detected in each strain's OMVs. B) Cellular localization of proteins from CA-adapted OMVs as determined by pSORTb v.3.0.2 (https://www.psort.org/psortb/). C) Gene ontology enrichment analysis of proteins identified in all samples by DAVID v.6.8 (https://david.ncifcrf.gov).

- CA-adapted OMVs contain more unique proteins than WT OMVs
- The most abundant proteins in all samples were outer membrane-associated proteins, involved in outer membrane assembly, protein transport and efflux, stress response and protein folding.

- Supplementation with CETR and CHXR OMVs had little to no effect on growth or susceptibility (CHXR-OMVs enhanced WT tolerance to CHX by 2-fold).
- Supplementation of COLR OMVs to WT cultures had a detrimental effect on growth (concentration dependent decrease in OD_{600nm}) and antimicrobial susceptibility (COLR-OMV increased WT susceptibility to COL by 2-fold).
- Supplementation of COLR or WT OMVs to COLR cultures appears to be protective (2-fold increase in MIC).

Table 1. Antimicrobial susceptibility testing against CET, CHX, and COL with OMV supplementation

Sample	OMV Supplementation	CET MIC (µg/mL)	CHX MIC (µg/mL)	COL MIC (µg/mL)
WT	-	30	2	1
WT	2.0 µg/mL WT OMVs	30	2	4
WT	2.0 µg/mL CETR OMVs	30	-	-
WT	2.0 µg/mL CHXR OMVs	-	4	-
WT	2.0 µg/mL COLR OMVs	-	-	0.5
CETR	-	120	-	-
CETR	2.0 µg/mL WT OMVs	120	-	-
CETR	2.0 µg/mL CETR OMVs	120	-	-
CHXR	-	-	16	-
CHXR	2.0 µg/mL WT OMVs	-	16	-
CHXR	2.0 µg/mL CHXR OMVs	-	16	-
COLR	-	-	-	256
COLR	2.0 µg/mL WT OMVs	-	-	512
COLR	2.0 µg/mL COLR OMVs	-	-	512

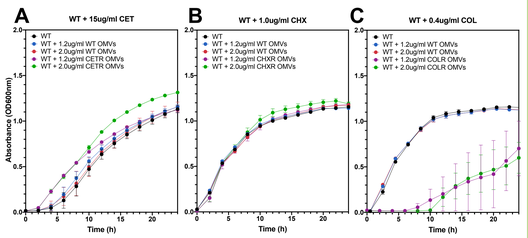
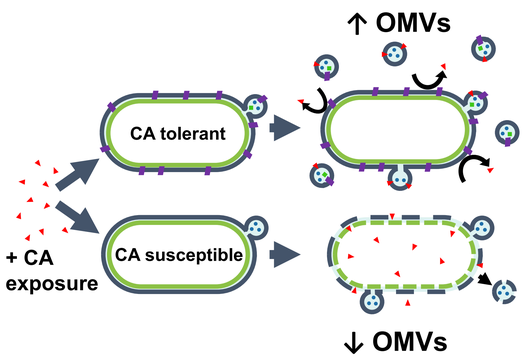


Figure 2. Growth curves of WT cultures in sub-MIC concentrations of CAs supplemented with either WT or CA-adapted OMVs. A) WT cultures grown in 15 µg/mL CET with WT and CETR OMVs. B) WT cultures grown in 1.0 µg/mL CHX with WT and CHXR OMVs. C) WT cultures grown in 0.4 µg/mL COL with WT and COLR OMVs.

5 CONCLUSIONS

- CA-adapted bacteria produce greater amounts of OMVs that are larger and have unique morphologies compared to WT.
- Phenotypes, proteomic analysis and antimicrobial susceptibility are CA-dependent, indicating that each CA-adapted strain has distinct membrane alterations that affect their OMV formation.
- OMVs have important consequences for survival and pathogenesis during infection, especially considering that *E. coli* can exist in diverse environments within hosts, often exposed to low levels of antimicrobials, host defenses and other stressors.



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7 ACKNOWLEDGEMENTS

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