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A horse called B12: Exploring the potential of vitamin B12-antibiotic conjugates to combat antimicrobial resistance in Gram-negative bacteria of WHO critical concern.

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Antimicrobial resistance is a significant global health risk, with priority bacteria (mostly Gram-negative) being identified for development of new antibiotics (World Health Organisation, 2017). More importantly, novel treatment approaches for currently available drugs are an ongoing research area.

In this presentation, I will describe a novel drug delivery method that utilises bacterial uptake of vitamin B12 (B12), an essential bacterial co-enzyme. Bacteria efficiently take up environmental B12/B12 analogues, therefore hijacking B12 uptake could circumvent the intrinsic resistance of the Gram-negative outer membrane - a key resistance mechanism. This is the "Trojan horse" principle at the core of our research. This principle has demonstrated previous success, with B12 conjugates demonstrating efficient intracellular transport of diverse substances (Równicki et al., 2017). Clinical success has also been demonstrated, with approval of the siderophore-cephalosporin conjugate Cefiderocol (U.S. Food and Drug Administration, 2025).

A key part of this research was identifying the localisation of B12. B12- fluorophore localisation in *Escherichia coli* (*E. coli*) was assessed by separating cell compartments via fractionation. B12-fluorophore conjugates were found in the combined outer membrane and periplasmic space fraction but absent from the cytosol. Additional research is now being done to better understand localisation to the outer membrane versus the periplasmic space.

We have also synthesised a range of B12-antibiotic conjugates. The efficacy of B12-conjugated versus unconjugated antibiotics was investigated using minimum inhibitory concentration (MIC) testing in *E. coli*. Conjugates of Gram-positive drugs Rifampicin and Vancomycin were tested, but B12 conjugation did not result in efficacy. Further research is underway to determine if B12 conjugation enhances efficacy of Gram-negative antibiotics such as ampicillin.

The applications of "Trojan horse" research in enhancing antimicrobial efficacy could have significant global health benefits. Restoration of susceptibility to common antibiotics and finer targeting of pathogens aid in minimising resistance, as well as reducing side effects.

Keywords
antibiotic resistance, antimicrobial resistance, vitamin B12

References

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