Assessing efficacy and mode of action of cefiderocol, a novel antibiotic conjugate, on multi-drug resistant *Pseudomonas aeruginosa* in the absence and presence of bound metal ions

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Introduction

The alarming rise of multidrug antibiotic resistance in *Pseudomonas aeruginosa,* which causes infections ranging from urinary tract infections to septicaemia, burn wound colonization and chronic colonization of the lungs of cystic fibrosis patients, necessitates the development of strategies to efficiently target antibiotic delivery.

One possibility is to exploit existing membrane transporters that are required for the uptake of metal ions which bacteria acquire from the host environment. Upon infection, the host-defence strategy of 'nutritional immunity' refers to the sequestration of essential metal nutrients within the host to restrict the growth of pathogenic bacteria. There is a tug-of-war for metal ions between host and pathogen, compounded by the presence of siderophores, which are low-molecular-weight chelators synthesized by bacteria for metal ion acquisition; some bacteria even produce siderophores tethered to antibiotics to harm competitors which express the siderophore receptors on their surfaces.

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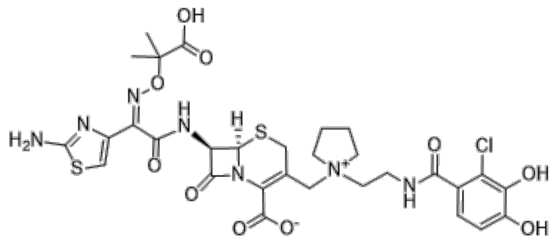

This discovery has inspired synthetic conjugation of antibiotics to siderophores in order to 'trick' siderophore uptake machinery for antibiotic delivery. Cefiderocol (fig. 1) is one such conjugate, composed of the antibiotic cephalosporin chemically bound to a small organic compound called a catechol group, which is known to be recognized by specific outer membrane receptors called TonB-dependent receptors (such as fepA) used endogenously by *P. aeruginosa* for iron transport[1] (fig. 2).

Objectives and Methods

The aim of the project was to understand how the antibiotic enters bacterial cells. It is currently in phase II and III clinical trials for complex antibiotic-resistant bloodstream infections, hospital-acquired pneumonia and sepsis, however little is known about the method of entry, other than the proposed 'Trojan-horse' style method based on its structure. My guiding research questions were:

→ To what extent is cefiderocol effective against wild-type *P. aeruginosa*, and several mutants lacking key membrane transporters?

→ Is cefiderocol complexed with a transition metal ion more effective at gaining entry into the bacterial cell, and does pre-formation of the metal-cefiderocol complex affect activity?

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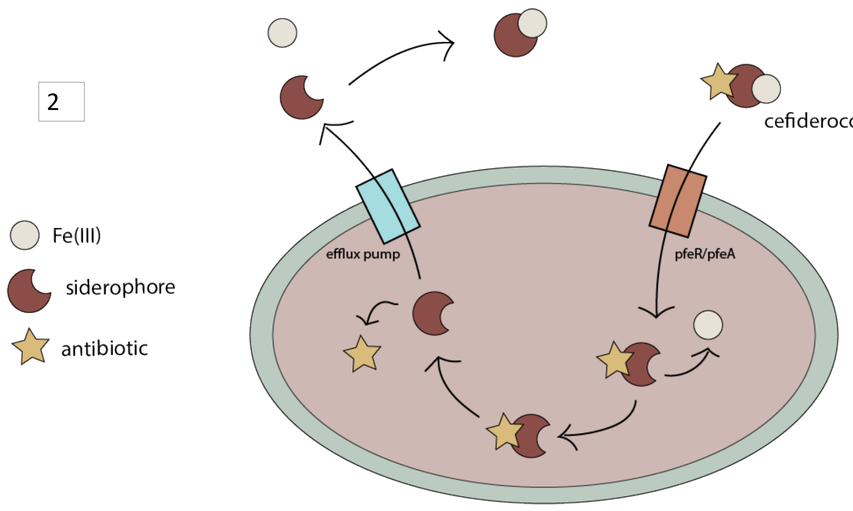

The first step was to assess the purity and composition of the compound cefiderocol, using analytical HPLC (fig. 3) and Liquid Chromatography-Mass Spectrometry (LC-MS).

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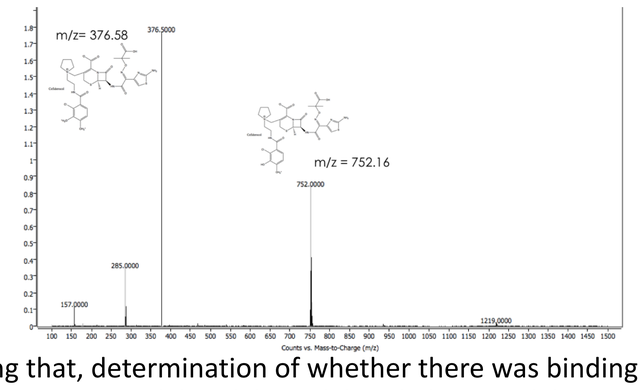
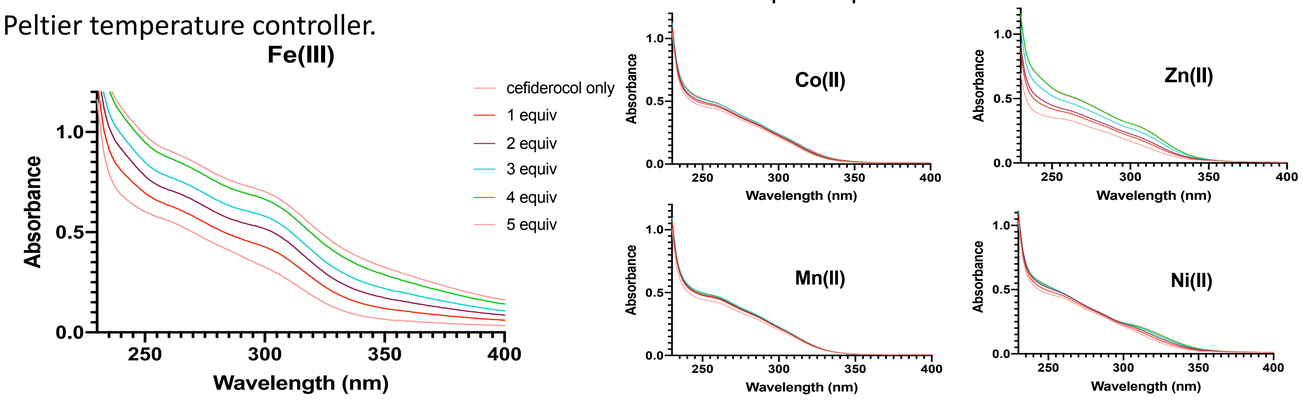


Following that, determination of whether there was binding to the transition metal ions Co(II), Ni(II), Mn(II), Fe(III) and Zn(II), and if so, with what thermodynamic parameters such binding occurs, was assessed using UV-Vis spectroscopy.

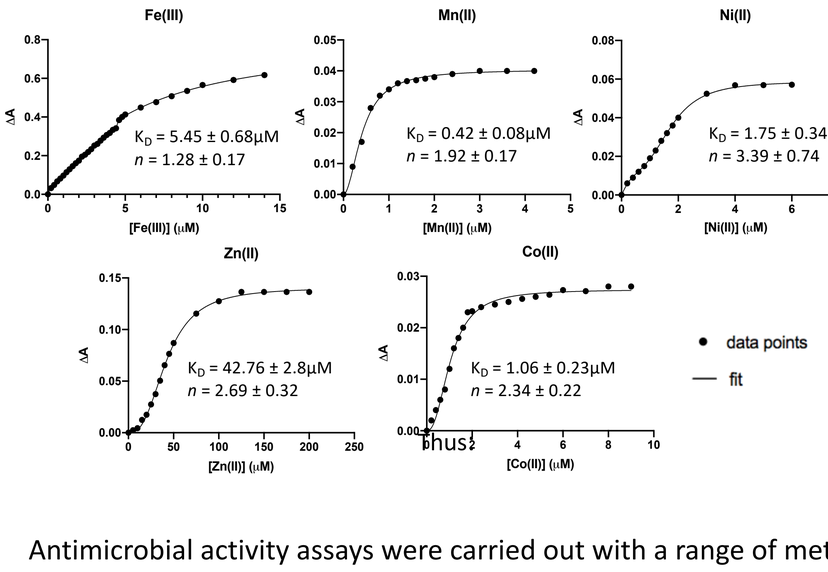
In order to assess the efficacy of the antibiotic, antimicrobial activity assays were performed in 96-well plates with several strains each of *Pseudomonas aeruginosa* and *Escherichia coli,* including mutants deficient in the synthesis of outer membrane ferric siderophore receptors. Culture turbidity readouts were used to assess the antibacterial activity of cefiderocol. Metal-cefiderocol complexes in varying ratios were also tested to assess the means by which the compound enters bacterial cells.

Results and Discussion

UV-Vis metal-binding titrations (25µM cefiderocol titrated with 2.5µL additions of 20mM metal ion solutions at pH7.0 in 2mL quartz cuvettes (Starna), 75mM HEPES, 100mM NaCl buffer at 25°C). Optical absorption spectra were collected from $\boldsymbol{\lambda}$ = 200-800nm on a Beckman Coulter DU 800 spectrophotometer thermostatted at 25 °C with a Peltier temperature controller.



The change in absorbance at 259 nm was plotted versus the metal ion concentration. Stoichiometry of binding was determined from the inflection points of the graphs, and the K_D of binding was estimated using the equation shown, with fitting based on the following model[3], where *n* is the Hill coefficient, which provides a quantitative measure of the degree of cooperativity between binding sites. The calculated thermodynamic parameters and the deduced stoichiometries informed my choices of concentrations of metal ions to use for the antimicrobial activity assays.



Let M = metal and P = cefiderocol

$$P + nM \leftrightharpoons PM_n$$

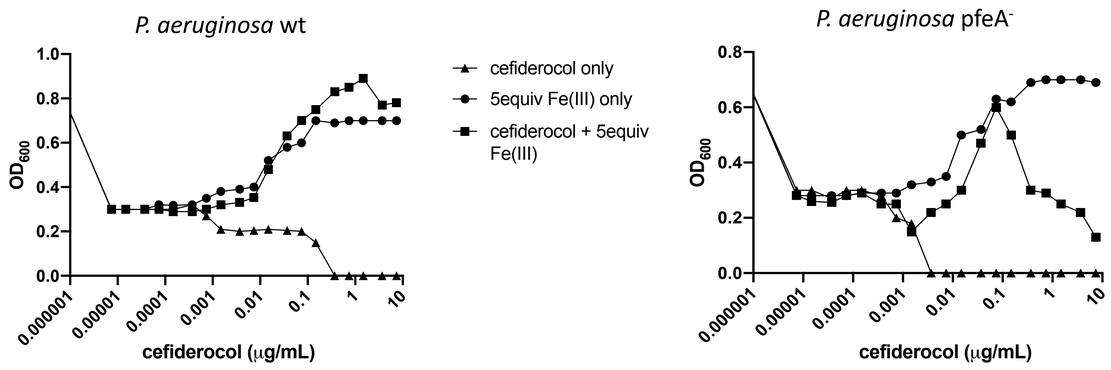
$$K_D = \frac{[P][M]^n}{[PM_n]}$$

The change in absorbance as a fraction of the maximal change observed reflects the proportion of cefiderocol that is ligand-bound.

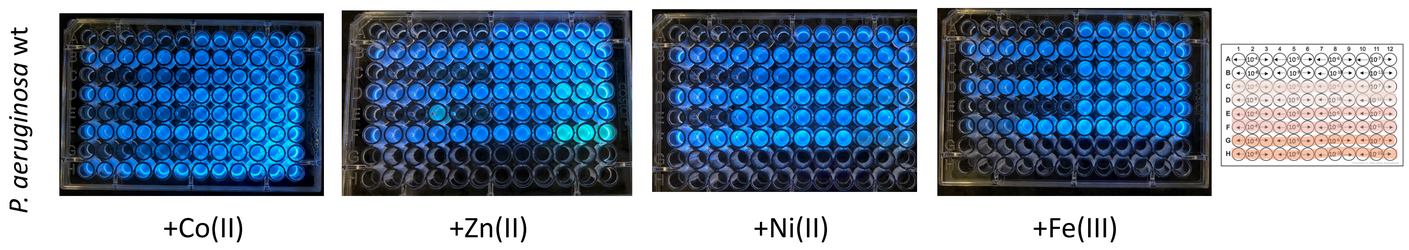
$$\frac{\Delta A}{\Delta A_\infty} = \frac{[PM_n]}{[P]+[PM_n]}$$

$$\Delta A = \frac{A_\infty([M]^n)}{(K_D + [M]^n)}$$

Antimicrobial activity assays were carried out with a range of metal concentrations, cefiderocol concentrations, and metal-cefiderocol complexes, in order to determine whether pre-formation of a metal complex affects the activity of the compound. In contrast to the predicted increase in MIC upon knockout of iron transporters in *P. aeruginosa*, suggesting that iron transporters contribute to the permeation of cefiderocol across bacterial outer membranes[2], my results display the opposite pattern, and elucidate that this antibiotic's means of entry is entirely different than current theory suggests.



The fluorescence of the bacteria at *t*=20h can give qualitative indications of the types of endogenous siderophores being produced, which can be informative about stress responses and nutrient sources used.[4]



For instance, in all cases, more pyocyanin, an endogenous siderophore, is produced at higher metal ion concentration; this likely indicates better growth. With Zn(II) addition, the metal-only control has a more aquamarine tint, indicating a higher ratio of pyoverdine (green) to pyocyanin (blue) compared to the wells in which only antibiotic was added. Cefiderocol may inhibit pyoverdine production, or may interfere with its production pathway, whilst metal addition has been shown to stimulate pyoverdine and pyochelin pathway genes in *P. aeruginosa*.[4] Moreover, whilst cefiderocol usually inhibits growth up to 10^{-6}M, as Ni(II) is added, cefiderocol inhibits growth up to 10^{-5} M, thus increased nickel availability improves drug efficacy.

Conclusions

- The mechanism by which cefiderocol acts does not in fact appear to be a Trojan horse mechanism
- The siderophore moiety does not appear to contribute to mode of entry
- The addition of metal ions improves bacterial growth and vastly outcompetes any potential effect observed due to complexation of metal ions with cefiderocol

These observations warrant further studies, such as an in vitro comparison of pure β-lactamase activity and pure cephalosporin activity in comparison to cefiderocol.

References:

1. Aoki et al, European Journal of Medicinal Chemistry (2018) 155:847-868. 2. Zhanel et al, Drugs (2019) 79:271-289. 3. Shalk, I.J. (2018). European Society of Clinical Microbiology and Infectious Diseases, 24(8):801-802. 4. Choi, J.J., McCarthy M.W. (2018) Expert Opinion on Investigational Drugs, 27(2), pp.193-197.

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