Written responses to open questions of the webinar 'Efflux inhibitors: A strategy to tackle multidrug resistance' by Helen Zgurskaya (speaker), Ruben Hartkoorn (speaker), Timothy Opperman (speaker), Timothy Opperman (speaker) and Laura Piddock (moderator) originally broadcast on 23 April 2024.

See webinar recording here: https://revive.gardp.org/efflux-inhibitors-a-strategy-to-tackle-multidrug-resistance/

Question asked	Response from the speakers
Ruben: Is the efflux pump strategy attributed to only Gram-negative bacteria or does this strategy exist also in Gram-positive bacteria?	Antibiotic efflux plays an important role in many bacteria, but the RND-efflux pumps of Gram negative bacteria are particularly important as they are often constitutively expressed and provide broad spectrum resistance. Saying this, there is significant research into inhibitors of other classes of antibiotic efflux pumps (particularly from the MFS family) that have the potential to improve antibiotic activity in resistant Gram positive bacteria.
Ruben: Have you acquired any in vitro data?	The published in vitro data can be found in: https://doi.org/10.1038/s41467-021-27726-2 https://doi.org/10.1038/s44321-023-00007-9 https://doi.org/10.1093/jacamr/dlad112
While studying the structure of efflux pumps (narrow or broad spectrum), have the extracellular domains been studied or found to act as receptors to bacteriophage (natural viruses of bacteria) binding?	This has been done for TolC, please see: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2832399/
How does conformational change occur for the efflux of the toxic substances? Is a mutation possible at these sites without harming the bacteria?	Yes, please see: https://pubmed.ncbi.nlm.nih.gov/25737552/
Ruben: Most of the MDR strain gets selected due to target modification. In that case, efflux inhibitors will not be effective. Isn't it?	Efflux is required for some drug resistance genes to confer resistance. Please see the section 'Clinical Relevance of MDR Efflux' of this article: https://pubs.acs.org/doi/10.1021/acsinfecdis.4c00091 This is in part correct. When mutations in an antibiotic target result in a very high level of resistance, the inhibition of antibiotic efflux may still allow for boosting antibiotic activity, but not sufficiently to attain the antibiotic breakpoint. The use of EPI to treat drug-resistant bacteria therefore will depend on the genotype of the bacteria, and the antibiotic to be boosted.

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Question asked	Response from the speakers
To anyone on the panel or Laura, how feasible is using an efflux pump as a diagnostic target in clinical practice to determine the need to use efflux inhibitors?	The discovery method used in this paper could be adapted for a clinical lab. Please see figure 4 of this article: https://journals.asm.org/doi/10.1128/mbio.00465-20 In addition to genotyping, a phenotypic screen could be imagined where standard drug susceptibility testing is performed with and without EPI.
If antibiotics also theoretically interact with these hydrophobic traps before being pumped out, what is special about the pump inhibitors that lock the pump conformation? Is it just that affinity is much higher for the hydrophobic trap in the DBP?	Correct. Efficient transporters must balance substrate affinity and specificity with fast turnover. Substrates interacting with the hydrophobic trap are less specific and have higher affinity.
Have you looked specifically at the synergism between biofilm formation in chronic infection and efflux-mediated resistance (as opposed to barrier-mediated efflux synergism in planktonic cells)?	Please see various publications: https://pubmed.ncbi.nlm.nih.gov/?term=Webber+M+AND+efflux+AND+biofilm&sort=date
Studies have shown how mutations in some efflux proteins can result in resistance to other antibiotics (besides those they originally conferred resistance to). With this, do you have cases where an efflux inhibitor will confer resistance to one antibiotic vs. not another?	Yes, these are multi-drug resistance efflux pumps. Please see: https://www.nature.com/articles/nrmicro3380
Helen: Why did you choose MexB for generating interaction descriptors?	MexB is a constitutively expressed efflux pump of *P. aeruginosa* and has very broad substrate-specificity.
Helen: Some antibiotics are taken into cells by active uptake transporters. Will efflux pumps affect such antibiotics?	It depends on the relative contributions of the two fluxes: if uptake is more efficient than efflux, an antibiotic will accumulate inside the cells.
Ruben: What would be the best-accompanying antibiotic for EPI?	This was discussed in the webinar. I believe the preferred antibiotics were tetracyclines (such as minocycline or tigecycline) and macrolides (such as azythromycin)
Ruben: Could EPI be realistically introduced to treat resistant bacteria before the emergence of carbapenem / ESBL resistance, or only as a last resort?	I think this question is still up in the air and requires much more research. Depending on the EPI/antibiotic combination, one could imagine treating either intermediately resistant bacteria (to protect carbapenems), or to treat ESBL/carbapenemase resistant bacteria (last resort, but perhaps better than colistin).

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