Written responses to open questions of the webinar 'Biofilms: What are they and why do we care?' originally broadcast on 21 October 2021. See webinar recording here: https://revive.gardp.org/biofilms-what-are-they-and-why-do-we-care/

Question asked	Response from the speaker
Freya, in your work with Andrew, was the assay done before the biofilm matrix formed or afterwards (that is treatment with colistin)? And if it was afterwards, since colistin does not target biofilm matrix, would you expect to observe reduced drug penetration?	We treated with colistin after the biofilms had been formed. There are a few published studies looking at colistin susceptibility of P. aeruginosa biofilms, and the results vary – probably due to different platforms being used for biofilm growth, resulting in different biofilm thicknesses, different amounts of matrix and potentially different matrix composition too. We expected to see relatively poor colistin penetration because the biofilm formed in the lung model seems to be particularly thick, but I was surprised it was so low. The main point of this study was to see if we could use a simple benchtop fluorimeter to measure the penetration of antibiotics, we chose colistin because it's clinically relevant for CF and Andrew's lab had already made the BODIPY-labelled version.
Mark, can you recommend a review paper providing an overview on *in vitro* biofilm models?	This is probably a useful start; https://www.sciencedirect.com/science/article/pii/S2589004221004119
Can you suggest any non-pharmaceutical interventions to inhibit biofilms?	In medical device settings, people are looking at non-pharmaceutical interventions in the sense that they are exploring how changing the materials or surface characteristics of devices can make it harder for biofilms to form on them. And in the case of chronic wounds, there are physical interventions such as wound debridement (physically scraping away infected/necrotic tissue) or maggot therapy (to remove necrotic tissue) which aim to remove infected tissue and promote healing. In CF, mucolytic agents are routinely given to break down the patient's lung mucus and make it easier to cough up – one of these is a DNase, this breaks down DNA in the mucus which is an important part of the pathogen biofilm matrix.

Remaining audience questions from the webinar 'Biofilms: What are they and why do we care', broadcast on 21 October 2021.

Question asked	Response from the speaker
Efflux pump upregulation seems to be a recurrent theme for explaining increased antibiotic tolerance and the emergence of resistance. It is interesting to hear about the potential fitness cost coming with decreased biofilm formation when ramA is mutated. Do you think this is generalizable to other organisms? Does efflux upregulation have other fitness costs (for example, energetic costs)?	The relation between efflux pump regulation and biofilm gene regulation seems pretty conserved and has been observed in many species now – upregulation of efflux in general is tolerated but only to a degree (pump expression tends to increase 2-3 fold rather than 10 fold for example), probably due to energetic costs or impacts on the membrane etc.
Freya, do you have any clue on what is making the PA14 biofilm in a wound setting resistant to the eye salve, when compared to the sensitive PA14 biofilm in the CF setting?	I think the physiology of the bacteria is going to be very different in the two settings, as the physical and chemical environments are very different. We have completed a transcriptomics study of PA14 in the lung model over 7 days which is already telling us a lot about physiology in that environment, but I would love to see more studies that explicitly compare the physiology of a given pathogen in some of the different host environments it inhabits. (The PA14 transcriptomics study is on bioRXiv and should be published soon as we have just submitted minor revisions).
Mark, it seems antibiotic resistance decreases biofilm production, but we find biofilm resistant bacteria are multidrug resistant. How? According to cost effectiveness resistant bacteria should not produce biofilm.	I think this shows that evolution of multiple phenotypes is complex – whilst you may incur a cost from one mutation (e.g. the one giving AMR) this can often be alleviated later by another compensatory mutation so cells can eventually accumulate both phenotypes
Mark, where selection for resistance in a biofilm has a fitness cost, is that stable or can compensatory mutations occur over time to allow both resistance and fitness?"	Probably depends on the specifics of the mutation but when we tested this in our resistant populations by re passaging them with no drug pressure they maintained resistance but gained other mutations to give them resistance
Mark, what potential is there for specific anti-biofilm inhibitors and how might they be used clinically?	Potentially good I think – some anti-biofilm treatments have been developed (matrix degrading enzymes etc.) which look promising, what hasn't really been done a lot is to look for specific biofilm essential targets but there is potential I think

Question asked	Response from the speaker
As we know the resistance of microbes to antibiotics is increasing day by day, it has been seen that traditional medicines have been explored from the last few decades as antimicrobials because of the presence of secondary metabolites. I want to know about the use of secondary metabolites whether this will be a good alternative approach for biofilm inhibition or not?	Most antibiotics derive from secondary metabolites already so no reason why there may not be biofilm inhibitors to be found…..
Mark, could you clarify the difference between persister cells and dormant cells?	Persisters are a result of active inhibition of cellular machinery, e.g. ribosome being inhibited, different to cells which are metabolically more active but nutrient stressed
Mark, how did you distinguish biofilms with the planktonic cell in-vitro, is there any in-silico packages or simulation available for modelling pathogenic bacterial biofilm in 3D space	In the biofilm evolution model we take planktonic cells from the liquid phase – biofilm cells are recovered from beads after they have been washed to remove any loosely adhered planktonic cells. Visualising biofilms is easy enough in situ by microscopy and there are lots of packages people use to so this