Antibiotic resistance and pathogenesis of Streptococci with focus on Group A Streptococci

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Errata

Page 16:
~~Multi-d~~ Drug resistant ~~(MDR)~~ infections remain one of the leading cause of death worldwide (WHO, 2019).

Page 17:
~~Multi-drug~~ Drug resistant infections (human diseases caused by bacteria resistant to ~~more than one~~ antibiotics) remain one of the leading cause of death worldwide resulting in ~ 700 000 deaths every year (2014).

Page 21:
~~Around~~ ~~65%~~ More than 60% of bacterial infections are associated with biofilm formation.

Page 23:
This mechanism is initiated by direct contact between bacterial ligands (virulence factors) and cell receptors ~~that may or may not require lipid rafts (depending on the expressed bacterial ligand and cell receptors)~~, consequently initiating cell membrane zippering around the bacteria that activate a set of signalling cascades, finally leading to bacterial uptake

Page 23:
However, when contaminating skin wounds, the commensal starts expressing a set of virulence factors and becomes pathogenic thereby causing cell damage and initiating skin infections that in some cases lead to sepsis ~~if the pathogen reaches the blood stream~~ (systemic ~~infection of vital organs needed for human survival~~ inflammatory response of the human body to a local infection or secreted toxic molecules by bacteria).

Page 27:
Pneumococcal infections range from being mild middle ear infections in children (otitis media) to lethal infections such as lung- (pneumonia), ~~blood- (sepsis), and~~ brain-infections (meningitis) or sepsis.

Page 30:
ErmB is the widely spread~~, pre-dominant~~ Erm enzyme that is present in most streptococci (such as Spn, GAS, or GBS) and is associated with high resistance levels to MLS antibiotics.

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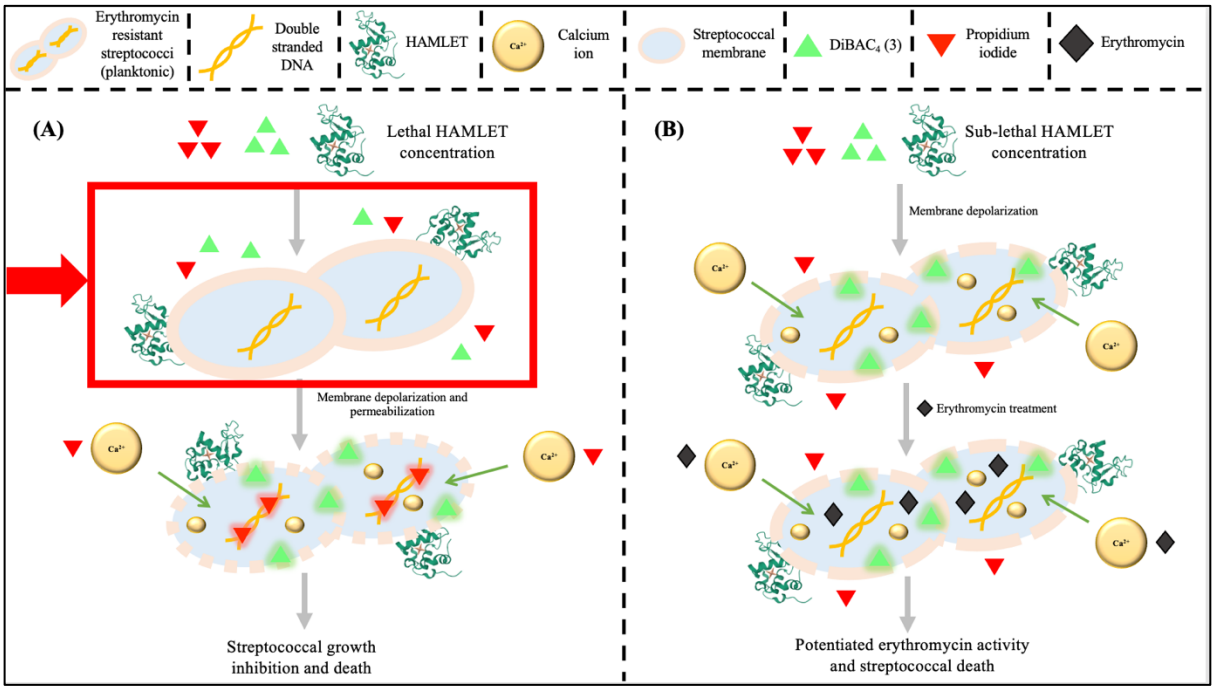
Page 40: $DiBAC_4$ does not enter intact membranes, it only emits fluorescence when bound to depolarized membranes. Therefore, in panel A, $DiBAC_4$ should be located outside the bacterial cell together with PI (as indicated by the red arrow):



Page 40:
Despite resistance, HAMLET exerts depolarization ~~and permeabilization~~ of the bacterial membrane which is needed to potentiate the effect of antibiotics.

Page 47:
In contrast to these findings, **paper 2** showed no correlation between auto-aggregation in broth and cellular adhesion to biofilm formation in GAS, in vitro. The M1T1 and M5 isolates that highly auto aggregated in broth, adhered in high numbers to cells, but formed biofilms with different functionalities. On the other hand, the M3 isolate that had a low auto-aggregation ability and adhered less to cells, was still able to develop mature and functional biofilms. ~~Therefore, other factors, not related to auto-aggregation and cell adhesion, play a role during biofilm formation in GAS.~~ Therefore, auto-aggregation in broth might not well represent the biofilm forming ability *in vitro*.

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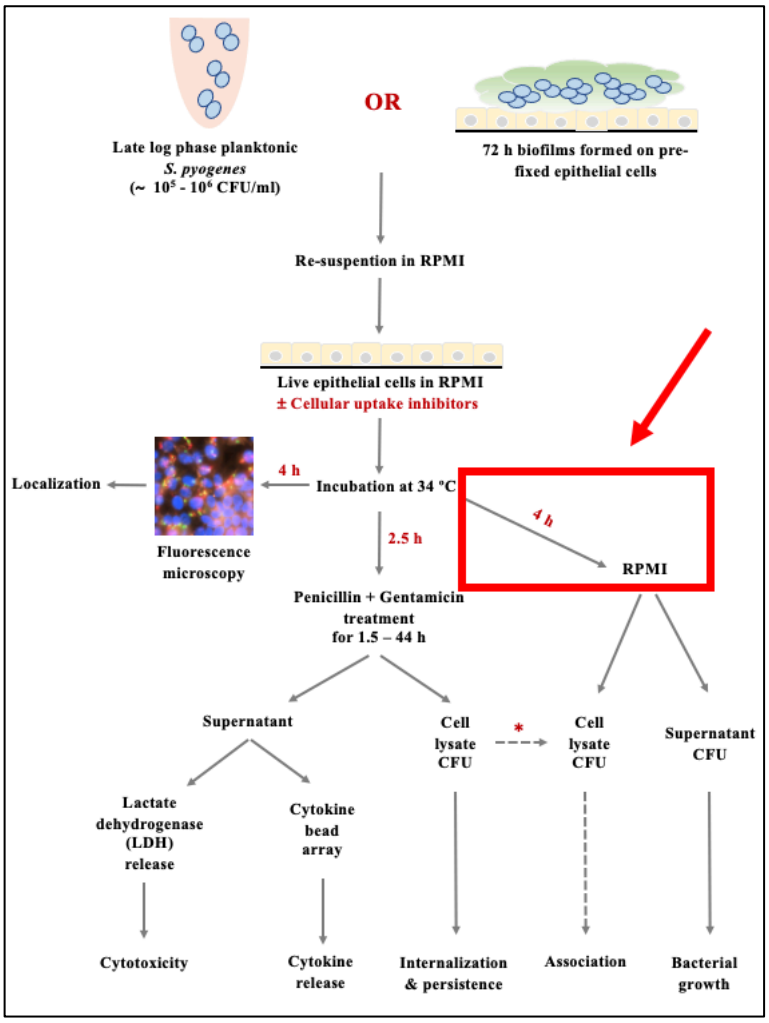
Page 51: cells were grown in RPMI for 4h (as indicated by the red arrow):



Page 52:
Along with earlier findings, planktonic bacteria utilized a more specific uptake mechanism via clathrin by which blocking this protein ~~partially~~ blocked planktonic uptake, however this was not seen in biofilm bacteria.

Page 53:
However, this was not dependent on the bacterial ability to auto aggregate in broth or adhere to cells *in vitro*, instead the biofilm forming ability of each strain was the deciding factor.

Page 54:
In **paper 4**, using *in vitro* settings, we showed that bacterial association is not ~~essential~~ directly involved during uptake into respiratory epithelial cells. We confirmed earlier studies and showed a ~~partial~~ role of clathrin during uptake of small clumps or individual planktonic bacteria into epithelial cells that were localized in the cytoplasm, suggesting a similar uptake mechanism (clathrin-mediated uptake) as *L. monocytogenes*. However, further live cell imaging is required to confirm the planktonic localization in the cytoplasm of epithelial cells before any conclusion can be drawn.

Page 60-61:
Similar to the LLO function in *L. monocytogenes*, SLO is also a CDC that forms pores in membranes when being ~~injected by the CMT in~~ secreted by GAS. ~~, thereby facilitating~~

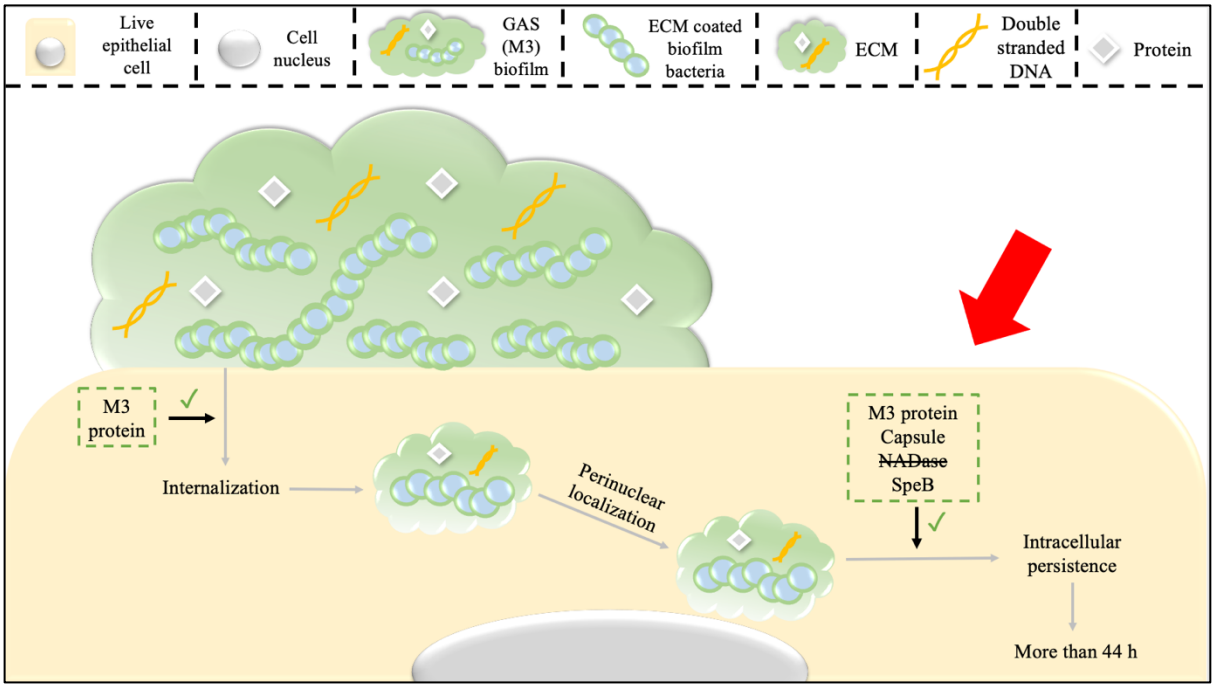
~~entrance of its co-expressed NADase toxin.~~ It is co-expressed with its co-toxin NADase that translocates to the intracellular environment using a pore-independent mechanism (Magassa et al. 2010).

Page 61:
As compared to biofilms formed by the corresponding WT strain, ~~denser and highly functional biofilms were formed by Δ*slo*, which suggests SLO mediated inhibition of biofilm formation when expressed in GAS~~ no difference in biomass or gentamicin was detected in the presence or absence of SLO (**paper 2**). Therefore, this indicates no direct role of SLO during biofilm formation in GAS.

On the other hand, no role of SLO or NADase was detected during GAS uptake, still NADase ~~was needed to mediate prolonged~~ inhibited intracellular persistence of biofilm bacteria (**Fig. 8**).

Overall, in the M3 strain at least, these results suggest an essential role of NADase to maintain biofilm formation. ~~and prolonged~~ Additionally, NADase inhibited prolonged intracellular persistence of biofilm bacteria. On the other hand, SLO ~~inhibited~~ did not affect biofilm formation, but was needed for intracellular trafficking of GAS bacteria within epithelial cells.

Page 62: NADase does not promote prolonged persistence of biofilm bacteria intracellularly, it should be therefore removed from this figure (as indicated by the red arrow):



Page 63:
Once inside cells, intracellular biofilm bacteria re-arrange protein expression of some virulence factors by which capsule, ~~NADase,~~ SpeB, along with the M3 protein, are needed to maintain prolonged intracellular survival within epithelial cells. ~~These results confirm the role of the NADase to maintain intracellular survival of internalized GAS bacteria.~~

Page 65:
~~Multi-drug~~ Drug resistant streptococci (Spn, GAS, or GBS) have become a threat to global health by which limited treatment options to cure infections are present. In this thesis, we introduced HAMLET as a potential treatment alternative for ~~respiratory~~ infections caused by these species.

Page 66:
We showed that auto-aggregation in broth and adhesion to epithelial cells in vitro, are not major determinants of biofilm formation in GAS.

Page 67:
The prolonged intracellular persistence of biofilm bacteria required the expression of the M3 protein, hyaluronic acid capsule~~, NADase~~ and SpeB.

In paper 3, page 8: sentence 231:
Zinc-transporters (yellow color) and their regulators ~~(fuchsia)~~ (light pink) were up-regulated.

Also paper 3, Figure 5 legend:
Pathways regulated by these proteins include amino acid metabolism (green), carbohydrate metabolism (light blue), vitamin metabolism (orange), DNA/RNA metabolism (including replication and repair mechanisms, ~~dark pink~~ fuchsia), transcriptional regulation (~~fuchsia~~ light pink), translation (including tRNA enzymes; purple), and protein secretion and nutrient transport (yellow).