Exploring the mechanisms by which camel lactoferrin can kill Salmonella enterica serovar typhimurium and Shigella sonnei (#77102)

Lipopolysaccharide is the major constituent of the outer membrane of Gram-negative bacteria which when released from the bacterial surface into the bloodstream can cause inflammation via activation of monocytes and endothelial cells, leading to septic shock and even death. The discovery of new molecules that can bind and neutralize the toxicity of LPS is therefore of major interest in human therapy.
The authors have previously demonstrated that the purified camel lactoferrins from different Saudi camel clans, as well as human and bovine lactoferrins possess antimicrobial properties against Salmonella enterica serovar Typhi. The authors further showed that all cLfs showed superior antibacterial potentials in comparison to hLf or bLf. In this paper, the authors aim at exploring/understanding the molecular mechanisms by which camel lactoferrin can kill S. typhimurium and S. sonnei. For this, the authors show that the camel lactoferrin interacts with lipopolysaccharides and membrane proteins of S. typhimurium and S. sonnei inducing extracellular and intracellular morphological changes leading to antimicrobial action by lactoferrin. The reviewer believes the article requires some major modifications which are

1. The authors write in abstract "Exploring molecular mechanisms by which camel lactoferrin can kill S. typhimurium and S. sonnei revealed that cLf affects expression of various bacterial proteins. There is no evidence for the same in the article. "Besides, it interacts with bacterial lipopolysaccharides (LPS) and numerous membrane proteins of S. typhimurium and S. sonnei, with each bacterial strain
posessing distinctive binding membrane proteins for lactoferrin" The abstract has been overstated with clearly no scientific evidence for same.

2. The authors report in abstract that cLf can kill S. typhimurium and S. sonnei by four molecular mechanisms, such as iron chelation, induction of
the release, appearance, disappearance, or high expression of some bacterial proteins, binding to bacterial LPS and membrane proteins, and impairing the integrity of the bacterial cells and their membranes. However, the authors have not shown any evidence for the same. Although there are reports that show possible mechanisms of action of lactoferrin by sequestering iron or binding with Lipid A portion of LPS but the authors don't provide the evidence of the same. The authors need to rewrite the abstract mentioning or putting together their specific observations and conclusions they have drawn from later.

3. Line 231-237: The authors show by ELISA that cLF was more reactive than bLf and hLf against S. typhimurium or S. sonnei LPS, with no evident differences between the cLf sub-types. The authors have shown the same by indirect ELISA. However the authors could have performed a competitive ELISA which could have shown better that cLF is more reactive than hLF and bLF. The authors overstate their observation by saying that 4 different cLf sub-types have no structural differences in their glycosylation moieties that are involved in the interaction between LPS and

lactoferrin giving no scientific evidence for later. LPS is an amphipathic molecule with an overall negative net charge due to the negatively charged phosphoryl groups of LPS to which proteins and peptides with an exposed positively charged domain could interact via electrostatic forces. Hydrophobic interactions involving the fatty acid residues of lipid A and hydrophobic amino acids have also been postulated to participate in the mechanism of LPS binding.

4. The authors state the interaction of BMP with LF using western blotting, however the authors fail to provide an evidence or western blot image for the same. Line 247-248 Protein profile of S. typhimurium or S. sonnei cell clearly changed on SDS-PAGE over time (from 30 min to 150 min) depending on the used antibacterial agents in treatment (no image representation of the same).

5. Line 267-270 The authors show the interaction of BMP crude extract of S. typhimurium or S. sonnei exposed to cLf-biotin via ELISA. The authors exaggerate the data by quoting "we propose that cLf could kill S. typhimurium and S. sonnei via a bactericidal mechanism that involves binding to bacterial LPS and membrane proteins", however providing no evidence for the same. A mere interaction is not proof of a bactericidal mechanism.

6. Line 308-310 The authors performed MIC of antibacterial agents by broth microdilution assay. The authors have not mentioned the concentration range they used for LF. Also, the authors can represent the same in a bar graph showing higher inhibitory concentration for other LFs compared to cLF.

7. Furthermore the authors demonstrate the time-kill study of S. typhimurium and S. sonnei after 4 h and 8 h of incubation with each Lf alone, or in combination with different antibiotics. The authors should add more timepoints and represent the same in the form of a graph rather than a table.

8. The authors should also modify the title as although the authors show that LF interacts with LPS and BM but clearly they need to perform more experiments to justify the title wherein they state they are aiming at exploring the mechanism of action of lactoferrin.

9. Line 410-417-an exaggeration of obtained results of observations.

10. Line 281-284 The author shows significant growth inhibition of S.typhimurium and S.sonnei by disk diffusion assay however the authors should provide the image of disk diffusion assay to show the inhibition at mentioned concentration.

11. . Line 357-359 The author shows membrane distortion of bacteria by TEM image while the gold nanoparticles black dots are not clear into the image. I would

appreciate it if the authors could provide a good quality image with high magnification that should show the membrane distortion. Also, The control image in TEM analysis is missing.

12. Overall, the image representation is not upto the standard of journals. The authors prefer tables and values over graphs and image representations. The authors also use different fonts in the paper that should be changed to just one font. The overstatement in the abstract should be reduced to the author's own findings.