Review of: "Escherichia coli BarA-UvrY regulates the pks island and kills Staphylococci via the genotoxin colibactin"

Sam Nooij[1]

1 Leiden University Medical Center (LUMC)

Potential competing interests: The author(s) declared that no potential competing interests exist.

The authors describe a function of colibactin, a genotoxin produced by some *Escherichia coli* strains, and a novel mechanism of its gene regulation. Colibactin-producing *E. coli* strains have gained a lot of attention, both as a probiotic (*E. coli* Nissle 1917[1]) and as putative procarcinogenic bacterium[2][3]. In fact, both the probiotic and procarcinogenic effects appear to be linked to colibactin[4]. Previously, bacterial growth inhibition by molecules related to colibactin production have been shown in *Bacillus subtilis*[5] and *Staphylococcus aureus*[6]. However, to my knowledge there were no examples that directly related colibactin to the killing of other bacteria. Also, the gene regulatory mechanism of the *pks* island, responsible for colibactin production, was only partly described[7]. Therefore, I think the current manuscript is a valuable addition to our knowledge of this interesting *E. coli* variant.

The manuscript is well written and has a clear narrative. The experiments include the right controls and demonstrate the described effect both *in vitro* and *in vivo*. My general impression is that this is a very good manuscript and I have a few comments that can hopefully contribute to improving it further:

1. Figures 1 and 3A have no legend. I think it would be easier for the reader if the figure itself has a visual explanation of what the open bars and checkered bars represent, rather than a written explanation in the caption.
2. Figure 3 shows growth inhibition by different *E. coli* UTI89 mutants on *S. aureus*. The figure shows asterisks indicating highly significant differences between each mixed colonies and single macrocolonies. In the text it is pointed out that mutant UTI89 strains inhibit *S. aureus* growth less than the wild-type, but I see no statistical test supporting this comparison. It would be nice if the text would include the test used here and p-value.
3. I am not sure if I understand figures 4 and 5 correctly, where the expression of *clbA* and *clbB* are compared between two different situations. Expression levels were measured by RT-qPCR and normalized to calculate the fold change compared to the control state. I understand that this control state is then set to 1, but in my view the figures now suggest that qPCR measurements were always identical between the 3 or 5-6 indepenent replicates of the control state. I would appreciate if the authors could clarify this normalization method and show raw output values from the qPCR.

4. Also, it is not entirely clear to my why *clbA* and *clbB* were chosen as representative genes of the *pks* island, which comprises 19 *clb* genes.
5. It is claimed that *pks+ E. coli* kills *S. aureus* by DNA damage, which I also think is probably the case, but no test is done to directly show the DNA damage. There is only indirect evidence of DNA damage.
6. The methods section describes several bioinformatics tools used for the transcriptomic analysis, but not the parameters used. Were the default parameters used? It would be nice if a little more detail would be added here, or if the scripts could be shared.

Besides, I would like to share two comments on things I personally appreciate about the manuscript:

1. Most figures are in black and white, which are also easy to understand for colorblind people.
2. The authors have shared their RNA-seq data throught the Gene Expression Omnibus, which increases the replicability of the study.

References

1. ^*Trudy M. Wassenaar. (2016). Insights from 100 years of research with probioticE. coli. European Journal of Microbiology and Immunology, vol. 6 (3), 147-161. doi:10.1556/1886.2016.00029.*
2. ^*Cayetano Pleguezuelos-Manzano, Jens Puschhof, Axel Rosendahl Huber, Arne van Hoeck, et al. (2020). Mutational signature in colorectal cancer caused by genotoxic pks+ E. coli. Nature, vol. 580 (7802), 269-273. doi:10.1038/s41586-020-2080-8.*
3. ^*Paulina J. Dziubańska-Kusibab, Hilmar Berger, Federica Battistini, Britta A. M. Bouwman, et al. (2020). Colibactin DNA-damage signature indicates mutational impact in colorectal cancer. Nat Med, vol. 26 (7), 1063-1069. doi:10.1038/s41591-020-0908-2.*
4. ^*Maïwenn Olier, Ingrid Marcq, Christel Salvador-Cartier, Thomas Secher, et al. (2012). Genotoxicity of Escherichia coli Nissle 1917 strain cannot be dissociated from its probiotic activity. Gut Microbes, vol. 3 (6), 501-509. doi:10.4161/gmic.21737.*
5. ^*Maria I. Vizcaino, Philipp Engel, Eric Trautman, Jason M. Crawford. (2014). Comparative Metabolomics and Structural Characterizations Illuminate Colibactin Pathway-Dependent Small Molecules. J. Am. Chem. Soc., vol. 136 (26), 9244-9247. doi:10.1021/ja503450q.*
6. ^*T. Faïs, A. Cougnoux, G. Dalmasso, F. Laurent, et al. (2016). Antibiotic Activity of Escherichia coli against Multiresistant Staphylococcus aureus. Antimicrob Agents Chemother, vol. 60 (11), 6986-6988. doi:10.1128/aac.00130-16.*
7. ^*Alexander Wallenstein, Nadine Rehm, Marina Brinkmann, Martina Selle, et al. (2020). ClbR Is the Key Transcriptional Activator of Colibactin Gene Expression in Escherichia coli. mSphere, vol. 5 (4). doi:10.1128/msphere.00591-20.*