EBioMedicine 56 (2020) 102802

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.elsevier.com/locate/ebiom

Commentary

Salmonella intracellular adaptation is key to understand cephalosporin treatment relapse

Gaëtan Thilliez[a], Robert A. Kingsley[a,b,*]

[a] *Quadram Institute Bioscience, Norwich, United Kingdom*
[b] *University of East Anglia, Norwich, United Kingdom*

ARTICLE INFO

Article History:
Received 1 May 2020
Accepted 1 May 2020
Available online xxx

In this article of *EBioMedicine* Francisco García-del Portillo and colleagues report findings that shine a new light on a bacterial mechanism responsible for relapse from antibiotic treatment, and pave the way for developing new drugs to treat enteric fever with reduced relapse [1].

Due to the emergence of resistance to chloramphenicol in the 1990′s and more recently to fluoroquinolones, third generation cephalosporins (β-lactam antibiotics), along with azithromycin, are now the treatments of choice for enteric fever [2]. Resistance to cephalosporins is currently relatively low for *S. Typhi* and *S. Paratyphi* A, the causative agents of enteric fever, but as much as 17% of cases treated with this class of antibiotic end with relapse [3]. Relapse not only increases morbidity and mortality to enteric fever, but may also contribute to the emergence of resistance to this important first choice drug. Indeed, the first reports of resistance to third generation cephalosporins in addition to multi-drug resistance affecting other drug choices, a profile called extensively drug resistant (XDR), was reported in outbreaks in India and Pakistan in 2016 [4]. The emergence of the XDR profile is a serious escalation in the potential lethality of enteric fever in the coming years.

The processes leading to relapse are not fully understood, but have been linked to poor access of the drug to the intracellular compartment or survival of a fraction of a slow growing pathogen population due to persistence or heteroresistance [5,6]. While persistence is loosely defined as when a fraction of the population survives exposure to a drug, heteroresistance is more specifically a phenomenon where a portion of the population exhibits a temporary increase in resistance to a drug [7]. The study by García-del Portillo and colleagues describes a distinct mechanism that could explain relapse in *Salmonella* infection after treatment with the β-lactam antibiotic cephalosporin, that unlike persistence or heteroresistance, did not seem to be limited to a subset of the population. Instead, adaptation to the intracellular environment resulted in an increased resistance to β-lactam antibiotics, including a cephalosporin, ceftriaxone.

Previous work by the same group identified $PBP3_{SAL}$, a paralog of the penicillin binding protein PBP3, that was absent form closely related bacterial species. PBPs are essential proteins involved in peptidoglycan synthesis, but are also targets for β-lactam antibiotics that mimic the peptidoglycan subunits and act as a suicide substrate by covalently binding the catalytic site. $PBP3_{SAL}$ differs from PBP3 in two major aspects, it is specifically involved in cell division in the environment of the host cell phagosome, and has a reduced affinity to β-lactam antibiotics [8]. The current study shows that $PBP3_{SAL}$ replaces PBP3 in intracellular *Salmonella* during infection, and importantly, that *Salmonella* that were unable to express the alternative $PBP3_{SAL}$ did not cause relapse in experimentally infected mice treated with the β-lactam antibiotic ceftriaxone, a third-generation cephalosporin.

An important message to be taken from this study is that commonly used assays to determine antimicrobial resistance are not capable of identifying resistance resulting from adaptation that occur specifically in the host. This is a potentially confounding factor for the surveillance of resistance to a wide range of antibiotics used to treat a broad array of bacterial pathogens. Currently, most studies on antimicrobial resistance of bacterial pathogens focus on resistance under defined culture conditions that do not mimic conditions inside the host. The emergence of resistance may go unnoticed by standard surveillance in diagnostic labs and the opportunity to improve therapies or clinical management missed. Consequently, there is a need to evaluate antimicrobial resistance and the expression of the molecular target of antibiotics in growth conditions encountered within the host.

Perhaps the most exciting prospect arising from the present study is the opportunity to develop new antimicrobials that target the $PBP3_{SAL}$ paralogue expressed by *Salmonella* during infection, and therefore lower the rate of relapse. This may be possible by the modification of existing antibiotics or by screening for novel compounds capable of killing *Salmonella* cultured in medium that mimics the environment in the phagosome of host cells.

The findings of this study also raise important questions about the function of the alternative PBP proteins in host-pathogen interactions. It seems unlikely that the PBP3-$PBP3_{SAL}$ adaptive mechanism evolved as a mechanism of resistance to β-lactam antibiotics, as these compounds are not normally present during infection, except as a result of

DOI of original article: http://dx.doi.org/10.1016/j.ebiom.2020.102771.
* Corresponding author.
E-mail address: rob.kingsley@quadram.ac.uk (R.A. Kingsley).

https://doi.org/10.1016/j.ebiom.2020.102802

therapy. Rather, increased resistance to β-lactam antibiotics is more likely to be an inadvertent result of adaptation to the intracellular lifestyle. The authors speculate that the PBP3-PBP3$_{SAL}$ mechanism may be associated with specific adaptation to replicate more slowly in the intracellular compartment in the host, to avoid excessive damage to the host. Such adaptation would be expected to affect the likelihood of transmission by extending the duration of infection [9].

The presence of this adaptive mechanism in *Salmonella* and its absence in the closely related bacterium *Escherichia coli* that evolved predominantly as an extracellular commensal of the intestinal tract of mammals, is consistent with its acquisition by *Salmonella* as an important event in the evolution of this enteric pathogen. Interestingly, alternative PBPs encoded on plasmids along with other antimicrobial resistance genes have been reported in other species such as the *Enterococci* [10], suggesting one possible origin of PBP paralogues by horizontal gene transfer.

This study marks a significant advancement in the understanding of relapse following cephalosporin treatment of enteric fever and identifies a potential target for improved therapies through the discovery of a previously unrecognised *Salmonella* adaptation within the host.

Declaration of Competing Interest

Dr. Kingsley has nothing to disclose. Dr. Thilliez has nothing to disclose.

Acknowledgements

The authors are supported by the BBSRC Institute Strategic Programme Microbes in the food chain BB/R012504/1 and its constituent projects BBS/E/F/000PR10348 and BBS/E/F/000PR10349. The funder had no role in preparation of the manuscript or decision to submit for publication. The authors have not received any payment or funding from pharmaceutical companies or other agencies to write this article.

Authors' contribution

RK drafted the text, GT provided additional text and both authors edited and approved the final manuscript.

References

[1] Castanheira S, López-Escarpa D, Pucciarelli MG, Cestero JJ, Baquero F, García-del Portillo F. An alternative penicillin-binding protein involved in Salmonella relapses following ceftriaxone therapy. EBioMedicine 2020 https://doi.org/10.1016/j.ebiom.2020.102771.
[2] Britto CD, Wong VK, Dougan G, Pollard AJ. A systematic review of antimicrobial resistance in Salmonella enterica serovar Typhi, the etiological agent of typhoid. PLoS Negl Trop Dis 2018;12(10):e0006779.
[3] Butler T. Treatment of typhoid fever in the 21st century: promises and shortcomings. Clin Microbiol Infect 2011;17(7):959–63.
[4] Klemm EJ, Shakoor S, Page AJ, et al. Emergence of an extensively drug-resistant Salmonella enterica serovar Typhi clone harboring a promiscuous plasmid encoding resistance to fluoroquinolones and third-generation cephalosporins. MBio 2018;9(1).
[5] Helaine S, Cheverton AM, Watson KG, Faure LM, Matthews SA, Holden DW. Internalization of Salmonella by macrophages induces formation of nonreplicating persisters. Science 2014;343(6167):204–8.
[6] Kaiser P, Regoes RR, Dolowschiak T, et al. Cecum lymph node dendritic cells harbor slow-growing bacteria phenotypically tolerant to antibiotic treatment. PLoS Biol 2014;12(2):e1001793.
[7] Balaban NQ, Helaine S, Lewis K, et al. Definitions and guidelines for research on antibiotic persistence. Nat Rev Microbiol 2019;17(7):441–8.
[8] Castanheira S, Cestero JJ, Rico-Perez G, et al. A specialized peptidoglycan synthase promotes Salmonella cell division inside host cells. MBio 2017;8(6).
[9] Kingsley RA, Baumler AJ. Host adaptation and the emergence of infectious disease: the Salmonella paradigm. Mol. Microbiol. 2000;36(5):1006–14.
[10] Raze D, Dardenne O, Hallut S, Martinez-Bueno M, Coyette J, Ghuysen JM. The gene encoding the low-affinity penicillin-binding protein 3r in Enterococcus hirae S185R is borne on a plasmid carrying other antibiotic resistance determinants. Antimicrob Agents Chemother 1998;42(3):534–9.