AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy

AUTHOR REPLY

Reply to "The Curious Case of TEM-116"

Juergen Pleiss, Catharina Zeil

Institute of Technical Biochemistry, University of Stuttgart, Stuttgart, Germany

In their comment letter "The curious case of TEM-116" (1), Jacoby and Bush discuss the long-standing question of whether the TEM β-lactamase A184V V84I variant (TEM-116) is a natural variant. The fact that TEM-116 has a high centrality in the sequence similarity network of TEM β-lactamase variants (2) indicates that "TEM-116 is now a naturally occurring enzyme" (1). TEM-116 has been identified multiple times in clinical isolates since 2004 (3). However, in 2007, it was suspected that the detection of TEM-116 might just be a false-positive result, caused by a contamination in PCR reagents (4), since the TEM β-lactamase A184V V84I double mutant has been part of the plasmid pUC5 and its plasmid progeny since 1982 (5). Therefore, the question arose as to whether TEM-116 is a product of evolution or merely an engineered enzyme.

It would not have been the first time that naturally occurring TEM variants were first developed synthetically and detected in clinical isolates only later; an example is the E104K M182T G238S triple variant, which was developed by DNA shuffling in 1994 (6) and detected in a clinical isolate in 1998 (7). In the meantime, a large number of functional TEM β-lactamase variants were identified by directed-evolution experiments, and more than 200 naturally occurring TEM β-lactamase variants are listed in the TEM mutation table maintained at the Lahey Clinic (8). These data show that stabilization, ESBL activity, or inhibitor resistance is mediated by mutations at fewer than 15 of the most relevant hot spot positions.

In the literature as well as in the comment letter by Jacoby and Bush (1), a sharp distinction is made between natural and synthetic TEM β-lactamase variants. However, we expect that a large fraction of (if not all) functional variants are expressed by some of the 10^{30} cells in the contemporary biosphere (9), since genes conferring resistance to β-lactams existed long before the advent of antibiotics in clinical use 70 years ago, as shown by metagenomic analyses of 30,000-year-old permafrost sediments (10). While it is still unknown how many of the 20^{15} ($=3 \cdot 10^{19}$) possible variants resulting from variations at 15 hot spot positions are functional, they may easily have been screened by the 10^{40} cells that came into life during 4 gigayears of evolution (9), even if only a small fraction of them expressed a TEM β-lactamase variant.

Thus, we expect that each functional TEM β-lactamase variant that we find by mutating the hot spot positions is also a member of the large pool of natural variants. Our current knowledge of the network of functional TEM β-lactamase variants is still sparse (2), but we are gradually detecting new functional variants, either by sequencing of clinical isolates or by synthetic approaches, such as directed evolution. The scientific challenge is to understand (and predict) which of the $3 \cdot 10^{19}$ possible variants are functional.

REFERENCES

1. **Jacoby G, Bush K.** 2016. The curious case of TEM-116. Antimicrob Agents Chemother **60:**7000. http://dx.doi.org/10.1128/AAC.01777-16.
2. **Zeil C, Widmann M, Fademrecht S, Vogel C, Pleiss J.** 2016. Network analysis of sequence-function relationships and exploration of sequence space of TEM β-lactamases. Antimicrob Agents Chemother **60:**2709–2717. http://dx.doi.org/10.1128/AAC.02930-15.
3. **Jeong SH, Bae IK, Lee JH, Sohn SG, Kang GH, Jeon GJ, Kim YH, Jeong BC, Lee SH.** 2004. Molecular characterization of extended-spectrum β-lactamases produced by clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli* from a Korean nationwide survey. J Clin Microbiol **42:**2902–2906. http://dx.doi.org/10.1128/JCM.42.7.2902-2906.2004.
4. **Koncan R, Valverde A, Morosini MI, Garcia-Castillo M, Canton R, Cornaglia G, Baquero F, del Campo R.** 2007. Learning from mistakes: Taq polymerase contaminated with β-lactamase sequences results in false emergence of *Streptococcus pneumoniae* containing TEM. J Antimicrob Chemother **60:**702–703. http://dx.doi.org/10.1093/jac/dkm239.
5. **Vieira J, Messing J.** 1982. The pUC plasmids, an M13mp7-derived system for insertion mutagenesis and sequencing with synthetic universal primers. Gene **19:**259–268. http://dx.doi.org/10.1016/0378-1119(82)90015-4.
6. **Stemmer WPC.** 1994. Rapid evolution of a protein *in vitro* by DNA shuffling. Nature **270:**389–391.
7. **Poyart C, Mugnier P, Quesne G, Berche P, Trieu-Cuot P.** 1998. A novel extended-spectrum TEM-type β-lactamase (TEM-52) associated with decreased susceptibility to moxalactam in *Klebsiella pneumoniae*. Antimicrob Agents Chemother **42:**108–113.
8. **Bush K, Jacoby G.** 1997. Nomenclature of TEM beta-lactamases. J Antimicrob Chemother **39:**1–3.
9. **Dryden DT, Thomson AR, White JH.** 2008. How much of protein sequence space has been explored by life on Earth? J R Soc Interface **5:**953–956. http://dx.doi.org/10.1098/rsif.2008.0085.
10. **D'Costa VM, King CE, Kalan L, Morar M, Sung WW, Schwarz C, Froese D, Zazula G, Calmels F, Debruyne R, Golding GB, Poinar HN, Wright GD.** 2011. Antibiotic resistance is ancient. Nature **477:**457–461. http://dx.doi.org/10.1038/nature10388.

Citation Pleiss J, Zeil C. 2016. Reply to "The curious case of TEM-116." Antimicrob Agents Chemother 60:7001. doi:10.1128/AAC.01786-16.

Address correspondence to Juergen Pleiss, Juergen.Pleiss@itb.uni-stuttgart.de.

This is a response to a letter by Jacoby and Bush (doi:10.1128/AAC.01777-16).