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Importance of Sequencing To Determine Functional bla_{TEM} Variants

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According to Jacoby and Bush, TEM-116 extended-spectrum beta-lactamase is now a naturally occurring enzyme (1). In 2005, Chiang and collaborators described the possibility of commercial *Taq* polymerase contamination with bla_{TEM-1} gene (2). One year later, Song et al. demonstrated the contamination of *Taq* polymerase with the bla_{TEM} gene and how to decontaminate it (3). This was due to the use of the TEM gene in vectors that are widely used, leading to the contamination of *Taq* polymerase preparations, which are used for the research of this gene, resulting in false-positive results (4, 5). This would not be the first β-lactamase TEM developed synthetically, since in 1994 after DNA shuffling, a variant with mutations at three sites developed (6).

We investigated bla_{TEM} in 26 clinical isolates of *Klebsiella pneumoniae* from Brazil and also in two reference strains, *K. pneumoniae* ATCC BAA 1705 and *Stenotrophomonas maltophilia* ATCC 13673. PCRs were performed thrice using the primers described by Dallenne et al. (7) and JumpStart *Taq* DNA polymerase (Sigma-Aldrich).

All of the 26 isolates, the two ATCC strains, and also the negative control (UltraPure DNase-RNase-free distilled water; Invitrogen, Carlsbad, CA) amplified the bla_{TEM} gene and were submitted to sequencing. The analyses showed that one clinical isolate, the reference strain *S. maltophilia* ATCC 13673, and the negative control presented the two mutation sites (A184V and V84I) related to the variant TEM-116. The remaining clinical isolates and the reference strain *K. pneumoniae* ATCC BAA 1705 did not have these mutations but presented another bla_{TEM} variant.

A network analysis was performed to verify the presence of the bla_{TEM} gene in the reference strains, and the data demonstrated that *K. pneumoniae* ATCC BAA 1705 (GenBank accession no. AOGQ00000000.1) harbors bla_{TEM-1}; however, *S. maltophilia* ATCC 13673 (GenBank CP008838.1) did not have any bla_{TEM} variant. Thus, our results suggest that when the bacterium in fact has the bla_{TEM} gene, the two mutation sites are not observed. However, when the isolates do not have the gene, the mutation sites regarding TEM-116 variants are detected, showing false-positive results.

The Lahey Clinic table describes 223 variants of β-lactamase TEM (http://www.lahey.org/Studies/temtable.asp); however, not all are naturally occurring. Pless and Zeil indicate that new β-lactamases TEM variants are increasingly being described, and it is a great challenge to understand which of them are functional (8).

In conclusion, the present study corroborates previous work (2, 3, 5) showing that the contamination in *Taq* polymerase interferes in the detection of bla_{TEM} since false-positive results were found; however, for some reason, when the bacterium really has the bla_{TEM} gene, the mutations characteristic of TEM-116 are not observed. Therefore, detection only by PCR is not enough and sequencing is essential for the determination of functional β-lactamase TEM variants.

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REFERENCES

1. Jacoby G, Bush K. 2016. The curious case of TEM-116. Antimicrob Agents Chemother 60:7000. https://doi.org/10.1128/AAC.01777-16.
2. Chiang CS, Liu CP, Weng LC, Wang NY, Liaw GJ. 2005. Presence of beta-lactamase gene TEM-1 DNA sequence in commercial Taq DNA polymerase. J Clin Microbiol 43:530–531. https://doi.org/10.1128/JCM.43.1.530-531.2005.
3. Song JS, Lee JH, Lee JH, Jeong BC, Lee WK, Lee SH. 2006. Removal of contaminating TEM-1a beta-lactamase gene from commercial Taq DNA polymerase. J Microbiol 44:126–128.
4. 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. https://doi.org/10.1016/0378-1119(82)90015-4.
5. 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 beta-lactamase sequences results in false emergence of *Streptococcus pneumoniae* containing TEM. J Antimicrob Chemother 60:702–703. https://doi.org/10.1093/jac/dkm239.
6. Stemmer WPC. 1994. Rapid evolution of a protein in vitro by DNA shuffling. Nature 270:389–391. https://doi.org/10.1038/370389a0.
7. Dallenne C, Da Costa A, Decré D, Favier C, Arlet G. 2010. Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in *Enterobacteriaceae*. J Antimicrob Chemother 65:490–495. https://doi.org/10.1093/jac/dkp498.
8. Pleiss J, Zeil C. 2016. Reply to "The Curious Case of TEM-116." Antimicrob Agents Chemother 60:7001. https://doi.org/10.1128/AAC.01786-16.