Piperacillin/tazobactam resistance in a clinical isolate of *Escherichia coli* due to IS*26*-mediated amplification of bla_{TEM-1B}.

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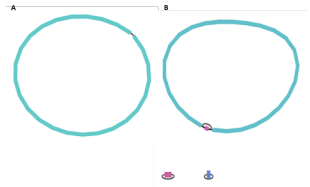
Resistance to piperacillin/tazobactam (TZP), but susceptibility to carbapenems and cephalosporins, has been proposed to be mediated by hyperproduction of the β-lactamases Bla_{TEM-1} or AmpC due to mutations within the promoter regions[1]. However, the mechanism of hyperproduction in isolates that lack promoter region mutations is not well understood but has recently been linked to the amplification of bla_{TEM-1}[2] and the presence of IS*26*[3]. Following identification of a pair of *Escherichia coli* clinical isolates displaying within-patient evolution to TZP resistance, we sought to further understand this mechanism

1

A TZP-susceptible and carbapenem/cephalosporin-susceptible *Escherichia coli* isolate was identified from a blood culture sample at the Royal Liverpool University Hospital in Liverpool, UK. The isolate contained a pseudo-compound transposon (PTn*6762)* with two IS*26* flanking multiple antimicrobial resistance genes, including bla_{TEM-1B}



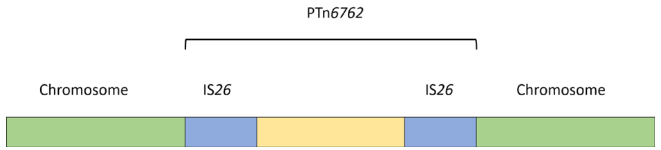


Figure 1: Visualisation of the completed genome of the **A**) TZP-susceptible, carbapenem/cephalosporin-susceptible isolate and the **B**) TZP-resistant, carbapenem/cephalosporin-susceptible isolate

2

When the isolate is exposed to TZP, PTn*6762* is excised from the chromosome with a single IS*26*, leaving the second IS*26* in the chromosome

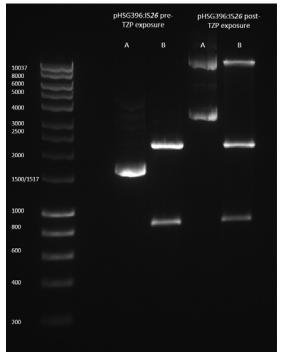


Figure 2: **A**) Undigested pHSG396:IS*26* and **B**) pHSG396:IS*26* digested with EcoRI and XhoI pre and post exposure to TZP revealing a >10kb insertion post exposure

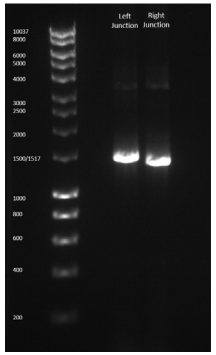
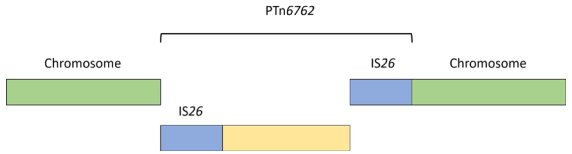


Figure 3: Amplification of the left and right junctions of the inserted translocatable unit into pHSG396:IS*26* following TZP exposure



3

The excised PTn*6762* circularises forming a translocatable unit

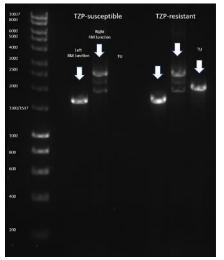
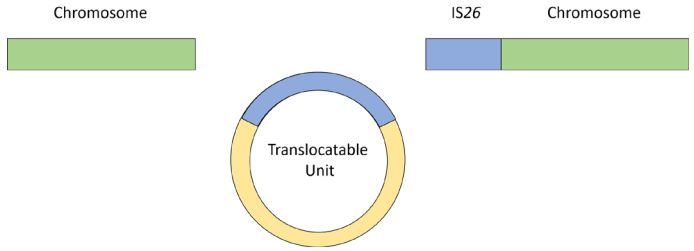


Figure 4: Amplification of the left and right junctions of the integrated, chromosomally located transposon and the translocatable unit



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The translocatable unit can re-insert into the chromosome multiple times, creating a tandem array and increasing the copy number of bla_{TEM-1B}, resulting in hyperproduction of the β-lactamase

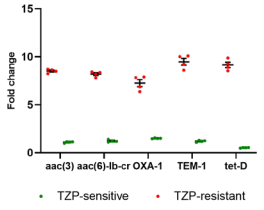


Figure 5: Copy number of the antibiotic resistance genes present on PTn*6762*/translocatable unit compared to the housekeeping gene *uidA*

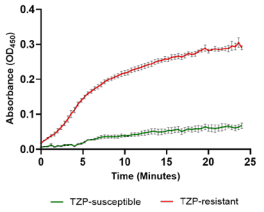
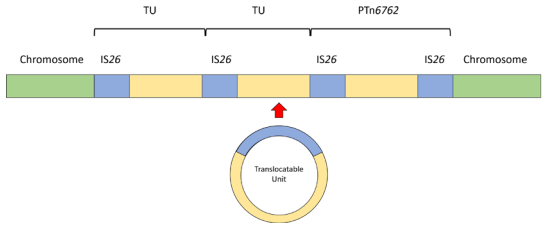


Figure 6: Hydrolysis of nitrocefin by bla_{TEM-1B} in the TZP-susceptible and TZP-resistant isolate



References:

1. *Lartigue, et al.* 2002. Promoters *P3*, *Pa*/*Pb*, *P4*, and *P5* Upstream from bla_{TEM} Genes and Their Relationship to β-Lactam Resistance. Antimicrobial Agents and Chemotherapy.
2. 2. *Schechter, et al.* 2019. Extensive Gene Amplification as a Mechanism for Piperacillin-Tazobactam Resistance in *Escherichia coli*. Mbio.
3. *Hansen, et al.* 2019 Resistance to piperacillin/tazobactam in *Escherichia coli* resulting from extensive IS*26*-associated gene amplification of bla_{TEM-1}. Journal of Antimicrobial Chemotherapy.