TEM-E4: A β-LACTAMASE WHICH CONFERS TRANSFERABLE RESISTANCE TO CEFTAZIDIME

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When third generation cephalosporins (3GCs), such as ceftazidime and cefotaxime, were first introduced they were resistant to hydrolysis by all the plasmid-mediated β-lactamases known at that time. However, in the last four years a number of plasmid mediated β-lactamases have evolved which have the ability to hydrolyse ceftazidime and cefotaxime and, consequently, promote transferable resistance to 3GCs (Philippon et al 1989). Many of the 3GC hydrolysing β-lactamases have been shown to have evolved from the ubiquitous TEM-1/2 and SHV-1 resistance genes.

The ceftazidime resistant isolate of Serratia marcescens 7919 was isolated in Belgium in 1987. Conjugation of strain 7919 with Escherichia coli J53-2 resulted in ceftazidime-resistant E. coli J53-2 transconjugants. Analysis of the plasmid DNA in these transconjugant strains revealed a single plasmid band of 56Kb, which was designated pUK724. This plasmid was also visualized in the S. marcescens strain. With the exception of imipenem, the S. marcescens 7919 strain was resistant to all penicillins as well as first, second and third generation cephalosporins but the E. coli J53-2 transconjugant was resistant only to ceftazidime and remained sensitive to all the other third and second generation cephalosporins tested. The ceftazidime and ampicillin resistances, expressed by the E. coli transconjugant, were diminished with the addition of clavulanic acid (2mg/L). This illustrated that the β-lactam resistance of the E. coli J53-2 transconjugant of S. marcescens 7919 must be β-lactamase mediated. Iso- electric focusing of bacterial extracts and visualisation with the chromogenic cephalosporin, nitrocephin revealed that both the original S. marcescens isolate and the E. coli J53-2 transconjugant produced a novel β-lactamase, designated TEM-E4. This enzyme focused marginally above the TEM-2 (pI 5.6) and below the TEM-6 (pI 5.85) enzyme. In comparison with standards, TEM-E4 was allocated a pI of 5.61. The TEM-E4 β-lactamase exhibited low rates of hydrolysis for ceftazidime and cefotaxime although it hydrolysed cefotaxime more efficiently than ceftazidime. It was also shown that TEM-E4 and TEM-1 had similar molecular weights and ID_{50} values for clavulanic acid.

In separate experiments, challenging E. coli J53-2 (RP4), a TEM-2 β-lactamase producing strain, with ceftazidime (2mg/L) resulted in spontaneous mutants capable of resisting the drug. In these mutants, the TEM-2 enzyme had mutated so that it was now capable of hydrolysing ceftazidime (Mutant β-lactamase D). Close comparison of Mutant β-lactamase D and TEM-E4 showed that they had similar resistance profiles to β-lactam antibiotics, they had identical pIs and similar kinetic constants for five different β-lactam substrates. These results infer that TEM-E4 resulted from direct mutation of the TEM-2 gene. Further evidence for this has been obtained by DNA-DNA hybridization studies which showed that the TEM-E4 gene hybridised with a radiolabelled TEM gene probe.

In conclusion, the TEM-E4 β-lactamase is a unique plasmid-encoded enzyme, capable of hydrolysing 3GCs, which is present in clinical bacteria. TEM-E4 could be obtained by spontaneous mutation of the common TEM-2 gene and seems to have derived directly from it.

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