An insight into antibacterial resistance emphasizing beta-lactamase induction and its physiological impact in *Escherichia coli*

Abstract

Gram-negative bacteria like *E. coli* is one of the common nosocomial pathogens encountered today. The bacteria evade antibiotic action by mechanisms like modifications of drug targets, active efflux pumps, outer membrane permeability changes, biofilm formation and drug hydrolysing enzymes. NGM9 was observed as a multidrug resistant strain among an array of *E. coli* clinical isolates. It revealed the simultaneous presence of four different active resistance mechanisms indicating the co-existence of drug resistance mechanisms. Beta-lactamase expression is the most common for beta-lactam resistance. In the induction pathway of AmpC, the first few steps of induction and peptidoglycan recycling are common after which the two pathways bifurcate depending upon the circumstance encountered by the bacterial cell. The genes implicated in the initial steps of the pathway, namely, *ampD*, *ampE* and *ampG* were cloned in pBAD-18Kan to study their roles in the expression of Ambler class A and D beta-lactamase. The representative beta-lactamases chosen were CTX-M-15, TEM-1 and OXA-2 on the basis of their relative prevalence and were cloned in pBAD-18Cam for *in vivo* studies. A two-fold increased resistance was observed in *E. coli* Δ*ampC* mutants harbouring beta-lactamases as compared to the wild type cells possessing the same beta-lactamases. This indicated the possibility of expression restriction of other serine beta-lactamase in presence of AmpC in *E. coli*. The effects of each of the three induction pathway genes were ascertained by inducing the expression of beta-lactamase in the deletion mutants and the ectopically complemented deletion mutants by determining beta-lactam sensitivities and nitrocefin hydrolysis. AmpD showed variable result depending upon the beta-lactamase under study. The deletion *ampE* enhanced beta-lactamase expression while *ampG* deletion enhanced susceptibility as compared to wild type harbouring beta-lactamase. The effect of beta-lactamase expression on biofilm formation was checked in presence and absence of the presumptive beta-lactamase inducing genes. CTX-M-15 and AmpC enhanced *E. coli* biofilm formation while TEM-1 and OXA-2 reduced it and results were similar to those obtained for beta-lactamase induction assays. Therefore, it can be inferred that AmpD has a variable role, AmpE probably acts as a negative regulator while AmpG directly influences *E. coli* biofilm formation.

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(10BT90F04)