Chromosomally-mediated antibiotic resistance and virulence

Although the relationship between plasmid-mediated antibiotic resistance and alterations in virulence is established, the association of chromosomally-mediated resistance and virulence has received less attention. Spontaneous mutations in chromosomal genes occur at a frequency of $10^6-10^8$/cell division. In relation to antibiotic resistance, such mutations usually involve genes encoding the target site, or cell structures affecting access to the target site. In practice, chromosomal mutations are found less frequently in natural bacterial populations probably because they reduce the fitness of bacteria to survive. In the field of medical microbiology this may result from the loss or alteration of a virulence factor.

Chromosomal mutations conferring antibiotic resistance via alterations in outer-membrane porins of gram-negative bacteria may affect virulence. Porins allow the transport across bacterial membranes of nutrients, ions and certain antibiotics. A complex control mechanism regulates the quantitative production of porins and affects the sensitivity of the bacterial cell to various antimicrobial agents such as the quinolones, $\beta$-lactam antibiotics and tetracyclines. The presence of porins of different sizes will affect the range of substances entering the cell. Mutations that affect the transcriptional regulator that controls the ratio of different porins, Omp R, can result in strains with markedly reduced virulence.

Cyclic AMP (cAMP) and the cAMP receptor protein are essential for the transcription of many genes and operons concerned with the transport and breakdown of catabolites. The cAMP concentration in cells also influences the synthesis of pili, fimbriae, flagella and at least one outer-membrane protein; all these factors are related to pathogenicity. Deficiency in cAMP and cAMP receptor protein can also be associated with resistance to $\beta$-lactam antibiotics. Curtiss and Kelly developed strains of *Salmonella typhimurium* that were unable to synthesise adenyl cyclase and cAMP receptor protein and thus were deficient in cAMP. These mutant strains required significantly higher infective doses than the parent strain in a mouse model.

In studies of the relationship between antibiotic resistance and pathogenicity the direct comparison of sensitive and resistant bacterial strains in virulence assays has been a favoured approach. In one study, aminoglycoside resistant mutants of *Pseudomonas aeruginosa* were examined for alterations in virulence. The mechanism of aminoglycoside resistance was either a decrease in cytochrome and nitrate reductase activity or the presence of uncapped subunits of lipopolysaccharide (LPS). The possession of smooth LPS is an established virulence factor in gram-negative bacteria. All mutant strains were less virulent in a mouse model and the strains with altered cytochrome activity also demonstrated slower growth rates in vitro. In a similar study, 4-quinolone-resistant *P. aeruginosa* obtained by serial culture through sub-inhibitory concentrations of the quinolones, exhibited decreased virulence for mice and also increased sensitivity to aminoglycosides. *Escherichia coli* mutants resistant to the 4-quinolones failed to haemagglutinate because of a lack of surface pili. It was suggested that these 4-quinolone-resistant *E. coli* may be less capable of colonising the urinary tract and initiating infection.

Chromosomally-mediated resistance of *Staphylococcus aureus* to several antibiotics has been shown to be associated with altered virulence. As early as 1944, penicillin-resistant *S. aureus* strains were shown to have reduced pathogenicity in mice and reduced production of coagulase and protein A compared to sensitive strains.

Exposure of *S. aureus* to increasing concentrations of gentamicin often results in bacteria that grow as small non-haemolytic colonies and do not produce coagulase or DNAase or ferment mannitol. Some methicillin-resistant *S. aureus* produce more coagulase but less protein A than methicillin-sensitive strains and are more likely to produce $\alpha$-haemolysin and enterotoxin A. Rifampicin-resistant mutants of *S. aureus* exhibit altered exoprotein production and are less virulent for mice. Some strains of *S. aureus* that are resistant to oxolinic appear to lose the ability to produce coagulase. However, in a more extensive study, ciprofloxacin-resistant and ciprofloxacin-sensitive strains of *S. aureus* produced similar amounts of coagulase, protein A, $\alpha$-haemolysin and $\delta$-haemolysin, grew at a similar rate and were equally virulent in a subcutaneous abscess model in mice (unpublished observation).

Bacteria exhibiting changes associated with chromosomal mutations may show a reduction in pathogenicity and infectivity. Further studies examining the pathogenicity of antibiotic-resistant mutants will help assess their potential both to initiate infection and to sustain infection, if the resistance-conferring mutations arise during therapy.

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References