EFFECT OF TEMPERATURE ON BACTERIAL MUTATIONAL CIPROFLOXACIN RESISTANCE

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The commonest mechanism causing clinical resistance of bacteria to antibiotics and chemotherapeutic agents is the possession of transferrable drug resistance plasmids. However, four exceptional antibacterials which do not suffer from plasmid-mediated resistance are polymyxin, nitrofurantoin, metronidazole and the 4-quinolones. With these four the only possible mechanism of clinical resistance for bacteria is chromosomal mutation. Consequently studies of the development of mutational resistance *in vitro* with such drugs have more clinical relevance than such studies with other antibacterials.

With *E. coli* mutational resistance to ciprofloxacin has been shown to occur more frequently at 30°C than at 25 or 37°C (Smith 1986). It was not known whether this effect was species-specific, so other bacteria were investigated as follows. Minimum inhibitory concentrations (MIC's) of ciprofloxacin were determined using 2 x 10^5-10^6 colony-forming units of *Staph. aureus*, *Staph. epidermidis*, *Pseudomonas aeruginosa* and *E. coli* inoculated on nutrient agar incubated at 25, 30 and 37°C. After 1 to 3 days (depending on the temperature) the lowest concentration of ciprofloxacin inhibiting colony formation was recorded. Bacterial cultures concentrated 20-fold by centrifugation were spread on nutrient agar containing 5 times the MIC of ciprofloxacin for each organism at each temperature and the plates incubated for up to 6 days. Colonies were counted and their frequency of occurrence used to calculate mutation rates.

	MUTATION RATES AT 25°C	30°C	37°C
Escherichia coli	1.75×10^{-8} (0.005)	2.88×10^{-8} (0.0075)	6.24×10^{-9} (0.02)
Staphylococcus aureus	2.96×10^{-8} (0.1)	4.80×10^{-8} (0.15)	1.57×10^{-8} (0.2)
Pseudomonas aeruginosa	1.42×10^{-7} (0.15)	1.38×10^{-7} (0.15)	1.07×10^{-7} (0.2)
Staphylococcus epidermidis	4.05×10^{-10} (0.4)	2.48×10^{-9} (0.3)	2.70×10^{-8} (0.2)

MIC's in mg/L are shown in parentheses

The results (Table) show that as before *E. coli* exhibited a temperature optimum for mutation to ciprofloxacin resistance as the frequency was greater at 30°C than at 25 or 37°C; a similar effect was seen with *Staph. aureus*. However, with Pseudomonas the mutation frequencies were similar at 25 and 30°C, but significantly higher than that at 37°C. With *Staph. epidermidis* quite different MIC results were seen and its mutation rates were also different being greatest at 37°C and progressively less at 30 and 25°C. Interestingly, mutants of all species isolated at 25 and 30°C grew at 37°C.

Skin surfaces exhibit temperatures less than 37°C and as mutational resistance occurred more frequently at such temperatures with three out of four species studied, perhaps ciprofloxacin may be more prone to therapeutic failure when used to treat skin infections caused by such bacteria. It may be relevant that the development of mutational ciprofloxacin resistance in patients occurs rarely with most bacteria but is significant with *Staph. aureus* and *Pseudomonas aeruginosa*, (Kresken and Weidmann 1988; Blumberg et al 1989) and both cause skin infections. It is also possible that mutational resistance may occur on fomites at room temperature.

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