Increased susceptibility to antibiotics in Gram-negative and Gram-positive pathogens, including *Pseudomonas aeruginosa*, at lower temperature: is antibiotic resistance reversal possible?

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Antibiotic resistance (ABR) has now emerged as an important global threat to animal and human medicine.[1] Many antibiotic agents, including the sulphonamides and certain β-lactam agents, have now become redundant due to overwhelming issues of resistance, which now render these largely ineffective and thus unavailable for safe clinical use. In particular, one clinical area that has suffered from overwhelming antibiotic resistance issues is cystic fibrosis (CF).

The role of bacterial pathogens in CF pulmonary disease contributes greatly to the morbidity and mortality in patients with CF. These patients have recurrent and chronic respiratory tract infections and most morbidity and mortality is due to such infections throughout their life.[2] These infections are usually dominated by Gram-negative organisms, especially the pseudomonads, including *Pseudomonas aeruginosa*, *Burkholderia cenocepacia* and *Stenotrophomonas maltophilia*.

P. aeruginosa is the single most important pathogen in this patient population, Recent advances in treatment, including intensive physiotherapy and aggressive antibiotic treatment, have greatly improved the outlook for patients. However, with the improvement in survival rates in CF patients, a new range of pulmonary issues has arisen. These include the emergence of multidrug-resistant strains of *P. aeruginosa* and the appearance of organisms with increased virulence, such as the *Burkholderia cepacia* complex (BCC).

Simultaneously, the development of new antibiotics and classes of antibiotic has slowed to a pace whereby development of microbial resistance is faster than drug development. As a consequence of this, and to protect the existing antibiotic armoury for continued and future clinical use, strategies have been introduced, particularly around prudent use of existing antibiotics both in animal and human health, in an attempt to curb their misuse and subsequent resistance development.[3]

To date, relatively little work has focused on alternative strategies to reverse existing antibiotic resistance, thereby allowing the redeployment of old and exhausted antibiotic agents. One strategy would be to examine the profile of antibiotic activity as a function of temperature in order to determine if antibiotics have more potency at alternatives to body temperature, via innovative local temperature alternating technologies, which as yet have not been described.

Therefore, the aim of this study is to examine the susceptibility of clinical bacterial pathogens to antibiotics at ambient temperature and to compare this with their susceptibility at normal body temperature, in order to determine if there is any potential in exploring alteration of temperature and thermal biology, as a new modality in combating antibiotic resistance.

Five bacterial pathogens were employed in this study, including three Gram-negative organisms and two Gram-positive organisms, as detailed in Table 1. In addition, eight isolates of *P. aeruginosa* were also examined in a separate experiment. Four of these isolates (01/10, 98/01, 98/04 and 01/11) were from adult patients with a diagnosis of CF, whereas the remaining four isolates (09/531, 09/559, 08/661 and 08/810) were invasive isolates cultured from positive blood cultures from non-CF patients.

All isolates were retrieved from the Northern Ireland Health and Social Care (HSC) Microbiology Strain Repository (MicroARK), located at the Northern Ireland Public Health Laboratory, Department of Bacteriology, Belfast City Hospital. All isolates were passaged three consecutive times on Columbia blood agar (Oxoid CM0331) supplemented with 5% (v/v) defibrinated horse blood.

Each organism was grown separately for 17 h, as described above, prior to antibiotic susceptibility testing. For each organism, a bacterial suspension was prepared, equating to a 0.5 MacFarland standard. Briefly, a cotton swab was charged with inoculum of each organism and was inoculated on the surface of Mueller-Hinton agar (Oxoid CM0337). On drying, a standard disk-diffusion assay was performed, where antibiotic disks of the following four classes of antibiotic agent were placed on the surface with a semi-automated stamper, which dispensed different antibiotics to a single plate: β-lactam (meropenem 10 µg), chloramphenicol (30 µg), macrolide (erythromycin 5 µg) and tetracycline (30 µg). Plates were incubated at both 22˚C and 37˚C for 48 h prior to reading. Susceptibility for each organism/antibiotic combination at both temperatures was recorded by measuring the diameter of the resulting zone of inhibition by a metric ruler.

Each *P. aeruginosa* organism (n=8) was grown separately for 17 h, as described above, prior to antibiotic susceptibility testing, as detailed above. The following five antibiotic disks

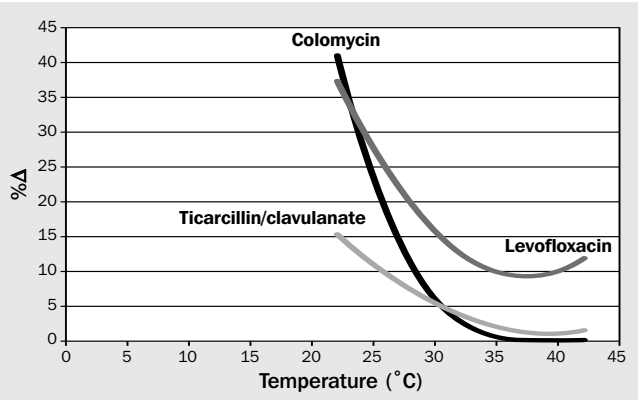

Fig. 1. Relationship between temperature and increased efficacy of antibiotic compared to susceptibility at 37˚C (%Δ) with three antibiotic agents.

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Table 1. Comparison of antibiotic susceptibility at 22˚C and 37˚C with five clinical bacterial pathogens and four classes of antibiotic.

	Zone of Inhibition (mm)											
Organism	**β-lactam***			**Chloramphenicol**			**Macrolide†**			**Tetracycline**		
	22˚C	**37˚C**	**%Δ**	**22˚C**	**37˚C**	**%Δ**	**22˚C**	**37˚C**	**%Δ**	**22˚C**	**37˚C**	**%Δ**
Gram –ve												
E. coli 0157:H7 NCTC12900	42	24	–75%	24	20	–20%	14	0	–100%	26	16	–63%
E. coli NCTC9001	40	26	–53%	26	23	–13%	16	0	–100%	30	22	–36%
Salmonella nottingham NCTC7832	38	28	–36%	22	28	+32%	14	0	–100%	22	25	+7%
Gram +ve												
Listeria innocua NCTC11288	39	29	–34%	24	23	–4%	26	25	–4%	32	24	–33%
Bacillus cereus NCTC7464	20	20	0%	26	17	–53%	28	24	–17%	23	10	–30%
Mean alteration in resistance between 22˚C and 37˚C	39.6% more susceptible at 22˚C			11.6% more susceptible at 22˚C			64.2% more susceptible at 22˚C			31.0% more susceptible at 22˚C		

*meropenem; †erythromycin

were employed: colomycin (25 µg), levofloxacin (5 µg), rifampicin (5 µg), tetracycline (30 µg) and timentin (ticarcillin + clavulanate, 85 µg). Plates were incubated at 22˚C, 30˚C, 37˚C and 42˚C for 48 h prior to reading. Susceptibility for each organism/antibiotic combination at each temperature was recorded by measuring the diameter of the resulting zone of inhibition by a metric ruler.

A comparison of antibiotic susceptibility of the five non-*Pseudomonas* clinical pathogens against four classes of antibiotic at 22˚C and 37˚C is shown in Table 1. The comparison of susceptibility of the eight *P. aeruginosa* isolates against three antibiotics with temperature is shown in Figure 1. All *Pseudomonas* isolates were resistant to rifampicin and there was no significant difference in susceptibility with the CF isolates compared to the non-CF isolates ($P=0.1158$). For all *Pseudomonas* isolates, tetracycline was not effective at 37˚C, but was markedly active at 22˚C, with antibiotic susceptibility poorest at 37˚C compared to 22˚C ($P<0.0001$), 30˚C ($P=0.002$) and 42˚C ($P=0.011$).

Antibiotic resistance is becoming an increasingly important public health problem globally, which requires interventions now in order to preserve the efficacy of antibiotic agents for future generations. Where this has now precipitated clinically is in the antibiotic management of adult patients with CF, predominantly with the Gram-negative pathogens *P. aeruginosa* and *B. cenocepacia*. Several CF centres now attempt to manage patients who are chronically colonised with these organisms and which are totally resistant (in vitro) to all available antibiotics, delivered either via an oral, nebulised or intravenous (iv) route. Therefore, it is imperative that antibiotic efficacy is re-established by some mechanism in order to provide a portfolio of antimicrobial agents to manage such extreme clinical scenarios.

Variation of physical parameters, including temperature, to elicit an increase in antibiotic efficacy has not been explored widely to date. The present study showed that antibiotic efficacy was greatest at lower temperature (22˚C) and worst at 37˚C. Presently, the authors do not have mechanistic data to explain this observation of the bacterium's phenotype and hence more experiments are urgently needed with thermal biologists to elucidate the findings of this preliminary short report.

One may speculate that at lower temperatures (22˚C) than the optimal temperature of the pathogen (37˚C), this creates an environmental stress on the bacterium, making it less capable of dealing with other cumulative stresses simultaneously; hence, the lowered susceptibility at suboptimal and lower temperatures.

In order to exploit such a finding and develop a clinical application, it is difficult to postulate a mechanism to lower body temperature safely to such extremes. There may be some potential application associated with manipulation of body temperature, particularly in neonates. Additionally, it may be possible to manipulate body temperature in a very localised region without marked systemic adverse effect; for example, the first few millimetres of tissue of a vascular leg ulcer could be cooled independently of the remainder of the leg/foot.

Overall, while a calamitous situation with regard to antibiotic efficacy has yet to be reached, it is now time to think of innovative ways to surmount inevitable increases in antibiotic resistance. In conclusion, with increasing rates of ABR in clinical pathogens, the data presented here suggest that antibiotic resistance reversal may be possible in certain clinical scenarios, where it is feasible to lower localised temperature (e.g., skin temperature) in order to gain this decrease in antibiotic resistance and thus salvage the antibiotic agent. Manipulation of temperature may be an interesting modality in an attempt to salvage efficacy of already exhausted antibiotics, but it is not without its challenges.

This work was financially supported through HSC R&D Office commissioned grant: Antimicrobial Resistance Action Plan (AMRAP) (COM/2730/04).

References

1 Gould IM. The epidemiology of antibiotic resistance. *Int J Antimicrob Agents* 2008; **32** (Suppl 1): S2–9.

2 Rajan S, Saiman L. Pulmonary infections in patients with cystic fibrosis. *Semin Respir Infect* 2002; **17** (1): 47–56.

3 Davey P, Brown E, Charani E *et al*. Interventions to improve antibiotic prescribing practices for hospital inpatients. *Cochrane Database Syst Rev* 2013; **4**: CD003543.