Effect of temperature on antimicrobial susceptibilities of *Pseudomonas maltophilia*

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SUMMARY After a case of peritonitis caused by *Pseudomonas maltophilia* had occurred 20 strains of the organism were investigated and the minimum inhibitory concentrations of a variety of antibiotics determined at 30°C and 37°C. There was a significant difference in susceptibility between 30°C (most resistant) and 37°C (most susceptible) for aminoglycosides and polymyxin B. No difference was seen with the other agents or in strains of *Ps aeruginosa* and *Enterobacteriaceae* tested under similar conditions. The possible mechanisms of this phenomenon are discussed below.

Since the first description by Hugh and Ryschenkow in 1961¹ *Pseudomonas maltophilia* has become recognised as the second most common pseudomonad implicated in clinical disease,² causing severe infections such as meningitis³ and septicæmia.⁴ Variation in the antimicrobial susceptibility of different strains of *Ps maltophilia* has been reported.^{2,5-12} On examining some of the methods used, however, it was found that different incubation temperatures had been used—30°C,^{7,8} 35°C,^{9,10} and 37°C^{11,12}—and this could account for the variation. A recent case of infection with *Ps maltophilia* prompted further investigations in which minimum inhibitory concentrations of a variety of antibiotics were determined on 20 strains of this species at 30°C and 37°C.

CASE REPORT

A 71 year old man presented with a five week history of increasing tiredness, nausea and vomiting, and decreased urine output. On admission his blood urea concentration was 70 m mol/l (421 mg/100 ml), serum creatinine concentration 1889 µmol/l (21 mg/100 ml) and urine output 20 ml/day. Continuous ambulatory peritoneal dialysis was started after a Tenckhoff catheter had been inserted under sterile conditions. Subsequent renal biopsy showed myeloma kidney secondary to a γ light chain paraprotein. Thirty one days after starting continuous ambulatory peritoneal dialysis he developed peritonitis, and culture of the peritoneal fluid by our standard method¹³ yielded a heavy growth of a

Gram negative bacillus identified as *Ps maltophilia* (API-2ONE gallery, API Code 2005). As the original isolate was cultured in larger numbers at 30°C than at 37°C, antibiotic disc susceptibility was determined by the Stoke's method on diagnostic sensitivity test agar (DST Oxoid) at 30°C and 37°C.¹⁴ The strain was resistant to gentamicin at 30°C but susceptible at 37°C and was susceptible to cefotaxime at both temperatures (Fig. 1).

Treatment was originally started with intra-peritoneal gentamicin, 20 mg/2 l dialysate bag, and subsequently supplemented with cefotaxime, 250 mg/2 l bag, with four changes daily. Despite intensive antibiotic treatment the peritonitis became chronic. Two months after continuous ambulatory peritoneal dialysis was started haemodialysis was started, and he subsequently remained well.

Material and methods

ORGANISMS

In addition to the index strain, 19 other isolates of *Ps maltophilia* were investigated: four from our own laboratory (two blood, two continuous ambulatory peritoneal dialysis isolates); five from the National Collection of Type Cultures (NCTC 10257, 10599, 10258, 10498, and 10259); and 10 clinical strains sent to us by Dr B Holmes, National Collection of Type Cultures, Central Public Health Laboratory, London. All strains were confirmed as *Ps maltophilia* by API-2ONE galleries. Eight clinical strains of *Ps aeruginosa* and 11 *Enterobacteriaceae* (six *Escherichia coli*, one *Citrobacter freundii*, two *Serratia marcescens*, and two *Klebsiella pneumoniae*),

Effect of temperature on sensitivity of index strain of *Ps maltophilia* to gentamicin and cefotaxime

identified by standard laboratory methods were also examined for comparison.¹⁵

MINIMUM INHIBITORY CONCENTRATIONS
Plate minimum inhibitory concentrations were determined on diagnostic sensitivity test agar medium (Oxoid). The antibiotics tested were aminoglycosides; gentamicin, tobramycin, and amikacin; other antibiotics with action on ribosomes; nalidixic acid and chloramphenicol; cell wall antibiotics; piperacillin and ceftazidime; and an antibiotic with cytoplasmic membrane action, polymyxin B sulphate. From a purity plate two colonies were touched with a sterile loop into 2 ml tryptone water (Oxoid) and used as the inoculum (roughly 10⁵ colony forming units/ml) using a multipoint inoculator (Denley Ltd). The minimum inhibitory concentration for each antibiotic was defined as the lowest concentration of antibiotic that completely inhibited growth after incubation overnight at 30°C or 37°C.

Results

The table shows the results obtained. The aminoglycoside antibiotics and polymyxin B showed a significant difference in minimum inhibitory concentration range and minimum inhibitory concentration₅₀ values between the two temperatures of incubation. For *Ps aeruginosa* and *Enterobacteriaceae* no such difference was shown. None of the other antibiotics tested showed a difference in values after incubation at 30°C or 37°C for both *Ps maltophilia* and other strains of bacteria. Growth was the same at both temperatures, as shown by purity plates.

Discussion

Ps maltophilia is now recognised as an important cause of nosocomial infection.^{2,5,16} Variation in the susceptibility to antibiotics of different strains of *Ps maltophilia* is common.⁵ In this report we have shown a considerable difference in the susceptibility

Comparison of minimum inhibitory concentration (MIC) (range, minimum inhibitory concentration₅₀ and minimum inhibitory concentration₉₀) (mg/l) at 30°C and 37°C

Antibiotic	Temperature of incubation (°C)	<i>Ps maltophilia</i> (n = 20)			<i>Ps aeruginosa</i> (n = 8)			<i>Enterobacteriaceae</i> (n = 11)		
		Range	MIC ₅₀	MIC ₉₀	Range	MIC ₅₀	MIC ₉₀	Range	MIC ₅₀	MIC ₉₀
Gentamicin	30	8->128	128	>128	0.125-16	0.25	0.25	0.25-128	0.5	2
	37	0.25->128	4	64	0.125-16	0.25	0.25	0.25-128	0.5	2
Tobramycin	30	4->128	128	>128	0.125-0.25	0.125	0.25	0.5-16	1	8
	37	0.25->128	2	64	0.125-0.25	0.125	0.25	0.5-16	1	8
Amikacin	30	8->128	128	>128	0.125-0.25	0.125	0.25	0.5-4	0.5	8
	37	0.25->128	2	64	0.125-0.25	0.125	0.25	0.5-4	0.5	8
Polymyxin B	30	8->128	128	>128	0.125-8	0.25	8	0.125-16	0.125	8
	37	4-128	32	64	0.125-8	0.25	8	0.125-16	0.125	8
Chloramphenicol	30	8-64	16	32	64->128	64	>128	8->128	8	16
	37	8-64	16	32	64->128	64	>128	8->128	8	16
Nalidixic acid	30	0.5->128	16	>128	32->128	64	>128	2->128	2	8
	37	0.5->128	16	>128	32->128	64	>128	2->128	2	8
Piperacillin	30	8->128	16	>128	4-8	4	8	1->128	4	8
	37	8->128	16	>128	4-8	4	8	1->128	4	8
Ceftazidime	30	0.5->128	2	>128	0.5-2	1	2	0.06-0.125	0.06	0.125
	37	0.5->128	2	>128	0.5-2	1	2	0.06-0.125	0.06	0.125

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to the aminoglycoside antibiotics and, to a lesser degree, polymyxin B at the incubation temperatures of 30°C and 37°C. With strains of *Ps aeruginosa* and *Enterobacteriaceae* no such difference could be shown. All other antibiotics tested gave similar minimum inhibitory concentrations at both temperatures.

It is unlikely that the difference in susceptibility to gentamicin played an important part in the development of chronic peritonitis in our patient. The dialysis fluid is warmed before administration and is run in over five to 10 minutes, so the temperature of the dialysate would have rapidly reached that of the body. In patients receiving continuous ambulatory peritoneal dialysis peritonitis caused by *Pseudomonas* spp is hard to eradicate. In a recent report by Krothapali *et al* 10 cases of *Pseudomonas* peritonitis failed to respond to up to four weeks of adequate aminoglycoside treatment, and in all cases removal of the Tenckhoff catheter was required.¹⁷

The question remains as to why these temperature effects occurred, especially with the aminoglycosides. Several explanations are possible: Annear and Macculloch suggested that there may be an association between resistance to gentamicin and delayed growth of a strain of *Ps aeruginosa* at 22°C.¹⁸ Seal and Strangeways believed that neither the acquisition of a resistance factor nor a single mutation leading to resistance to gentamicin affected growth at extremes of temperature.¹⁹ Recent studies have examined the susceptibility of *Ps aeruginosa* and *E coli* to aminoglycosides and shown an association between resistance and decreased permeability of the bacteria to the aminoglycosides.²⁰ This type of mechanism as the source of resistance rather than a change in ribosomal property is supported by our observation that those antibiotics that also have ribosomal activity—that is, chloramphenicol and nalidixic acid—showed no change in their *in vitro* activity.

In view of the fact that cell wall lipids, especially phospholipids, probably help exclude certain lethal agents, it seems likely that variations in the temperature at which growth occurs has some effect. In general, organisms grown at a lower temperature in the growth range contain an increased proportion of unsaturated fatty acid residues in their lipids,²¹ and the composition of the phospholipid has also been shown to vary.²² Dunnick and O'Leary examined the composition of lipids from a variety of *Enterobacteriaceae* susceptible to and resistant to antibiotics and showed that there was a higher concentration of unsaturated acids and a lower concentration of cyclopropanic acids in lipids from resistant strains.²³ Almost nothing, however, is known of the biochemical basis of this effect of temperature on

the fatty acid composition of bacteria.²¹ K Shannon (personal communication) could detect no acetyl transferase or adenylyl transferase activity in the original isolate of *Ps maltophilia* at 30°C. He commented that this resistance to a broad spectrum of aminoglycoside is seldom associated with enzymes that modify aminoglycoside. He had also not noticed this effect of temperature on susceptibility to aminoglycoside before.

Why this temperature effect seems to be so pronounced in *Ps maltophilia* with regard to susceptibility to aminoglycosides remains to be explained. In view of the increasing importance of the organism as a hospital pathogen it is imperative that susceptibility to tests for antibiotics be standardised, using uniform laboratory conditions with special regard to temperature.

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