Effects of test conditions on the susceptibility of staphylococci *in vitro* to cephradine, cephaloridine, cephalixin, and cefuroxime

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SUMMARY Methicillin-resistant staphylococci were tested for susceptibility to cephradine, cephaloridine, cephalixin, and cefuroxime at 30°C and 37°C on ordinary media and on media of enhanced osmotic strength. The coagulase-negative strains were divided into *Staphylococcus epidermidis* and *Staphylococcus hominis*. Generally the number of susceptible strains decreased with low incubation temperature and osmotic support. When *Staphylococcus aureus* was tested against cephalixin and cefuroxime, most were susceptible at 37°C whereas all were resistant at 30°C. A similar trend was found in the case of cephradine, where it was accentuated by osmotic support. While almost all strains of *Staph. epidermidis* were sensitive to all four drugs under all test conditions, the susceptibility of *Staph. hominis* was generally decreased by either lower incubation temperature or osmotic support or both.

The use of an incubation temperature of 30°C rather than 37°C when testing *Staphylococcus aureus* for susceptibility to methicillin, or alternatively the addition of sodium chloride to the test medium if the higher temperature is used, results in the detection of more resistant strains.^{1,2} Though cephalosporins also exert their effects on the cell walls of bacteria, investigation into the use of low incubation temperature when testing for susceptibility to these drugs, with or without osmotic support, has been limited.³⁻⁵

Coagulase-negative staphylococci (*Staphylococcus albus*) are now recognised as potential causes of sepsis in patients whose immune responses are compromised or who have surgically implanted devices such as cardiac valve replacements, hydrocephalus shunts, and artificial joints. Strains of *Staph. albus* have been shown to be multi-resistant^{6,7} and often resistant to methicillin.⁸ Because of this, the use of cephalosporins in the treatment of infections due to methicillin-resistant staphylococci has been considered, though cross resistance between methicillin and cephalosporins has been suggested.⁹

We therefore investigated the susceptibility of methicillin-resistant strains of *Staph. aureus* and *Staph. albus* to cephradine, cephalixin, cephaloridine, and cefuroxime, using low incubation tem-

peratures and osmotic support in addition to a conventional agar plate method. All coagulase-negative staphylococci were fully identified as it was considered possible that differences in antibiotic susceptibility might exist between the species constituting the *Staph. albus* group.

Material and methods

Eighteen strains of *Staph. aureus* and 69 strains of *Staph. albus*, which were resistant to 12.5 mg/l of methicillin when tested at 30°C on agar containing the antibiotic, were studied. The organisms were isolated from a variety of patients at the Children's Hospital and the Northern General Hospital, Sheffield.

All strains were tested for production of coagulase and deoxyribonuclease, and were further identified using the 'API Staph.' system (API Laboratories Ltd), which is based on the classification of Kloos and Schleiffer.¹⁰ Strains classified as *Staphylococcus epidermidis* and *Staphylococcus hominis* under this scheme correspond to Baird-Parker's subgroups II and V, respectively, of *Staph. epidermidis*.¹¹

Overnight peptone water cultures of the organisms were diluted by the addition of one drop from a Pasteur pipette to 3 ml of phosphate-buffered saline, pH 7.4, and inoculated on to the surfaces of agar

plates with a multipoint inoculator. The antibiotics were added to the agar before being poured in doubling dilutions based on the attainable serum levels of each drug using accepted dosage. Columbia agar (LAB M) was used throughout, and, where osmotic support was required, this was provided by the addition of polyvinylpyrrolidone (PVP, mol wt 44 000, BDH) to give a final concentration of 40 g/l.^{4,5} Each strain was inoculated on to four series of Columbia agar plates containing antibiotics. Two series of plates consisting of plain Columbia agar and two containing PVP were used. One series of plain and one series of PVP plates were incubated at 37°C, and one series of plain and one of PVP plate were incubated at 30°C. The plates were incubated for 18 hours in each case. The methodology is summarised in the Figure. The ranges of final concentrations of antibiotics tested were cephradine, cephaloridine, and cefuroxime, 2-64 mg/l, and cephaloridine, 0.5-16 mg/l.

Summary of methodology.

Table 1 Number of strains of *Staph. aureus* susceptible to various concentrations of cephradine, cephaloridine, cefuroxime, and cephaloridine under different conditions of temperature and osmolality (N = 18)

Drug	Medium	Temp (°C)	MIC (mg/l)			
			≤ 8	16	32	64
Cephradine	PVP	30	3	2	12	1
		37	14	4	0	0
	COL	30	4	5	9	0
		37	17	1	0	0
Cefuroxime	PVP	30	0	0	15	3
		37	6	7	5	0
	COL	30	0	0	18	0
		37	7	8	3	0
Cephalexin	PVP	30	0	0	18	0
		37	0	18	0	0
	COL	30	0	0	18	0
		37	5	13	0	0
			MIC (mg/l)			
			≤ 1	2-4	≥ 8	
Cephaloridine	PVP	30	1	1	16	
		37	1	1	16	
	COL	30	0	18	0	
		37	2	6	10	

COL = Columbia agar.

PVP = Columbia agar + polyvinylpyrrolidone.

After viability had been checked the minimum inhibitory concentrations (MIC) of the organisms were read in the usual way. Strains were considered to be susceptible when the MIC did not exceed 16 mg/l in the case of cephradine, cephaloridine, and cefuroxime, and 4 mg/l in the case of cephaloridine.

Results

All of the strains of *Staph. aureus* produced coagulase and deoxyribonuclease. Thirty-eight of the 69 coagulase-negative strains were shown to be *Staph. epidermidis* and 31 were shown to be *Staph. hominis*.

The results of the MIC determinations are shown in Tables 1 to 3 and are summarised in Table 4. When methicillin-resistant strains of *Staph. aureus* were tested on plain Columbia agar at 37°C all were found to be susceptible to cephradine and cephaloridine, but 56% were resistant to cephaloridine and 17% were resistant to cefuroxime. Using the same test conditions, all methicillin-resistant strains of *Staph. epidermidis* and *Staph. hominis* were susceptible to cephradine, cephaloridine, and cephaloridine but 8% of *Staph. epidermidis* and 61% of *Staph. hominis* were resistant to cefuroxime.

The use of the same medium at 30°C increased the percentage of strains of *Staph. aureus* that were susceptible to cephaloridine from 44% to 100% but decreased the percentage of strains susceptible to cephradine to 50%. At this temperature all strains of *Staph. aureus* were resistant to cephaloridine and cefuroxime. Under the same test conditions there

Table 2 Number of strains of *Staph. epidermidis* susceptible to various concentrations of cephradine, cephalixin, cefuroxime, and cephaloridine under different conditions of temperature and osmolality (N = 38)

Drug	Medium	Temp (°C)	MIC (mg/l)		
			≤ 8	16	32
Cephhradine	PVP	30	35	3	0
		37	35	3	0
	COL	30	36	2	0
		37	35	3	0
Cefuroxime	PVP	30	35	1	2
		37	36	0	2
	COL	30	34	2	2
		37	34	1	3
Cephalexin	PVP	30	35	3	0
		37	35	3	0
	COL	30	35	3	0
		37	35	3	0
			MIC (mg/l)		
			≤ 1	2-4	≥ 8
Cephaloridine	PVP	30	30	7	1
		37	36	2	0
	COL	30	36	2	0
		37	38	0	0

COL = Columbia agar.

PVP = Columbia agar + polyvinylpyrrolidone.

Table 3 Number of strains of methicillin-resistant *Staph. hominis* susceptible to various concentrations of cephradine, cephalixin, cefuroxime, and cephaloridine under different conditions of temperature and osmolality (N = 31)

Drug	Medium	Temp (°C)	MIC (mg/l)		
			≤ 8	16	≥ 32
Cephradine	PVP	30	16	13	2
		37	26	4	1
	COL	30	29	2	0
		37	29	2	0
Cefuroxime	PVP	30	7	6	18
		37	8	1	22
	COL	30	14	3	14
		37	8	4	19
Cephalexin	PVP	30	13	8	10
		37	16	15	0
	COL	30	15	13	3
		37	20	11	0
MIC (mg/l)					
			≤ 1	2-4	≥ 8
Cephaloridine	PVP	30	12	18	1
		37	12	19	0
	COL	30	15	16	0
		37	25	6	0

COL = Columbia agar.

PVP = Columbia agar + polyvinylpyrrolidone.

was no significant change in the susceptibilities of *Staph. epidermidis* to all four drugs but 10% of strains of *Staph. hominis* were resistant to cephalixin, and the percentage susceptible to cefuroxime rose from 39% to 55%.

The addition of PVP to the medium with the use of an incubation temperature of 37°C led to a fall of 10% in the number of strains of *Staph. aureus* sus-

ceptible to cefuroxime, and of 33% in those susceptible to cephaloridine, but there was no change in their susceptibility to cephradine or cephalixin.

When both PVP and 30°C were used, all strains of *Staph. aureus* showed resistance to cephalixin and cefuroxime, and there was a sharp decrease in the percentages of strains susceptible to cephradine and cephaloridine. Under these conditions the numbers

Table 4 Percentage of strains of methicillin-resistant staphylococci susceptible to cephradine (16 mg/l), cephaloridine (4 mg/l), cefuroxime (16 mg/l), and cephalixin (16 mg/l) under different conditions of temperature and osmolality

Drug	Medium	Temp.	<i>Staph. aureus</i> n = 18	<i>Staph. epidermidis</i> n = 38	<i>Staph. hominis</i> n = 31
Cephradine:	PVP	30°C	17	100	94
		37°C	100	100	97
	COL	30°C	50	100	100
		37°C	100	100	100
Cephaloridine:	PVP	30°C	11	97	96
		37°C	11	100	100
	COL	30°C	100	100	100
		37°C	44	100	100
Cefuroxime:	PVP	30°C	0	95	42
		37°C	72	95	29
	COL	30°C	0	95	55
		37°C	83	92	39
Cephalixin:	PVP	30°C	0	100	68
		37°C	100	100	100
	COL	30°C	0	100	90
		37°C	100	100	100

COL = Columbia agar.

PVP = Columbia agar + polyvinylpyrrolidone.

of strains of *Staph. hominis* susceptible to all four drugs decreased.

Discussion

Test conditions are known to influence the susceptibility *in vitro* of staphylococci to methicillin. Conventionally, staphylococci, in common with other organisms, are tested for susceptibility to cephalosporins at 37°C on media without added osmotic support. Recently, they have been tested for susceptibility to certain cephalosporins on media containing sodium chloride⁹ and PVP,⁴ and in some cases the effect has been found to be similar to that seen with methicillin. As multiple drug resistance, including methicillin-resistance, among coagulase-negative staphylococci is now widely recognised, and as cephalosporins have been used with varying results to treat infections due to these organisms,¹² we decided to examine the susceptibility of methicillin-resistant strains of both coagulase-positive and coagulase-negative staphylococci to various cephalosporins and cefuroxime, and to attempt to define the most suitable test conditions for each drug. The results in the Tables suggest that methicillin-resistant strains of *Staph. aureus* may be expected to be susceptible to cephradine and cephalixin when tested on ordinary medium at 37°C, but when tested at 30°C in the presence of PVP the opposite is indicated. The same is true to a lesser extent in the case of cephaloridine and cefuroxime. With the exception of cephaloridine, the results at 37°C are not much affected by PVP, and incubation temperature appears

to be the most important factor, although there is some apparent enhancement of the temperature effect by PVP at 30°C in the case of cephradine and at 37°C in the case of cephalixin. No explanation is offered for the finding that all strains are susceptible to cephaloridine on ordinary media at 30°C without PVP while the majority are resistant under other test conditions.

The results of *Staph. epidermidis* suggest that most methicillin-resistant strains can be expected to be susceptible to cephradine, cephalixin, cefuroxime, and cephaloridine, irrespective of which test method is used. The number of strains of *Staph. hominis* susceptible to cephradine, cephaloridine, and cephalixin was found to decrease when they were tested at 30°C with osmotic support, whereas the lowest number of strains susceptible to cefuroxime was demonstrated at 37°C in the presence of PVP. These results show that staphylococci cannot be assumed to be homogeneous in their behaviour in the presence of these drugs. They also show the necessity for testing individual drugs in this family.

Unless tests for susceptibility to drugs have some value in predicting what will happen *in vivo*, they are irrelevant in the clinical laboratory. The effect of osmotic support is shown to be variable, but we suggest that the testing of cell-wall-active drugs in a medium whose colloid osmotic pressure is similar to that of proteinaceous body fluids^{4,5} may represent a step towards greater relevance. In addition, the drug-resistant members of a methicillin-resistant culture grow more slowly at 37°C than at 30°C, and if the same is true for cultures resistant to cephalosporins, the use of lower incubation temperatures

would also be justified as it would hasten their detection in the laboratory.

The significance of these laboratory studies with regard to their ability to predict therapeutic efficacy requires further investigation, but in a recent study of *Staph. aureus* in burns,¹³ 100% of burns showed elimination or reduction of strains that had been shown to be sensitive at both 30°C and 37°C, whereas only 80% showed elimination or reduction of strains shown to be sensitive at 37°C but resistant at 30°C, using 500 mg flucloxacillin six-hourly in each case. The demonstration of 'resistance' of strains to cell-wall-active antibiotics *in vitro* at 30°C may indicate that successful therapy with the drug tested is more likely if a higher dosage is used.

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References

- ¹ Annear DI. The effect of temperature on resistance of *Staphylococcus aureus* to methicillin and some other antibiotics. *Med J Austral* 1968;1:444-6.
- ² Churcher GM. A screening test for the detection of methicillin-resistant staphylococci. *J Clin Pathol* 1968; 21:213-7.
- ³ Vernon GN, Russell AD. Effects of methicillin, cephaloridine and cephalothin on the growth, lysis and viability of some methicillin-resistant strains of *Staphylococcus aureus* at different temperatures. *J Antimicrob Chemother* 1976;2:41-8.
- ⁴ Bayston R. Use of polyvinylpyrrolidone in the testing of staphylococci for sensitivity to methicillin and cephradine. *J Clin Pathol* 1978;31:434-6.
- ⁵ Bayston R. The use of polyvinylpyrrolidone in sensitivity testing. *J Antimicrob Chemother* 1978;4:283-94.
- ⁶ Corse J, Williams REO. Antibiotic resistance of coagulase-negative staphylococci and micrococci. *J Clin Pathol* 1968;21:722-8.
- ⁷ Sabath L, Garner C, Wilcox C, Finland M. Susceptibility of *Staphylococcus aureus* and *Staphylococcus epidermidis* to 65 antibiotics. *Antimicrob Ag Chemother* 1976;9:962-9.
- ⁸ Sabath L, Barrett FF, Wilcox C, Gerstein DA, Finland M. Methicillin resistance of *Staphylococcus aureus* and *Staphylococcus epidermidis*. *Antimicrob Ag Chemother* 1969;1968:302-6.
- ⁹ Lavadiere M, Peterson PK, Verhoef J, Williams DN, Sabath LD. In vitro activity of cephalosporins against methicillin-resistant coagulase negative staphylococci. *J Inf Dis* 1978;137:245-50.
- ¹⁰ Kloos WE, Schleiffer KH. Simplified scheme for routine identification of human staphylococcus species. *J Clin Microbiol* 1975;1:82-8.
- ¹¹ Baird-Parker AC. Staphylococci and their classification. *Ann New York Acad Sci* 1965;128:4-25.
- ¹² Acar JF, Courvalin P, Chabbert YA. Methicillin-resistant staphylococcaemia: bacteriological failure of treatment with cephalosporins. *Antimicrob Ag Chemother* 1970; 10:280-5.
- ¹³ Kidson A, Lilly HA, Lowbury EJJ. Flucloxacillin treatment of methicillin-'resistant' and sensitive staphylococcal infection. *J Antimicrob Chemother* 1979;5:359-64.

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