Comparison of culture media for detecting methicillin resistance in *Staphylococcus aureus* and coagulase negative staphylococci

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SUMMARY The detection of methicillin resistance was examined in 51 strains of *Staphylococcus aureus* and 135 strains of coagulase negative staphylococci using Isosensitest, Diagnostic Sensitivity Test (DST), Mueller-Hinton (MH), Columbia, and Sensitest agars. MH agar with 5% added sodium chloride incubated at 35°C was the most effective in detecting resistance in *S aureus*, and Columbia agar with 5% added sodium chloride incubated at 35°C was most effective for coagulase negative staphylococci. For clinical purposes, a provisional report of sensitivity for *S aureus* could be issued after 18 hours; with coagulase negative staphylococci, only resistant strains could be reported at this time. For definitive results cultures must be examined after 40 hours of incubation.

Resistance to methicillin in *Staphylococcus aureus* is usually heterogeneous and due to the presence of a component which sometimes only manifests resistance if grown on osmotically supportive media¹ or if cultured at 30°C² for up to 48 hours.³ Snell *et al*⁴ recommended that incubation at 30°C or adding 5% sodium chloride to the medium, or both, be used for methicillin testing. Despite the heterogeneous nature of the resistance in *S aureus* to methicillin it has been shown to be clinically relevant in that it impairs the response to treatment with methicillin.⁵ Resistance to methicillin in coagulase negative staphylococci has also been shown to be due to heterogeneous populations and to be enhanced by growth on hypertonic medium or incubation at 30°C or for 48 hours.⁶

In addition to these factors, the detection of methicillin resistance can be affected by the basal test medium used. Hindler and Inderleid⁷ showed that when MH, an undefined medium, was used from five different manufacturers there were differences in the detection of resistance of *S aureus* to methicillin.

Brown and Kothari⁸ determined the minimum inhibitory concentration (MIC) of strains of *S aureus* using MH, DST, Sensitest, and Wellcotest agars, and all gave similar results when incubated at 30°C for 18 hours. In view of the increasing clinical importance of

coagulase negative staphylococci we examined several media to determine the optimal test conditions for detecting the methicillin resistance of both *S aureus* and coagulase negative staphylococci.

Material and methods

The 186 strains of staphylococci tested consisted of eight reference strains from national collections, three strains from quality control schemes, and 175 clinical isolates. Many of the clinical isolates were selected because of difficulty in the interpretation of the disc sensitivity test.

Columbia (CM331), Diagnostic Sensitivity Test (DST) (CM261), Isosensitest (CM471), Mueller-Hinton (MH) (CM337), and Sensitest (CM409) agars were obtained from Oxoid Limited. The media were prepared according to the manufacturer's instructions, except that initial suspension of the powder was made in nine tenths of the recommended volume of water. After sterilisation and cooling to 45°C the remaining one tenth volume was made up by the addition of appropriate sterile aqueous solutions of methicillin (Sigma Chemical Company) to give final concentrations of 0, 0.5, 1, 2, 4, 8 and 16 µg/ml. A duplicate set of media containing an additional 50 g/l sodium chloride was prepared at the same time. The poured plates were refrigerated overnight before inoculation the next day.

All strains were examined by the method of Harvey

and Gilmour.⁹ Seventeen strains, which failed to provide good identification, were further tested using the API STAPH system (API Laboratory Products Limited).

The MIC tests were performed according to the National Committee for Clinical Laboratory Standards (NCCLS) Tentative Standard M7-T (1983), using a multipoint inoculating device. Plates containing added sodium chloride were incubated at 35°C, and those without sodium chloride at 30°C. Readings were made at 18 hours and 40 hours, the MIC being recorded as the lowest concentration of antimicrobial agent that completely inhibited growth. A single colony or a faint haze caused by the inoculum was disregarded. Strains were considered to be resistant to methicillin if their MIC was ≥ 8 µg/ml on two separate tests and sensitive if their MIC was < 8 µg/ml. The MIC test was repeated on any strain producing a result that could not be interpreted by NCCLS criteria—that is, gave an unclear end point or insufficient growth on the control medium without added antibiotic.

The eight reference strains and three quality control strains were tested and designated as sensitive or resistant by Dr RC George, Division of Hospital Infection, Central Public Health Laboratory, Colindale.

Results

Table 1 shows that all 51 strains of *S aureus* grew satisfactorily on each medium by 18 hours. Twenty strains were sensitive under all test conditions. Of the remaining 31 strains, 28 showed resistance on more than one medium. The other three strains were resistant (MIC = 8 µg/ml) only on MH agar with 5% added sodium chloride after 40 hours of incubation.

Sensitest and Isosensitest media performed considerably worse than the others—notably after 18 hours. All media detected more strains as resistant after 40

hours than at 18 hours. After 40 hours MH with 5% added sodium chloride detected 31 (100%) of the 31 resistant strains and Columbia and DST with 5% added sodium chloride detected 26 (84%). After 40 hours the performance of MH, DST, and Columbia incubated at 30°C were similar in that they detected 87%, 90%, and 81%, respectively, of the resistant strains.

Table 2 shows the results obtained with 135 strains of coagulase negative staphylococci. At 18 hours 25 strains failed to show adequate growth on one or more media. At 40 hours six strains failed to grow adequately on some of the media, but all grew on Columbia with or without added sodium chloride. Seventy two strains were designated as sensitive on all media after 40 hours' incubation. Forty eight strains showed resistance on at least one medium after 18 hours incubation and 63 after 40 hours. Again, Sensitest and Isosensitest media performed inadequately. After 40 hours of incubation the use of 5% sodium chloride in the Columbia and DST media was shown to be advantageous. Columbia with 5% added sodium chloride detected 55 (87%) of the 63 resistant strains and DST with 5% added sodium chloride, 47 (75%).

In contrast to the results with *S aureus*, MH with 5% added sodium chloride detected only 35 (56%) of the resistant strains. The media without additional sodium chloride detected a lower percentage of resistant strains at 40 hours, Columbia agar 73%, MH 54%, and DST 44%. Eight resistant strains were not detected using Columbia with 5% added sodium chloride at 35°C. Six of these were detected using Columbia agar at 30°C. MH at 30°C detected four of these eight strains and MH with 5% added sodium chloride, one. DST at 30°C detected three, and DST with 5% added sodium chloride, none.

Table 3 shows more detailed results of the 11 reference and quality control strains. None of the media showed a sensitive strain as resistant. Resistant strains, however, were found to be sensitive under

Table 1 MICs of methicillin for 51 strains of *Staphylococcus aureus* recorded after 18 hours and 40 hours of incubation

	18 hours			40 hours		
	≥ 8	< 8	NG/ISG	≥ 8	< 8	NG/ISG
Col + 5% sodium chloride	24	27	0	26	25	0
Col	24	27	0	25	26	0
DST + 5% sodium chloride	22	29	0	26	25	0
DST	20	31	0	28	23	0
MH + 5% sodium chloride	24	27	0	31	20	0
MH	27	24	0	27	24	0
Sens + 5% sodium chloride	1	50	0	13	38	0
Sens	13	38	0	21	30	0
Iso + 5% sodium chloride	0	51	0	17	34	0
Iso	9	42	0	19	32	0

Abbreviations: Col = Columbia agar, DST = Diagnostic sensitivity agar, MH = Mueller-Hinton agar, Sens = Sensitest agar, Iso = Isosensitest agar, NG = No growth, ISG = Insufficient growth

Table 2 MICs for methicillin for 135 strains of coagulase negative staphylococci* recorded after 18 hours and 40 hours of incubation

	18 hours			40 hours		
	≥8	<8	NG/ISG	≥8	<8	NG/ISG
Col + 5% sodium chloride	36	97	2	55	80	0
Col	32	100	3	46	89	0
DST + 5% sodium chloride	25	109	1	47	87	1
DST	23	108	4	28	106	1
MH + 5% sodium chloride	20	109	6	35	98	2
MH	21	103	11	34	100	1
Sens + 5% sodium chloride	3	120	12	11	121	3
Sens	14	110	11	18	115	2
Iso + 5% sodium chloride	1	126	8	7	124	4
Iso	10	112	13	19	115	1

Abbreviations: Col = Columbia agar, DST = Diagnostic sensitivity agar, MH = Mueller-Hinton agar, Sens = Sensitest agar, Iso = Isosensitest agar, NG = No growth, ISG = Insufficient growth

**Staphylococcus epidermidis* 93, *S. haemolyticus* 19, *S. simulans* 11, *S. warneri* 6, *S. capitis* 3, *S. xylosum* 2, *S. hominis* 1

several test conditions, especially after 18 hours—for example, strain 12 was sensitive on DST with 5% added sodium chloride after 18 hours, but resistant at 40 hours. Isosensitest and Sensitest agars, with or without 5% added sodium chloride were unreliable and yielded erroneous results with nine of these 11 strains. Columbia and MH agars with or without 5% added sodium chloride were the most reliable media and when incubated for 40 hours detected all the resistant strains.

Discussion

Any method of antibiotic sensitivity testing adopted by a clinical laboratory must produce a reliable result as early as possible. There have been many attempts to define how best to test for methicillin resistance,¹⁻³ and the large number of reports clearly reflects the difficult nature of the problem. The recent re-emergence of epidemic strains of methicillin resistant

*S. aureus*¹⁰ and of methicillin resistant coagulase negative staphylococci in prosthetic heart valves, orthopaedic implants, and cerebrospinal fluid shunts has highlighted the need for a reliable method of laboratory testing. Previous studies of media have dealt only with *S. aureus*^{7,8} or have examined both coagulase positive and negative species as a single group.¹¹ It is clear from tables 1 and 2 that these two groups behave differently in methicillin sensitivity tests and must be considered separately.

All strains of *S. aureus* grew on each medium examined by 18 hours. After 18 hours of incubation Columbia and MH agars, whether or not 5% sodium chloride was included, performed better than the other media because they detected more of the resistant isolates. After 40 hours' incubation MH with 5% added sodium chloride was the best medium for detecting all resistant strains. We therefore recommend MH agar with 5% added sodium chloride when using the breakpoint method for the detection of

Table 3 Results of quality control and reference strains

Strain No	Identification	Reference result	Agar		Columbia		DST		MH		Iso		Sens	
			Incubation temp °C and time (hours)		35 30		35 30		35 30		35 30		35 30	
			18	40	18	40	18	40	18	40	18	40	18	40
9	<i>S. aureus</i>	R	r	r	r	r	r	r	r	r	r	r	r	r
10	<i>S. aureus</i>	R	r	r	r	r	r	r	r	r	r	r	r	r
12	<i>S. aureus</i>	R	r	r	r	r	r	r	r	r	r	r	r	r
13	<i>S. aureus</i>	S	s	s	s	s	s	s	s	s	s	s	s	s
14	<i>S. aureus</i>	R	r	r	r	r	r	r	r	r	r	r	r	r
15	<i>S. aureus</i>	S	s	s	s	s	s	s	s	s	s	s	s	s
24	<i>S. aureus</i>	R	r	r	r	r	r	r	r	r	r	r	r	r
181	<i>S. aureus</i>	R	r	r	r	r	r	r	r	r	r	r	r	r
182	<i>S. aureus</i>	R	r	r	r	r	r	r	r	r	r	r	r	r
183	<i>S. haemolyticus</i>	R	r	r	r	r	r	r	r	r	r	r	r	r
185	<i>S. aureus</i>	R	r	r	r	r	r	r	r	r	r	r	r	r
Total No of incorrect results			1	0	1	0	3	1	2	0	2	0	9	3

Media incubated at 35°C contained 5% sodium chloride

r = resistant strain (MIC ≥ 8 µg/ml) determined as resistant, s = sensitive strain (MIC < 8 µg/ml) determined as sensitive, S = resistant strain determined as sensitive, ISG = insufficient growth.

resistance to methicillin in *S aureus*. A reading at 18 hours would detect nearly all the resistant strains and a provisional report could be issued at that time. In only a few strains would this have to be changed after 40 hours.

The greatest difference between the media was evident with coagulase negative staphylococci. The use of a more nutritious agar than the semidefined media specially formulated for sensitivity testing is advisable, and only Columbia agar supported the growth of all strains after 40 hours of incubation. MH agar with or without added sodium chloride was not satisfactory for detecting methicillin resistance in coagulase negative staphylococci. The medium of choice for coagulase negative staphylococci is Columbia agar with 5% added sodium chloride incubated at 35°C. After 18 hours of incubation only resistant coagulase negative staphylococci should be reported and the remaining strains incubated until 40 hours incubation has been completed.

In conclusion, none of the five media with or without added 5% sodium chloride, and irrespective of incubation temperature, provided complete detection of methicillin resistance in both *S aureus* and coagulase negative staphylococci. For *S aureus* we recommend MH agar with 5% added sodium chloride and for coagulase negative staphylococci, Columbia agar with 5% added sodium chloride, both incubated at 35°C for 40 hours.

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