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Selection of *Staphylococcus aureus* in cultures from air samples

By LYNN HARDING and R. E. O. WILLIAMS
Wright-Fleming Institute, St Mary's Hospital Medical School, London, W.2

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Although a good many selective culture media have been described for the isolation of *Staphylococcus aureus*, none of them has proved useful for the examination of cultures from air. This is partly because, in examining such cultures, one is trying to select the *S. aureus* from among a great variety of other members of the *Micrococcaceae*. Media depending on a high concentration of sodium chloride (e.g. Chapman, 1944), which are principally inhibitory for enterobacteria and similar Gram-negative bacilli, are not, therefore, of much use. Other media, such as those containing potassium tellurite (Zebovitz, Evans & Niven, 1955) or polymyxin B (Davis & Davis, 1965), though often satisfactory for examination of material from infected lesions or carrier sites, prove too inhibitory for the isolation of staphylococci from air. It has been a common experience that bacteria are often difficult to cultivate when collected from the airborne state. The recent studies on the taxonomy of staphylococci and micrococci published by Baird-Parker (1963, 1965) suggest a new approach, for he showed that *S. aureus* is able to grow at a higher temperature than most of the micrococci. Moreover members of the genus *Staphylococcus* are able to grow under anaerobic conditions, while those of the genus *Micrococcus* cannot, and other studies have shown that a substantial proportion of the airborne cocci fall into *Micrococcus* (Corse & Williams, 1968). We therefore explored the use of anaerobic culture at 41° C. as a selective method for the isolation of *S. aureus*. After a number of preliminary experiments with pure cultures, which need not be discussed here, all our studies were made by the exposure of culture plates containing the media under test in the wards of St Mary's Hospital.

METHODS

Air sampling

Culture plates 14 cm. in diameter were exposed in hospital wards for periods varying between 8 and 16 hr. The plates were stacked in the holder illustrated in Plate 1, which provides about 5 cm. separation between plates. Preliminary experiments showed that there were no significant differences in the numbers of colonies developing on the four plates in the stack. The culture plates with the different media were placed at random on the four layers of the stack. One or more stacks of plates were exposed each day but each stack included at least one 'control' plate.

The culture media used are listed in Table 1.

The 14 cm. diameter culture plates were poured with about 80 ml. of medium to give thick plates which resist desiccation during exposure in the ward.

For anaerobic incubation the plates were placed in 20 l. stainless-steel milk churns (R. J. Fullwood and Bland Ltd.) with a metal tube through the lid for evacuating the air, and three cold-catalyst sachets (Baird and Tatlock Ltd.), two at the top and one midway down the churn.

Table 1. *Composition of culture media*

	Control TY	Baird-Parker (Oxoid CM 275) BP	Modified Baird-Parker MBP
Tryptone	10 g.*	10 g.	10 g.*
Lab-Lemco beef extract	—	5 g.	—
Yeast extract	5 g.†	1 g.	5 g.†
K_2HPO_4	5 g.	—	—
NaCl	5 g.	—	—
Sodium pyruvate	—	10 g.	10 g.
Lithium chloride	—	5 g.	5 g.
Glycine	—	12 g.	12 g.
Agar	15 g.†	20 g.	15 g.†
1 % phenolphthalein diphosphate	10 ml.	10 ml.	10 ml.
Horse serum	50 ml.	—	50 ml.
Distilled water to	1000 ml.	1000 ml.	1000 ml.

*Oxoid brand. †Difco brand.

Further media were constituted as follows: TYG was TY + 1 % glucose, BPG was BP + 1 % glucose, BPS was BP + 5 % horse serum, TYM was TY + 1 % mannitol.

NOTE. The Oxoid CM275 medium is based on Baird-Parker's (1962) description.

Examination of cultures

After incubation the plates were exposed to ammonia vapour and the bright pink colonies counted. All, or a sample, of these were subcultured for coagulase testing; coagulase-positive strains are referred to as *Staphylococcus aureus*. The total number of *S. aureus* colonies on the plates was estimated from the proportion of the sample found to be coagulase-positive.

RESULTS

In each experiment we had the results from the parallel exposure of one or more test media and one or more control plates on each of a series of days. The total number of colonies from any series of test plates was compared with that from the series of an equal number of control plates exposed simultaneously. Totals were also derived for *S. aureus* colonies. Thus in Expt. 1 (Table 2) seventeen plates of TYM medium were exposed on a total of 5 days and incubated anaerobically at 37° C. for 48 hr.; an equal number of control TY plates were exposed simultaneously and incubated aerobically at 37° C. for 18–20 hr. On the latter plates we counted a total of 44,284 colonies of which 1802 were estimated to be *S. aureus*. The counts of total colonies and *S. aureus* on the TYM plates were 60 and 93 % respectively of the control counts.

Table 2. *Comparison of various selective media for air samples*

Expt. no.	Test conditions*: Medium (see Table 1)	Incubation: Temp. °C.	Incubation: Time hr.	No. days of test	No. plates	Total colonies: On TY control†	Total colonies: On test as % of control	*Staphylococcus aureus*: On TY control†	*Staphylococcus aureus*: On test as % of control
1	TYM	37	48	5	17	44,284	60	1,802	93‡
	TYM	41	48	5	17	45,530	31	1,854	77‡
2	TY	41	48	7	22	61,688	22	243	97‡
	TYG	41	48	7	22	61,688	25	243	96
3	TYG	41	48	10	28	83,301	31	447	81
	BP	41	48	10	28	83,301	17	447	54‡
	BPG	41	48	10	28	83,301	16	447	45‡
4	TYG	41	48	9	28	55,733	34	161	96
	BPS	41	48	9	28	55,733	34	161	104
	MBP	41	48	9	28	55,733	28	161	103
5	TYG	41	24	6	24	50,071	22	824	92
	BPS	41	24	6	24	50,071	19	824	78‡
	MBP	41	24	6	24	50,071	23	824	76‡
6	TYG 8 hr. exp.	41	24	18	44	101,028	26	2,872	90
	TYG 16 hr. exp.	41	24	18	44	139,410	34	5,752	103

* All test media were incubated anaerobically.

† Control TY plates were incubated aerobically at 37° C. for 18–20 hr.

‡ *S. aureus* colonies were small and the phosphatase reactions difficult to read on these media.

Experiment 1 showed that with the mannitol agar (TYM) plates there was a substantial advantage from anaerobic incubation at 41° C. for the suppression of the general air flora; however, the *Staphylococcus aureus* colonies, especially on the 41° C. plates, were very small. In Expt. 2 both the media tested gave a good numerical yield of *S. aureus* but the colonies on the control (TY) medium incubated at 41° C. were very much smaller than those on the glucose-containing (TYG) medium incubated at 41° C.

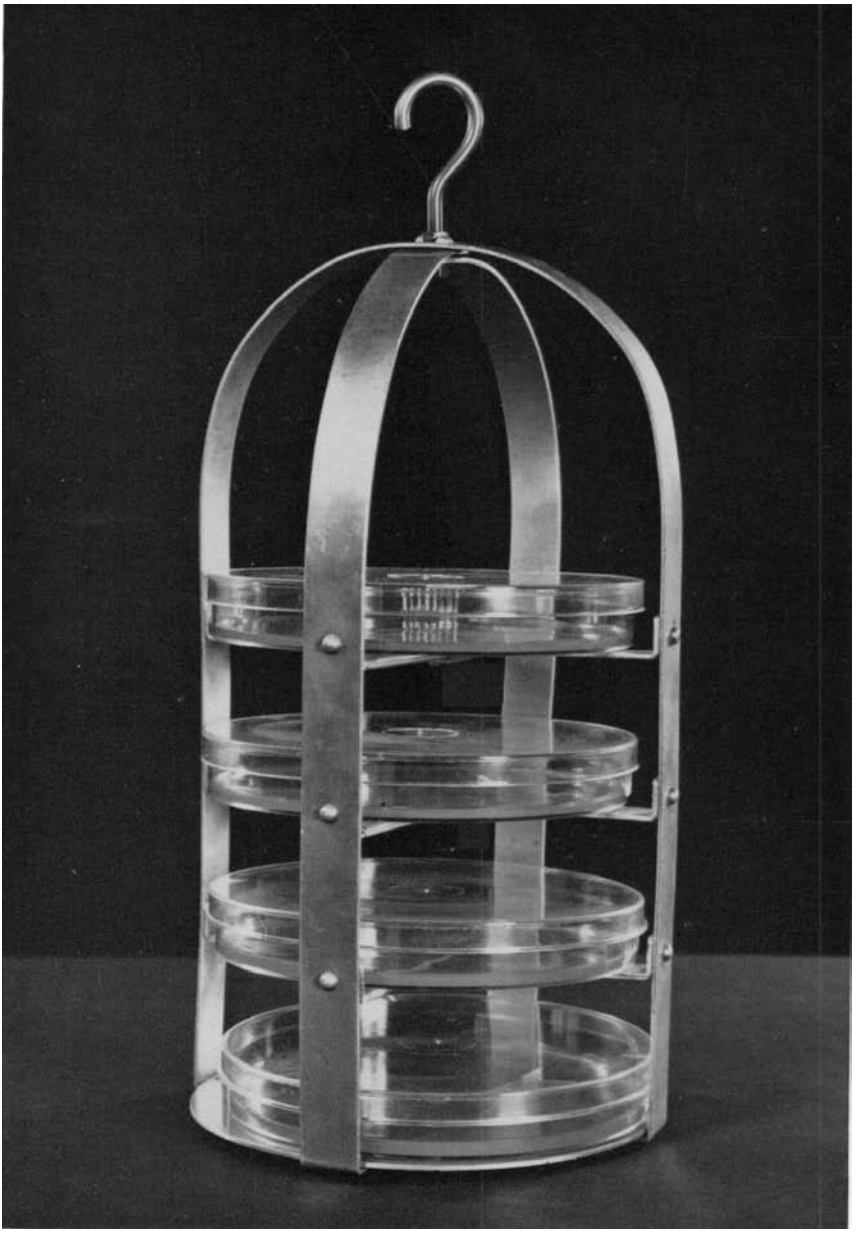
Various experiments were carried out with Baird-Parker's medium, alone, enriched with glucose or serum, or modified as shown in Table 1 (Expts. 3–5). Although in some cases the results appear from the counts to have been satisfactory, the phosphatase reaction was often difficult to read, and there were unexplained variations in the proportion of the *S. aureus* colonies that grew on the medium, as is seen in the comparison of the three experiments cited in Table 2.

The TYG medium incubated at 41° C. anaerobically for 48 hr. seemed to yield, reasonably consistently, *S. aureus* counts at about 90% of the number on the control plates (TY incubated aerobically), while reducing the total bacterial count to around 20–30% of the control count. Further experiments (Expts. 5, 6) indicated that 24 hr. incubation was equally good.

In Expts. 1–5 all the plates were exposed in the wards for 12 hr. A final test was made in which the plates exposed for 8 hr. were compared with those exposed for 16 hr., the latter being a convenient 'overnight' exposure time. As will be seen, the yield of *S. aureus* on the 16 hr. TYG plates incubated anaerobically at 41° C. for 24 hr. was virtually the same as that on the control TY plates (incubated at 37° C. aerobically for 24 hr.); on the 8 hr. plates the *S. aureus* count was slightly lower than on the corresponding control plates. The total colony count was less reduced on the 16 hr. plates than on the 8 hr. plates.

COMMENT

The enumeration of *Staphylococcus aureus* in the air of hospital wards may be of some value for routine monitoring in hospital hygiene (Alder & Gillespie, 1964) and also in the exploration of the hygienic consequences of particular sorts of hospital design or practice (Lidwell *et al.* 1966; Williams, 1967). A truly selective medium for collection of *S. aureus* from the air would be a great advantage for either of these uses, but none of the selective media devised in the past has been found suitable. The method described here, involving the use of a relatively simple medium enriched with 1% glucose and 5% horse serum incubated anaerobically at 41° C., appears to us to be a distinct advance over the previous methods. Phosphatase-positive colonies are not by any means always easy to distinguish, and a method that substantially reduces the number of colonies that have to be scanned certainly eases the reading of the plates. The technique described has now been in regular use in a survey in the isolation ward of St Mary's Hospital for a period of several months and has greatly reduced the labour and time involved in searching the culture plates for *S. aureus*.



LYNN HARDING AND R. E. O. WILLIAMS (*Facing p.* 39)

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SUMMARY

A tryptone–yeast extract medium enriched with glucose and serum incubated anaerobically at 41° C. was found to give a good yield of *Staphylococcus aureus* from air samples while suppressing the growth of 70–80 % of the other airborne bacteria.

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EXPLANATION OF PLATE

Plate holder used for the simultaneous exposure of four culture plates. One of the vertical strips moves laterally to facilitate insertion of the plates and removal of the lids.