Cloxacillin-Resistant *Staphylococcus aureus* in a Children's Hospital

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SUMMARY

A group of cultures of cloxacillin-resistant *Staphylococcus aureus* was collected over a period of 6 months. The method of determination of resistance to cloxacillin is described. The use of the disc diffusion test for cloxacillin was found to be unsatisfactory.

The incidence of cloxacillin-resistant *Staph. aureus* at the Red Cross War Memorial Children's Hospital (inpatients as well as outpatients) is slightly less than 2% of all isolates of *Staph. aureus*. Of the total non-White ward patients with penicillin-resistant *Staph. aureus* infections, 6,5% have staphylococci resistant to cloxacillin.

Reaction of these isolates to Fucidin, clindamycin and erythromycin is described.

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During the second half of 1973 an attempt was made to investigate all cultures of penicillin-resistant *Staphylococcus aureus* isolated in the laboratory of the Red Cross War Memorial Children's Hospital. Strains resistant to methicillin and cloxacillin were sought and their incidence determined.

Annear[1] demonstrated that the incubation of cultures of *Staph. aureus* at temperatures below 37°C accentuated the difference between sensitive and resistant strains for methicillin and to a lesser extent for other antibiotics.

Hewitt *et al.*[2] examined a group of borderline cultures of which the sensitivity to methicillin had been in doubt. They performed disc sensitivity tests at 30°C and 37°C, as well as mean inhibitory concentration tests at 30°C and 37°C, and found that by the use of tests at 30°C they were able to divide their group of borderline cultures into clear-cut sensitive and insensitive groups. Their conclusions were that (*a*) disc diffusion tests for cloxacillin were unsatisfactory; (*b*) disc diffusion tests for methicillin at 30°C were satisfactory; and (*c*) the mean inhibitory concentration (MIC) test at 37°C for methicillin read at 48 hours gave equivalent results to the MIC test at 30°C for methicillin read at 18 hours, and both these gave equivalent results to the MIC test at 30°C for cloxacillin read at 48 hours.

Hallander *et al.*[3] also investigated sensitivity testing with methicillin at 30°C and 37°C and found that incubation at the lower temperature gave more accurate results.

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It thus becomes evident that if a strain of *Staph. aureus* can be demonstrated to be resistant to methicillin, it can be inferred that it will also be resistant to cloxacillin.

METHODS

Disc Diffusion Test

Methicillin 10-μg blotting paper discs, 6 mm in diameter, were prepared and dried *in vacuo* at 4°C. Fresh batches of discs were prepared every 3 - 4 weeks and stored with a desiccant at 4°C. Cloxacillin 5-μg blotting paper discs 6 mm in diameter were supplied by Beecham Research Laboratories together with a supply of methicillin. The disc diffusion tests were set up with Oxoid Blood Agar Base No. 2 and zone sizes were compared with those of an Oxford *Staphylococcus* used as a control with each batch. Cultures were incubated for 18 hours. The zone diameters stated are the total inhibitory zone diameters minus the diameters of the discs.

Mean Inhibitory Concentration Tests

Plate method: Nutrient agar plates (Oxoid Blood Agar Base No. 2) were prepared containing the appropriate concentration of methicillin. These were spot-inoculated with 0,02 ml (*c.* 10^6 organisms) of a fresh broth culture of each organism being tested. Controls with Oxford *Staphylococcus* were set up with each batch of tests.

Tube method: Rows of tubes containing 1-ml quantities of broth (Oxoid Nutrient Broth No. 2) and progressively doubling concentrations of antibiotic were set up. These were inoculated with 0,02-ml quantities of the broth cultures of test organisms as described above. Oxford *Staphylococcus* controls were set up with each batch. Sets of tubes containing either methicillin or cloxacillin were incubated at 30°C and 37°C. They were read after both 18 hours' and 48 hours' incubation.

Laboratory Investigations

As the cultures of penicillin-resistant *Staph. aureus* were isolated they were subcultured and disc diffusion tests for methicillin at 30°C were carried out. If zones of 5 mm or less were obtained, the test was repeated to exclude the possibility of an unsatisfactory disc, which did on occasion occur. In the event of an organism showing resistance to methicillin by the disc technique on two occasions, the culture was retained and, when a batch of such cultures

TABLE I. RESULTS

Specimen No.	Coagulase tube method	Disc sensitivity tests (18 h): Methicillin 10-μg discs — zone diameter in mm, 30°C	Disc sensitivity tests (18 h): Methicillin 10-μg discs — zone diameter in mm, 37°C	Disc sensitivity tests (18 h): Cloxacillin 5-μg discs — zone diameter in mm, 30°C	Disc sensitivity tests (18 h): Cloxacillin 5-μg discs — zone diameter in mm, 37°C	Mean inhibitory concentration tests: Methicillin (30°C) plate method—concentration (μg/ml), 18 h	Methicillin (30°C) plate method—concentration (μg/ml), 48 h	Methicillin (30°C) tube method—concentration (μg/ml), 18 h	Methicillin (30°C) tube method—concentration (μg/ml), 48 h	Methicillin (37°C) tube method—concentration (μg/ml), 18 h	Methicillin (37°C) tube method—concentration (μg/ml), 48 h	Cloxacillin (30°C) tube method—concentration (μg/ml), 18 h	Cloxacillin (30°C) tube method—concentration (μg/ml), 48 h	Cloxacillin (37°C) tube method—concentration (μg/ml), 18 h	Cloxacillin (37°C) tube method—concentration (μg/ml), 48 h	
Oxford staph.	+	17	20	24	24	1,6	1,6	1,6	3,1	1,6	3,1	0,8	0,8	0,8	0,8	
13136	+	0	8	15*	17	100	100+	100+	100+	25	100+	100	100+	3,1	25	Methicillin-resistant strains obtained from Colindale, London
9463	+	0	10	19	19	50	100+	100+	100+	12,5	100+	12,5	100+	1,6	25	
7270	+	0	10	22	18	100	100+	100+	100+	12,5	100+	25	100+	1,6	25	
11164	+	0	0	0	9*	100+	100+	100+	100+	100	100+	100+	100+	100+	100+	
22747	+	0	9	24	19	100	100	100+	100+	25	100+	12,5	25	0,8	6,25	
23425	+	0	8	21*	21	100	100+	100+	100+	50	100+	25	100+	1,6	25	Cultures from patients at Red Cross Children's Hospital
24489	+	0	8	22	19	100	100+	100+	100+	25	100+	12,5	25	0,8	6,25	
25316	+	0	10	20	20	100	100+	100+	100+	25	100+	25	50	3,1	6,25	
25540	+	0	9	22	19	100	100+	100+	100+	25	100+	25	100+	1,6	6,25	
26006	+	0	13	22	21	100	100+	100+	100+	25	100+	25	100	0,8	50	
Oxford staph.	+	17	20	24	24	1,6	1,6	1,6	3,1	1,6	3,1	0,8	0,8	0,4	0,4	
30195	+	0	11	20*	20	100+	100+	100+	100+	12,5	100+	100	100+	3,2	100	
31359	+	0	10	22	20	100+	100+	100+	100+	25	100+	50	100+	3,2	50	Cultures from patients at Red Cross Children's Hospital
31793	+	0	10	19*	19	100+	100+	100+	100+	25	100+	25	100+	3,2	50	
32140	+	0	12	21	22	100+	100+	100+	100+	12,5	100+	25	100+	3,2	50	
32137	+	0	7	21	21	100+	100+	100+	100+	25	100+	25	100+	3,2	50	
33464	+	0	10	19	19	100+	100+	100+	100+	25	100+	100	100+	12,5	50	
Oxford staph.	+	11	16	24	24	1,6	3,1	3,1	3,1	3,1	3,1	0,4	0,4	0,4	0,4	
34542	+	0	0	24	22	50	100	100+	100+	12,5	100+	6,25	100+	0,8	100+	
35223	+	0	8	0	17	100	100+	100	100+	12,5	100+	6,25	100+	0,8	100+	
35497	+	0	0	19	19	100	100+	100+	100+	12,5	100	6,25	100+	3,2	100+	Cultures from patients at Red Cross Children's Hospital
43522	+	0	0	21	19	100	100	100+	100+	50	100	12,5	100+	1,6	100	
43684	+	0	0	17	17	100	100	100+	100+	25	100+	25	100+	3,2	100	
44169	+	0	0	19	19	100	100+	100+	100+	50	100+	100+	100+	12,5	100	
45239	+	0	0	6	19	100	100+	100+	100+	50	100+	100+	100+	6,25	100	
45486	+	0	0	19	19	100	100+	100+	100+	50	100	100+	100+	12,5	100	
45816	+	0	0	19	19	100	100+	100+	100+	50	100+	100+	100+	25	100	
Oxford staph.	+	11	16	24	24	1,6	3,1	3,1	3,1	3,1	3,1	0,2	0,4	0,4	0,4	
39647	+	0	0	24	22	100	100+	100+	100+	25	100+	6,25	100+	0,8	50	
39835	+	0	0	24	22	100	100+	100+	100+	25	100+	50	100+	1,6	50	Cultures from patients at Red Cross Children's Hospital
42707	+	0	0	19	19	100	100+	100+	100+	25	100+	50	100+	1,6	100	
43198	+	0	0	19	19	100	100+	100+	100+	50	100+	50	100+	1,6	100	
42468	+	0	0	20	20	100	100+	100+	100+	100	100+	100+	100+	6,25	100+	
43285	+	0	0	24	20	100	100+	100+	100+	50	100+	12,5	100+	1,6	100+	
43469	+	0	0	19	24	100	100+	100+	100+	25	100+	3,2	100+	0,8	50	
Number resistant		32	29	3	1	32	32	32	32	25	32	22	32	2	28	MIC 25 — 100 unequivocally resistant
Number doubtful		—	3	5	3	—	—	—	—	7	—	5	—	3	—	MIC 12,5 doubtful

*Colonies within the zone of inhibition.

was obtained, they were submitted to disc diffusion tests with methicillin and cloxacillin, at both 30°C and 37°C, as well as MIC tests consisting of plate and tube methods for methicillin at 30°C, tube method for methicillin at 37°C, as well as tube method for cloxacillin at both 30°C and 37°C.

RESULTS AND DISCUSSION

Table I demonstrates the well-marked resistance of all the cultures to methicillin on disc sensitivity testing at 30°C and also shows the temperature-dependent difference in zone size of between 0 and 13 mm with methicillin. This pattern of difference in zone diameter was lacking with cloxacillin, for which the disc sensitivity tests were found to be unsatisfactory.

MIC tests with methicillin at 30°C after 18 hours' incubation as well as those at 37°C after 48 hours and those with cloxacillin at 30°C after 48 hours, all showed unequivocal evidence of resistance in all cultures.

Table II gives information regarding the type of specimen from which each culture was obtained, as well as brief clinical data. These patients are divided into three groups: (*i*) acute staphylococcal infections — 2 cases with pneumonia and empyema; (*ii*) patients contracting a hospital infection — 13 cases including neonatal tetanus, postoperative surgical cases, a burn case and a chronic enteritis case developing impetigo — illustrating how difficult it is to control hospital infection, especially in a children's hospital; (*iii*) 2 chronic debilitated patients probably admitted with cloxacillin-resistant staphylococci — including a case of kwashiorkor with otitis media and a case of pneumonia and possible pulmonary TB with otitis media. From 9 of the patients more than one specimen each was sent to the laboratory.

Incidence of Cloxacillin-Resistant Staphylococcus aureus

An analysis of cultures of penicillin-resistant *Staph. aureus* obtained is shown in Fig. 1, while an analysis of the patients producing resistant cultures is given in Fig. 2.

TABLE II. INFORMATION REGARDING SPECIMENS

Specimen No.	Patient's identification	Nature of specimen	Clinical data
13136			
9463			Methicillin-resistant strains
7270			from Colindale, London.
11164			
22747	1*	Swab scalp	Neonatal tetanus, skin lesion.
23425	2	Swab trachea	Neonatal tetanus, tracheostomy.
24489	1*	Swab skin	
25316	3	Swab trachea	Neonatal tetanus, tracheostomy.
25540	4	Swab wound	Nephrectomy, polycystic kidney.
26006	5	Swab wound	Gastrostomy, colonic interposition.
30195	6*	Sputum	Pneumonia.
31359	7*	Swab trachea	Neonatal tetanus, tracheostomy.
31793	6*	Sputum	
32140	7*	Swab trachea	
32137	6*	Sputum	
33464	8	Swab wound	Hirschsprung's disease.
34542	9	Swab burn	Burn case.
35223	10*	Swab eye	Volvulus developing skin lesions.
35497	10*	Swab skin	
43522	11*	Swab skin	Chronic gastro-enteritis developing impetigo.
43684	11*	Swab skin	
44169	12*	Swab ear	Pneumonia, ?pulmonary TB, chronic otitis media.
45239	12*	Swab ear	
45486	13*	Swab wound	Empyema.
45816	13*	Swab wound	
39647	14*	Swab skin	Post-tracheostomy, mass in trachea, carbuncle, skin lesion.
39835	14*	Swab carbuncle	
42707	15*	Swab skin	Chronic gastro-enteritis, skin pustules, staph. septicaemia.
43198	15*	Swab eye	
42468	16	Swab ear	Kwashiorkor with otitis media.
43285	11*	Swab skin	
43469	17	Swab trachea	Laryngeal mass with tracheostomy.

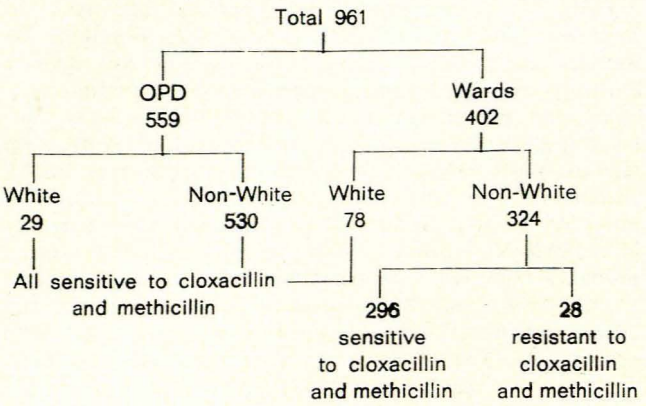

Fig. 1. Cultures of penicillin-resistant *Staph. aureus*.

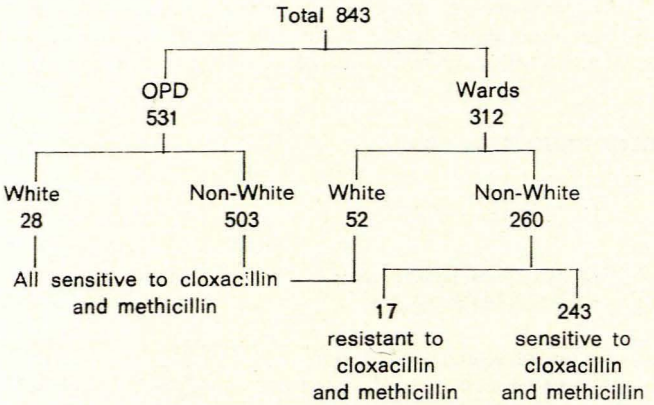

Fig. 2. Patients producing cultures of penicillin-resistant *Staph. aureus*.

From Fig. 1 it can be seen that 2,9% of the total cultures of penicillin-resistant *Staph. aureus* were resistant to cloxacillin; 3,3% of cultures of penicillin-resistant *Staph. aureus* from non-White patients were resistant to cloxacillin; and 7,0% of the total cultures of penicillin-resistant *Staph. aureus* from ward cases were resistant to cloxacillin.

As multiple specimens were received from a number of the patients, these figures alter on a patient basis (Fig. 2): 2,0% of the total patients with penicillin-resistant *Staph. aureus* infection had *Staph. aureus* resistant to cloxacillin; 2,2% of non-White patients with penicillin-resistant *Staph. aureus* infections had *Staph. aureus* resistant to cloxacillin; but the most noteworthy figure is that 6,5% of all non-White ward patients with penicillin-resistant *Staph. aureus* infections had staphylococci resistant to cloxacillin.

This group included patients with neonatal tetanus and burns, etc., and they were thus exposed to hospital infection for a prolonged period of time.

During the period of investigation penicillin-sensitive strains consisted of over 40% of the isolates of *Staph. aureus* in outpatients. If this is taken into account, the isolates of cloxacillin-resistant *Staph. aureus* probably consisted of slightly less than 2% of the total isolates of *Staph. aureus* in the laboratory.

In the UK Dyke *et al.*[4] found an incidence of methicillin-resistant *Staph. aureus* of 0,06% during 1960, and this rose to 0,97% in 1964. Also in the UK, Parker and Hewitt[5] found an incidence of 4,11% during 1969. Much higher figures have been reported from various countries in Europe.[6]

The advisability of retaining penicillinase-resistant penicillin for penicillin-resistant *Staph. aureus* infections is stressed, in the hope that the incidence of cloxacillin-resistant strains in the general population will increase as slowly as possible. The treatment of infections for an adequate period of time is also important.

Sensitivity to Other Antibiotics

Disc tests for sensitivity to other antibiotics, including Fucidin, clindamycin and erythromycin, were carried out on each of the 32 cultures resistant to cloxacillin. The following results were obtained:

No. 13136	This strain from the UK was sensitive to erythromycin, Fucidin and clindamycin.
No. 11164	This strain from the UK was sensitive to Fucidin but resistant to erythromycin and clindamycin.
No. 25540	This strain was sensitive to each of the three antibiotics.
No. 35223 No. 35497	Both cultures, from the same patient, showed sensitivity to all three antibiotics.

All the other cultures showed resistance to erythromycin and sensitivity to Fucidin and clindamycin.

Fucidin and clindamycin should not be widely used for staphylococcal infections, but should be reserved for selected cases, otherwise it is likely that a strain of *Staph. aureus* resistant to these antibiotics may replace the sensitive strains.

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