responded to bed rest, diuretics, and reduced food intake. Unexpected death in malnourished children during refeeding has been reported many times.¹⁴⁻¹⁶

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"Methicillin-resistant" *Staphylococcus aureus*: reassessment by controlled trial in burns unit

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Summary

A controlled trial of oral flucloxacillin (250 mg six-hourly for four days) was performed in 34 patients treated by the covered method whose burns had yielded a heavy or moderate growth of *Staphylococcus aureus* resistant to methicillin at 30°C but moderately sensitive at 37°C. *Staph aureus* was eliminated in nine of the 17 patients treated with flucloxacillin but in none of the 17 controls; the proportion of patients from whose burns sensitive *Staph aureus* was eliminated in an earlier trial of cloxacillin was greater than this. Strains of *Staph aureus* commonly described as methicillin-resistant and showing heterogeneous growth at 37°C of many sensitive and very few resistant bacterial cells should, in the light of these findings, be called moderately sensitive to flucloxacillin. Such "heteroresistant" strains showed consistent moderate sensitivity in replicate diffusion sensitivity tests at 37°C, but very inconsistent results in replicate dilution tests, especially with flucloxacillin. These studies showed that 18-hour diffusion sensitivity tests indicate the clinical value of treatment with flucloxacillin for staphylococcal infections of moderate severity more correctly at 37°C than at 30°C.

Introduction

Cultures of *Staphylococcus aureus* are usually tested for sensitivity to methicillin at 30°C, because many strains that appear sensitive at 37°C are found to be resistant at 30°C. The resistant cultures include a usually very large proportion of bacterial cells that are sensitive to methicillin at 37°C but resistant at 30°C.¹⁻⁶

As the site of infection is usually at or above the normal body temperature, the use of a test at a lower temperature (30°C) is anomalous. Moreover, many "heteroresistant" strains of *Staph aureus*, which appear sensitive to methicillin when tested at 37°C but resistant when tested at 30°C, seem, on diffusion tests, to be sensitive or relatively sensitive to cloxacillin and flucloxacillin at both 30°C and 37°C. The irregularity of results on testing such strains for sensitivity to cloxacillin has led to the further anomaly of using methicillin as the sensitivity test agent when cloxacillin or flucloxacillin are the antibiotics used for treatment.⁶

Increasing proportions of *Staph aureus* isolated from burns in our unit (57% in 1972) have been found to be resistant to methicillin when tested by a ditch plate diffusion test at 30°C.⁷ Controlled trials soon after the introduction of methicillin and cloxacillin showed that in about 80% of patients sensitive strains of *Staph aureus* were eliminated from burn sites covered with dressings after a course of subcutaneous methicillin⁸ or five days' oral treatment with cloxacillin.⁹ To assess the clinical sensitivity or resistance of strains of *Staph aureus* described as methicillin-resistant by standard tests at 30°C, we performed a controlled trial of oral flucloxacillin in patients whose burns were colonised by such staphylococci and were covered with dressings.

Method

The burns of patients in the burns unit were routinely examined¹⁰ for bacterial flora at every change of dressing. Strains of *Staph aureus* picked from horse blood agar plates and identified by a tube coagulase test were examined by a ditch plate test for their sensitivity to methicillin at 30°C and 37°C, with 25 µg of the antibiotic per ml of nutrient agar in the ditch. A few strains were also tested by a tube dilution method with methicillin and flucloxacillin at 30°C and 37°C; 0.02 ml of a 1/250 dilution of an overnight broth culture was used as the inoculum. They were also tested by a disc diffusion test, using Mast discs of methicillin (10 µg) and flucloxacillin (5 µg) at 30°C and at 37°C and a 1/250 dilution of an overnight broth culture as the plate inoculum. The tests were read after 18 hours' incubation; tests at 37°C were reincubated at 37°C and read again after 42 hours. The medium used for the tests was horse blood agar.

None of the patients studied had signs of clinical infection. Their burns were treated with dressings and were found to be heavily or moderately colonised with *Staph aureus* that was resistant to methicillin at 30°C, as shown by growth across the ditch, and of reduced or

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moderate sensitivity at 37°C, as shown by a zone of inhibition smaller than that of fully sensitive strains. If eligible on clinical grounds, they were allocated alternately to treatment groups in which they received either (a) four days' course of oral flucloxacillin (250 mg six-hourly by mouth in adults) or (b) no specific antimicrobial treatment (controls). Only covered burns were included in the trial, because in earlier trials^{8,9} burns treated by exposure showed less effective clearance of *Staph aureus* in response to a course of cloxacillin or methicillin than those that were covered with dressings. Any patients in the control group who, on clinical grounds, needed chemotherapy and those in the flucloxacillin treatment group who needed a different antibiotic were taken out of the trial and given the appropriate treatment.

Results and comment

Altogether 5302 isolates of *Staph aureus* from burns were tested by the ditch plate method, and 2476 were found to be moderately sensitive at 37°C but resistant at 30°C (heteroresistant strains); no strains were resistant at both 30°C and 37°C. With methicillin, disc sensitivity tests (four replicates) on each of the two heteroresistant strains showed zones of inhibition in the range 3.5–4.8 mm at 37°C and no inhibition at 30°C. With flucloxacillin, zones of inhibition were in the range 6.3–8.0 mm at 37°C and 4.8–7.3 mm at 30°C. Tube dilution tests with methicillin and cloxacillin at 30°C showed these strains to be resistant to both antibiotics at 30°C (minimum inhibitory concentrations (MICs) were mainly 64–256 mg/l for methicillin and 64 mg/l for flucloxacillin, though some individual tests showed very low MICs). Because of such variability and because the resistance shown in most of the tube dilution tests reflects the character of a small proportion of the bacteria at 37°C, this method seems to be less satisfactory and to have less relevance to clinical sensitivity than disc or ditch diffusion tests, which agree with the results of treatment (see below) in showing the heteroresistant strains to be moderately sensitive to flucloxacillin. The basic resistance—that is, concentration of the antibiotic tolerated by 20% of the cells—as used by Benner and Kayser,¹¹ avoids this difficulty but could not be used as a routine test.

The table shows the results of the controlled trial of flucloxacillin for *Staph aureus* moderately sensitive at 37°C and resistant at 30°C. In the 17 patients treated with flucloxacillin 10 out of 21 burn sites were cleared of *Staph aureus*, while none of the 18 burns in the controls were cleared of *Staph aureus* ($\chi^2=9.16$; $P<0.01$). Nine of the 17 patients treated with flucloxacillin and none of the 17 controls lost their staphylococci ($\chi^2=9.67$; $P<0.01$).

Results in controlled trial of flucloxacillin in patients whose burns were colonised by methicillin-resistant *Staph aureus* compared with those of an earlier trial of cloxacillin for methicillin-sensitive infections⁹

	No of patients	No of burns	No of covered burns from which <i>Staph aureus</i> was:		
			Eliminated	Reduced	Eliminated or reduced
Flucloxacillin:					
Treated group ..	17	21	10 (48%)*	3	13 (62%)‡
Control group ..	17	18	0	3	3 (17%)‡
Cloxacillin ⁹ :					
Treated group ..	14	17	14 (82%)+	3	17 (100%)§
Control group ..	16	17	3 (18%)+	2	5 (29%)§

* $\chi^2=9.16$; $P<0.01$. + $\chi^2=11.76$; $P<0.001$. ‡ $\chi^2=6.44$; $P<0.02$. § $\chi^2=15.58$; $P<0.001$.

The results of a similar⁹ trial of cloxacillin (500 mg six-hourly for five days) in patients with *Staph aureus* in covered burns, which was carried out when the antibiotic was new, are also shown in the table; all strains were found to be sensitive, and a larger proportion of burns (82%) was cleared of *Staph aureus* than in this trial. Nevertheless, the difference between the results of these trials is possibly less than the percentage clearance from antibiotic-treated cases would suggest, because three of the 17 burns in the control patients of the earlier trial lost their staphylococci, whereas none of the burns of the controls were cleared in this trial. Results similar to those in the cloxacillin trial were obtained in an earlier controlled trial of methicillin given by intramuscular injection, in which 32 out of 40 (80%) covered burns were cleared of *Staph aureus*.⁸

Comparability of groups in trial—The 10 males and seven females treated with flucloxacillin had a mean estimated area of burn of

16.5% (range 0.7–41.5%) and a mean age of 27.1 years (range 5 months–59 years). The corresponding means for the seven male and 10 female controls were 15.5% (range 0.5–42%) and 42.9 years (range 8 months–85 years).

Discussion

The incidence of methicillin-resistant *Staph aureus* increased during and after the 1960s, reaching proportions as high as 40–50% of the strains isolated in some hospitals.^{7,11–13} The potential clinical importance of such strains has been established, but there has been little evidence to show that methicillin and its analogues are ineffective in treating human infections caused by these strains, although the term “methicillin-resistant” (and, by implication, also “cloxacillin- and flucloxacillin-resistant”) would suggest this. Discouraging results have been reported in treating experimental infections with such strains in animals,^{14,15} and opinions vary on the potential response to treatment of human infections with such strains.^{11,16} It is well known that staphylococci described as resistant on the basis of tests at 30°C with methicillin was generally found to be sensitive to methicillin (at least by diffusion tests) and even more sensitive to cloxacillin and flucloxacillin when the tests are done at 37°C and read after 18 hours' incubation. At 37°C there are usually very few resistant cells in a predominantly sensitive culture. Cloxacillin or flucloxacillin, rather than methicillin, however, are the antibiotics in this group most often used for treatment, and the temperature of the infected lesion is likely to be about 37°C or above. Therefore tests with these antibiotics at 37°C, when consistent, must give a closer indication of their probable therapeutic value (except, probably, in septicæmia and other very severe infections) than the standard methicillin sensitivity tests at 30°C.

This view is supported by the results of our controlled trial of flucloxacillin. The clearance of methicillin-resistant *Staph aureus* from 10 out of 21 burns (in nine out of 17 patients) after four days' treatment with flucloxacillin, while no such organisms were cleared from any of the 18 burns in the 17 control patients, suggests that these strains should be called “moderately sensitive to flucloxacillin” rather than “methicillin-resistant.” The results were apparently not quite as good as those found in our earlier trials of methicillin and cloxacillin against methicillin-sensitive strains. But this difference is consistent with the difference in results of in-vitro diffusion sensitivity tests at 37°C on methicillin-sensitive and methicillin-resistant strains, in which the latter were shown to be sensitive but usually less so than the former.

The tube dilution method showed large discrepancies between replicate tests with flucloxacillin carried out on different occasions, presumably because different inocula had contained different proportions of flucloxacillin-sensitive and flucloxacillin-resistant bacteria. There was more consistency in tube dilution tests on such strains with methicillin, though MICs of methicillin were misleading in relation to flucloxacillin treatment, as they showed the strains to be resistant. There was diminished growth on two or more doubling dilutions beyond the MIC in most of the tube dilution tests. Dilution tests are generally regarded as giving the most useful in-vitro evidence of probable clinical sensitivity or resistance of an infecting organism, but this does not apply in the case of flucloxacillin, either at 30°C or at 37°C, in which the resistance indicates the character of a (usually) very small proportion of the bacteria present. Diffusion tests, on the other hand, provide consistent results, by which the heteroresistant strains are shown to be moderately sensitive, as they are in their response to systemic treatment with flucloxacillin. Though the resistant component of heteroresistant cultures may grow in the presence of flucloxacillin at concentrations that exceed the usual peak blood concentration, they grow more slowly in the presence of methicillin and its analogues than the sensitive bacteria,¹⁷ and they are likely to be at a disadvantage in competition with more vigorous

organisms colonising the wound and also, perhaps, with the natural defences.

Clinical sensitivity tests are required to give information on the possible effectiveness of treatment with an antibiotic, and for this purpose 18-hour diffusion tests with flucloxacillin at 37°C might be adequate. These would also detect the emergence of strains of *Staph aureus* highly resistant to flucloxacillin at 37°C—a type of resistance which we have not yet encountered but for which the microbiologist must be vigilant. Benner and Kayser¹¹ found an increasing proportion of clinical isolates of *Staph aureus* in which most cells in the culture were resistant to methicillin and its analogues, and infection with such strains was less responsive to treatment with these antibiotics. The heteroresistant state is best shown by parallel sensitivity tests with methicillin at 30°C and at 37°C, or by 18- and 42-hour methicillin sensitivity tests at 37°C, which would also show the reduced sensitivity of these strains at 37°C and detect the emergence of a strain highly resistant at 37°C. Methicillin sensitivity tests done only at 30°C would fail to detect the emergence of such highly resistant strains, as well as failing to recognise the potential value of flucloxacillin and its analogues in treatment of infections by heteroresistant strains of *Staph aureus*.

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A small cluster of Hodgkin's disease

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Summary

Nine cases of Hodgkin's disease and one of acute lymphoblastic leukaemia occurred in 1972-5 in an area of less than 1 km². Clinical, social, and drug histories provided no relevant information, and definite patient-to-patient contacts before the onset of disease were not established. There was a higher incidence of malignant neoplasms in first-degree relatives of the patients than in those of a small control group living in the same area. There was no apparent reason for this cluster of cases, but geographical, climatic, and entomological studies are being carried out.

Introduction

Reports of cases of Hodgkin's disease¹⁻⁴ and leukaemia⁵⁻⁷ occurring in a pattern similar to that seen in infectious disease have stimulated interest in space-time clustering in all types of

lymphoreticular and haematological malignancies. We recently heard about a cluster of five patients with Hodgkin's disease living close to each other, and further investigation disclosed a further four cases of Hodgkin's disease and one of leukaemia within an area of less than 1 km² (population about 2800). All these patients presented within a period of four years. We describe here the clinical and epidemiological findings.

Patients and methods

Full clinical and histological details were available for all 10 patients. All were interviewed and epidemiological inquiries made. The immune status of four patients was assessed by in-vivo and in-vitro techniques.

Results

The incidence of Hodgkin's disease in the area studied was greater than 70 cases per 100 000 population per year, although the expected incidence (for the Sheffield metropolitan area) was 4-5 cases per 100 000 per year. The clinical and epidemiological details of the patients are summarised in the table and their homes are marked on the map. For reasons of confidentiality we have not given precise details of roadways.

The patients had no common features in their medical histories, and the incidence of glandular fever, tonsillectomy, and appendicectomy was not increased. The drug and social histories of the patients were unremarkable but the incidence of malignant neoplasms among first-degree relatives seemed to be higher than in a group of 30 patients matched for age and sex who were attending the local general practitioner's surgery. Among first-degree relatives of the controls there were seven malignant neoplasms (three gastrointestinal, two lung, one haematological, and one where the primary site was undetermined), while among those of the 10 patients studied there were six (four gastrointestinal, one lung, and one haematological).

No definite patient-to-patient contacts were elicited, despite the fact that several of the patients lived very near to one another. No

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