Bacteriological characters of strains of *Staphylococcus aureus* submitted to a reference laboratory related to methicillin resistance.

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SUMMARY

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains present an increasing clinical problem. Analysis of 2679 strains submitted to a reference laboratory in the first quarter of 1983 and 3050 strains submitted in summer 1984 showed 479 and 593 multi-resistant strains. The proportion of methicillin-resistant strains classified as epidemic rose from 5·9 to 10·2 %. Other methicillin-resistant strains continued to occur but other methicillin-sensitive multi-resistant strains appeared to fall. A strain with defined characters could be recognized in the Thames regions.

INTRODUCTION

Methicillin resistance in strains of *Staphylococcus aureus* was detected shortly after the introduction of the first penicillinase-resistant penicillin, methicillin (Jevons, 1961). Despite an apparent rise in frequency of resistant strains during the first few years after introduction of this group of drugs (Parker *et al.* 1974), possibly associated with widespread use of ampicillin, little clinically important resistance was detected in England until 1981, when there was increasing concern about spread of these resistant strains (Shanson, 1981).

The Public Health Laboratory Service set up a retrospective study (Cooke *et al.* 1986) to attempt to determine the extent, nationally, of this problem, and strains submitted for typing to the Staphylococcus Reference Laboratory (a part of the Division of Hospital Infection, CPHL) were examined to complement this survey. Public interest during 1984 led us to examine strains submitted for typing in the first quarter of 1983, a period ante-dating this interest, and to compare them with strains during the holiday period, June, July and August of 1984, when interest had subsided somewhat.

MATERIALS AND METHODS

Strains submitted to the Division of Hospital Infection (DHI) in the two periods for phage typing formed the source for this study. Phage typing was performed by the standard method (Parker, 1972), using the present international set of phages plus 8 experimental phages; 4 to subdivide strains typed by group V phages

(94/96) and phages 88A, 90, 83C and a phage numbered 932 introduced in 1981 to divide group III strains.

All strains were screened for antibiotic resistance, though the method of screening was changed early in 1984. In 1983 a disc screen, of susceptibility to penicillin, 1 i.u.; tetracycline, gentamicin, erythromycin, fusidic acid and methicillin, all 10 μg discs, was used. All were tested on nutrient agar at 30 °C, from the broth used for phage typing. In 1984 the screening was by a break point method. Plates of Isosensitest agar (Oxoid CM471) containing penicillin 0·06 μg/ml, tetracycline 1 μg, gentamicin 2 μg, erythromycin 2 μg, fusidic acid 8 μg, methicillin 8 μg and ciprofloxacin 0·5 μg were inoculated with a multipoint inoculator using a diluted broth culture. The plates were incubated overnight at 37 °C and read by a Mastascan system. Methicillin containing plates were incubated at 30 °C for 2 days and read to the end of visible growth in the traditional way.

Strains sensitive to these antibiotics and strains resistant only to penicillin or to tetracycline or to both from both periods were considered 'sensitive' and not studied further. Strains resistant to additional antibiotics were defined as 'resistant' and tested by a disc method (Porthouse, unpublished) against 27 antibiotics and antibacterials in 1983 or, after assessing the 1983 results, to 16 antibiotics in 1984. The results of antibiotic testing, phage typing and source for resistant strains were re-tabulated under the classifications described below.

RESULTS

In the first quarter of 1983, 2679 strains of *S. aureus* were submitted to DHI for phage typing of which 479 were classified as resistant on the screening criteria; in the summer of 1984, 3050 were submitted and 593 were classified as resistant.

Tabulation of the strains quickly suggested that they could be classified into four groups: (1) EMRSA – epidemic methicillin-resistant *S. aureus*; (2) OMRSA – other methicillin-resistant *S. aureus*; (3) ORSA – other resistant (methicillin sensitive) *S. aureus*; (4) OSA – the remaining strains (Table 1).

Phage typing results

EMRSA were recognized on phage typing characters. Most isolates typed weakly with phage 85 but some also typed, occasionally strongly, with phage 84. Characteristically EMRSA typed strongly with experimental phages 88A and 932, a phage isolated in 1981 from a methicillin-resistant strain. Consequently typability in EMRSA was high, with only 1 of 139 strains in the first quarter of 1983 recorded as non-typable (N.T.) though 31 of 321 strains from summer 1984 gave no response to the international set.

In contrast, typability of OMRSA was poor. In 1983 29 % of 86 strains, and in 1984 nearly 44 % of 119 strains were untypable. For comparison ORSA were untypable in 19·7 % of 254 strains and 13·7 % of 153 strains in the respective periods.

The conventions of staphylococcal phage typing state that when an adequate result is found at routine test dilution (RTD) the strain is not retyped at 100 RTD. The relevant experimental phages are placed on the 100 RTD plate and were not

Table 1. *No.* (%) *of strains classified into each resistance group*

	1983	1984
EMRSA	139 (5·19)	321 (10·52)
OMRSA	86 (3·21)	119 (3·90)
ORSA	254 (9·48)	153 (5·02)
OSA	2200 (82·12)	2457 (80·56)
Total	2679	3050

recorded therefore for strains typing well at RTD. As can be seen in Fig. 1, EMRSA were less well typed by the international set in 1984 than in 1983, particularly by phage 84. Consequently the frequency of recording of strong reactions to the experimental phages increased in the 1984 study period. The strains grouped as OMRSA and ORSA showed a variety of typing patterns. From Fig. 1 it can be seen that group II strains were poorly represented, particularly in the methicillin-resistant groups and group V (94/96) was also reduced in number even though the latter group appear to display somewhat increased methicillin resistance as an intrinsic character. Overall, resistant strains most often fell into groups I + III or group III in typing pattern, consistent with earlier reports (Parker *et al.* 1974).

Antibiotic resistance results

The percentage of strains resistant to 12 antibiotics in each resistant group is shown in Fig. 2 for each of the two 3-month periods. EMRSA showed the same rather distinctive pattern in both periods. Strains in this group were universally resistant to tetracycline, minocycline and streptomycin and showed constitutive macrolide resistance. About 70 % of strains were resistant to gentamicin and 30 % to chloramphenicol. Resistance was very rare to fusidic acid, neomycin, and even rarer to rifampicin. OMRSA and ORSA showed similar frequencies of resistance in 1983 but the reduced phage typability of EMRSA in 1984 clearly had resulted in mis-classification of some EMRSA strains as OMRSA. Subsequent re-testing of 26 strains included as OMRSA with the resistance pattern of EMRSA, with 1000 × RTD phages gave the typing pattern of EMRSA in 22. The non-epidemic resistant strains were less frequently resistant to the tetracyclines but were more likely to show inducible macrolide resistance, fusidic acid resistance and, notably, neomycin resistance. Some were resistant to bacitracin. The frequency of chloramphenicol resistance was significantly lower than in the EMRSA group. Interestingly a few strains showed novobiocin resistance. All strains were sensitive to vancomycin and all strains tested, to ciprofloxacin. A few strains in each group were resistant to rifampicin.

In this small series only two strains of ORSA showed resistance to tobramycin without resistance to gentamicin; kanamycin resistance was detected in all strains resistant to neomycin or gentamicin.

Sources of the strains

The Division of Hospital Infection CPHL receives strains for typing from diverse sources but because it acts as a local typing centre for many hospitals in the London area, most strains received originate in the four Thames regions. A measure of this
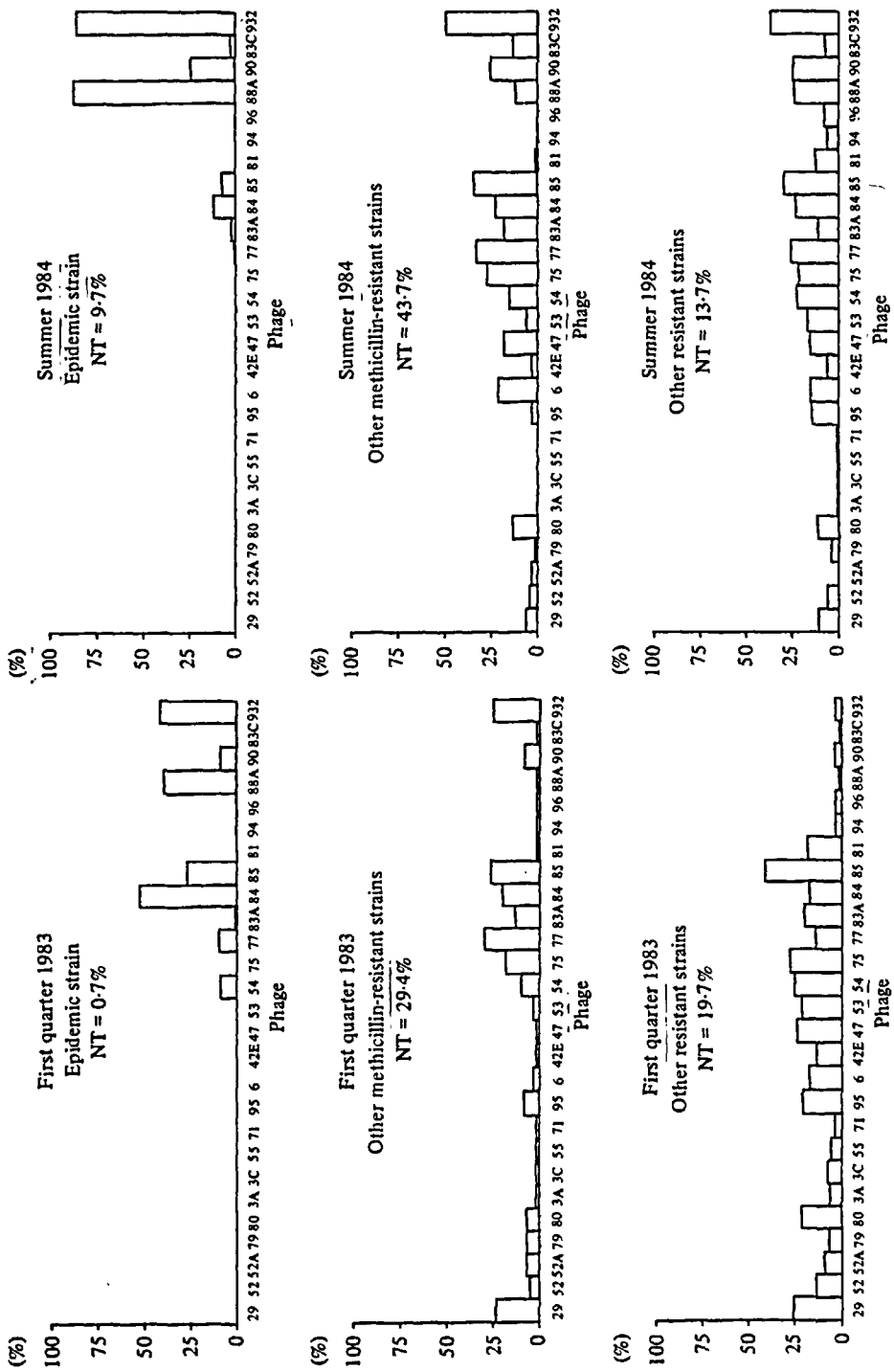
Fig. 1. Percentage of typable strains lysed by each phage. NT, not typable.
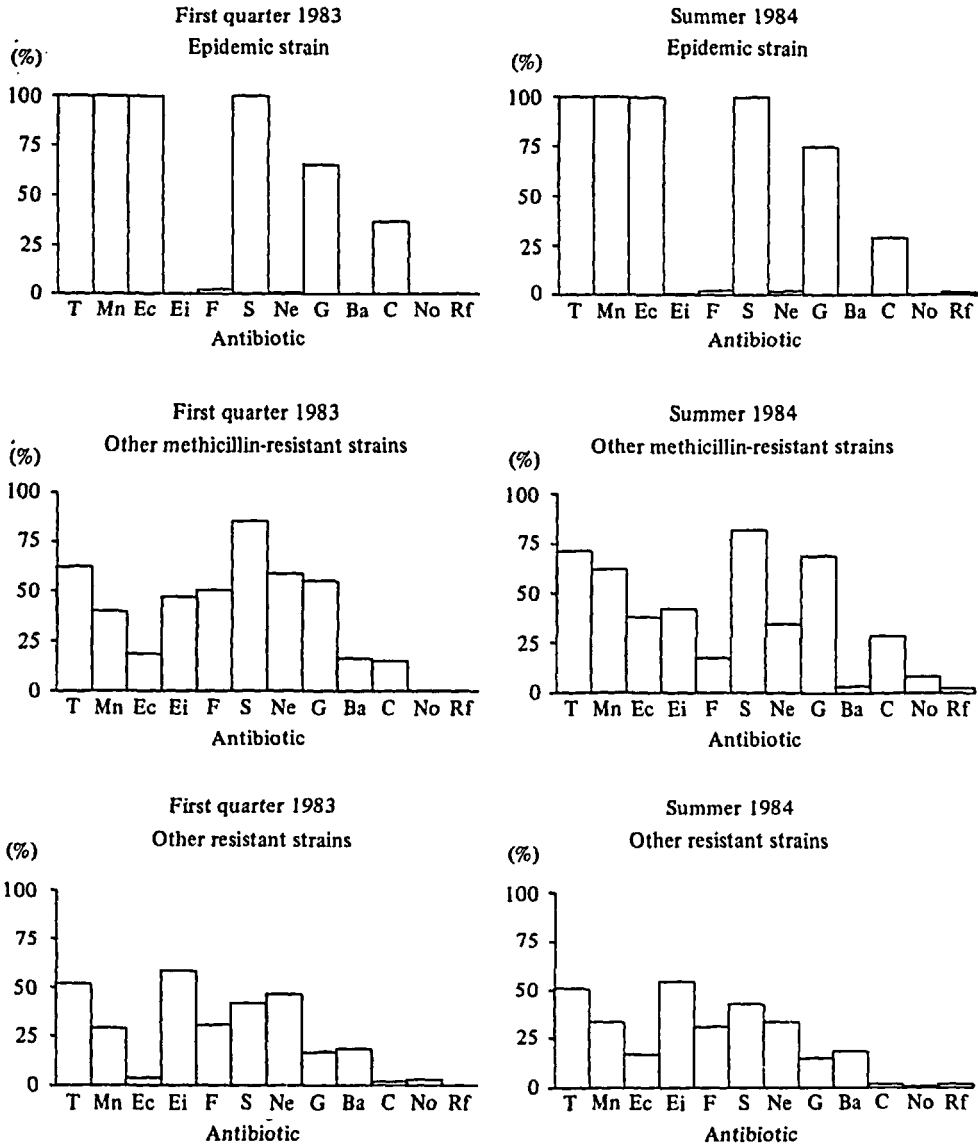
Fig. 2. Antibiotic resistances (%) of the three groups in the two time periods. T, tetracycline; Mn, minocycline; Ec, constitutive macrolide resistance; Ei, inducible macrolide resistance; F, fusidic acid; S, streptomycin; Ne, neomycin; G, gentamicin; Ba, bacitracin; C, chloramphenicol; No, novobiocin and Rf, rifampicin.

bias is that of 135 hospitals submitting strains during the study periods, 88 were in the four Thames regions. EMRSA showed a significantly more extreme distribution than the overall bias, being reported from 32 hospitals in the Thames regions with only 1 hospital referral from outside these regions, while OMRSA were submitted from 27 hospitals in the Thames regions and from 15 in other regions and ORSA from 29 and 31 respectively. There was therefore a significant excess of EMRSA from the Thames regions and a significant excess of ORSA from other

than the Thames regions; the latter finding probably reflecting the selection of unusual strains to be sent to a reference laboratory.

When the two study periods were compared it was noted that isolates of EMRSA were submitted from NE and NW Thames almost exclusively in the first quarter of 1983 but from all four Thames regions and Anglia in the summer of 1984 with the hospitals involved clearly delineating a larger area of occurrence than in 1983. Further work may clarify the time course of these changes.

Between the 1983 and 1984 periods EMRSA appeared to increase at the expense of ORSA while OMRSA remained steady. More than 80 % of strains were classified as OSA, i.e. sensitive or resistant only to penicillin, tetracycline or to both.

DISCUSSION

Strains of *S. aureus* resistant to several antibiotics have been recognized for many years and the changes in resistance and in the attitudes of microbiologists have been reviewed by Shanson (1981). Antibiotic resistance characters appear to add on to previously acquired resistances in hospital strains – the Barber effect (Parker *et al.* 1974) – either by new mutations, by selection of pre-existing genetic characters or by transfer of genetic information from the more resistant coagulase-negative staphylococci, principally *S. epidermidis*. The maintenance of antibiotic resistance characters, particularly if plasmid borne, is energetically expensive and counter-selective (Lacey & Chopra, 1975). The delayed emergence of first gentamicin-resistant strains, and then, very quickly, of methicillin and gentamicin-resistant strains was unexpected. In recent times apparent epidemics have been recorded in Newcastle (Selkon, Stokes & Ingham, 1980), Ireland (Cafferkey *et al.* 1983), Liverpool and, most well-documented in our sources, the London regions. The Australian epidemics have been extensively investigated, (Gedney & Lacey, 1982; Townsend, Ashdown & Grubb, 1985) and show considerable complexity.

This study was designed to compare strains of *S. aureus* submitted in two 3-month periods to a reference laboratory, the first quarter of 1983, slightly before MRSA publicity was apparent and the summer quarter of 1984, a period after the major publicity and a holiday period in which major investigations not driven by clinical needs were unlikely. In these results an epidemic of a strain of *S. aureus*, coincidentally methicillin resistant, here marked EMRSA, dominated the findings in the Thames NHS regions. This dominance possibly obscured other local epidemics in hospitals less forward in submitting strains to a reference laboratory than the London teaching hospitals. Further analyses and retesting of strains over a longer period than here reported are in progress; such studies may clarify the extent to which these results are representative of the national situation.

In the two 3-month periods, strains classified as EMRSA changed in phage-typing pattern from being readily typable with phages 84 and 85 to weak reactions with these phages although strong typing responses to two experimental phages persisted. Some of this change may be technical but similar changes have been described for other epidemic strains (Jevons & Parker, 1964). Antibiotic resistance of this strain showed little change between the two periods and was unusual in the inclusion of minocycline resistance and constitutive macrolide resistance as well as other characters. In 1983 this apparently epidemic strain was restricted to Essex

connections but by 1984 had spread widely in the London area. Retrospective and prospective monitoring of this strain and its distribution is in progress.

Other methicillin-resistant strains (OMRSA) in this study showed little evidence of inter-hospital spread but local epidemic within single hospital groups were apparent from both the phage typing and antibiotic resistance results. The extent to which this finding was affected by the selection of strains sent to a reference laboratory needs to be established. The problem of mis-classification of EMRSA strains as OMRSA was readily demonstrable, particularly in the 1984 period.

Methicillin-sensitive, resistant strains (ORSA) perhaps are the best test for bias in selection of strains sent to a reference laboratory. These strains showed a wider selection of phage patterns and antibiotic-resistance patterns than did EMRSA but were more comparable with OMRSA. The occurrence of apparently methicillin-sensitive variants of isolates otherwise indistinguishable from EMRSA was also noted.

The distributions and characters of the remaining strains (OSA) could also be analysed but this would involve a major investment in manpower so will take time.

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