

# Resistance of *Staphylococcus aureus* to sulphamethoxazole and trimethoprim

L. S. NAKHLA

*From the Cross-Infection Reference Laboratory, Colindale, London*

**SYNOPSIS** Strains of *Staphylococcus aureus* isolated from the lesions of hospital patients were surveyed for resistance to sulphamethoxazole and to trimethoprim. Of 675 strains tested, 18.5% were resistant to sulphamethoxazole and 1.6% to trimethoprim. All the trimethoprim-resistant strains were resistant to sulphamethoxazole and to a 1:20 mixture of the two drugs. Trimethoprim-resistant strains were on average more resistant to sulphamethoxazole than were trimethoprim-sensitive strains. They were all resistant to several other antimicrobial agents. Most of them had the phage-typing pattern 84/85, 84, or 85.

Trimethoprim, 2,4-diamino(2,3,5-trimethoxybenzyl)pyrimidine, and sulphamethoxazole came into general therapeutic use as a mixture in 1969 (Garrod and O'Grady, 1971). Favourable reports have appeared of their effect in chronic chest infections (Hughes, 1969) and in urinary-tract infections (Brumfitt, Faiers, Pursell, Reeves, and Turnbull, 1969; O'Grady, Chamberlain, Stark, Cattell, Sardeson, Kelsey, Fry, Spiro, and Waters, 1969). Garrod (1969) has suggested that they may be useful in the treatment of staphylococcal infections of the respiratory tract, and Craven, Pugsley, and Blowers (1970) advocated their use in cases of acute osteomyelitis due to penicillin-resistant staphylococci. Combined therapy with trimethoprim and sulphamethoxazole is now widely used in hospitals, and it therefore seemed desirable to find out whether a problem with resistance to these drugs was likely to appear. We therefore examined a collection of strains collected from septic lesions in hospital patients in the London area between September 1970 and October 1971.

## Materials and Methods

### STAPHYLOCOCCI

Each year since 1961, the Cross-Infection Reference Laboratory has received sets of 100 consecutively isolated cultures of *Staphylococcus aureus* from the lesions of patients in six to eight hospitals in the London area. The 1970-71 collection, from seven of

these hospitals, was used in the present survey. After the removal of duplicate isolates from the same patient, strains that may not have been obtained from staphylococcal lesions and others not qualifying for inclusion, we were left with 675 cultures. On receipt, the strains were subcultured from single colonies, stored on nutrient agar slopes at room temperature, and phage typed and tested for resistance to antibiotics in batches.

### SULPHAMETHOXAZOLE AND TRIMETHOPRIM SENSITIVITY TESTS

#### *Media*

Diagnostic sensitivity test agar (DST, Oxoid) with the addition of 5% lysed horse blood was used in tests for resistance to sulphamethoxazole and trimethoprim and to the mixture of the two drugs.

#### *Inoculum*

A 1 in 250 dilution of an 18-hour broth culture was used. This diluted culture when spread on a plate gave a growth which was just short of being confluent.

#### *Determination of the Minimum Inhibitory Concentration (MIC)*

Doubling dilutions of the drugs were incorporated into the medium, which was then poured in measured 25 ml amounts into 9 cm plastic petri dishes. The range of final concentrations, in  $\mu\text{g}$  per ml, were for sulphamethoxazole 3280 to 0.8 and for trimethoprim from 4 to 0.015. In tests with the mixture the ratio of trimethoprim to sulphamethoxazole was 1:20 and

the range was from 2 + 40 to 0.015 + 0.3 of the respective drugs. Drops (0.01 ml, about  $2 \times 10^4$  organism per drop) of the diluted inoculum were spotted on the plates of medium by means of a multiple inoculator, 25 cultures per plate. The plates were left with the lids open to dry for 30 minutes and then incubated at 37°C for 18 hours. The MIC was taken as the lowest concentration that completely inhibited the growth. All strains found to be resistant to 4 µg per ml of trimethoprim were retested in concentrations up to 256 µg per ml of trimethoprim.

#### Disk-diffusion sensitivity tests

The diluted cultures were spread on plates by means of cottonwool-tipped swabs (Garrod and Waterworth, 1971). Paper disks, 6 mm in diameter, containing the following concentrations per disk were applied: sulphamethoxazole 50 µg; trimethoprim 2.5 µg; sulphamethoxazole 25 µg + trimethoprim 0.15 µg. The plates were incubated for 18 hours at 37°C and the diameters of zones of inhibition were measured with calipers.

#### Antibiotic sensitivity tests

These were made on plates of nutrient agar, by means of Oxoid Multodisks; the plates were incubated overnight at 37°C. Tests for methicillin resistance were made separately, with single 10 µg disks, and were incubated at 30°C.

## Results

### RELATION OF MINIMUM INHIBITORY CONCENTRATION AND ZONE SIZE

The first 272 strains were examined in parallel by the plate dilution and the disk-diffusion sensitivity tests. The minimum inhibitory concentration of sulphamethoxazole for these strains covered a wide range (Fig. 1) and an arbitrary decision was taken to consider a value of 102 µg per ml or greater as an indication of resistance. With trimethoprim on the other hand, the cultures fell clearly into two groups: 269 for which the MIC was in the range of 0.12 to 0.5 µg per ml and three with an MIC of 4 or greater (Fig. 2).

Comparison with the results of the disk sensitivity test showed the following. All the strains classified as sulphamethoxazole sensitive (MIC ≤ 51 µg per ml) gave zones with a diameter of 18 mm or more, that is to say the organisms were inhibited for a distance of 6 mm or more from the edge of the disk (Table I). Most of the resistant strains grew right up to the disk, but a few, for which the MIC was 102-205 µg per ml, gave small zones with a total diameter of up to 14 mm. Trimethoprim-sensitive strains (MIC < 1.0 µg per ml) all gave zones 18 mm or more in

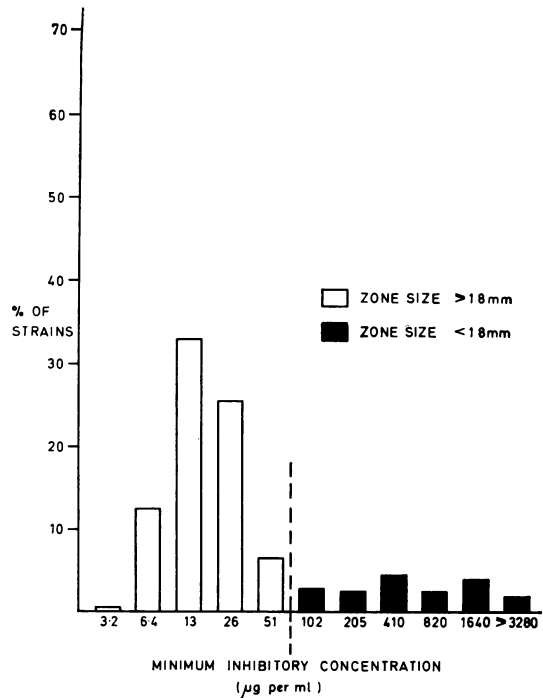


Fig. 1 Sulphamethoxazole sensitivity, MICs, and zone sizes

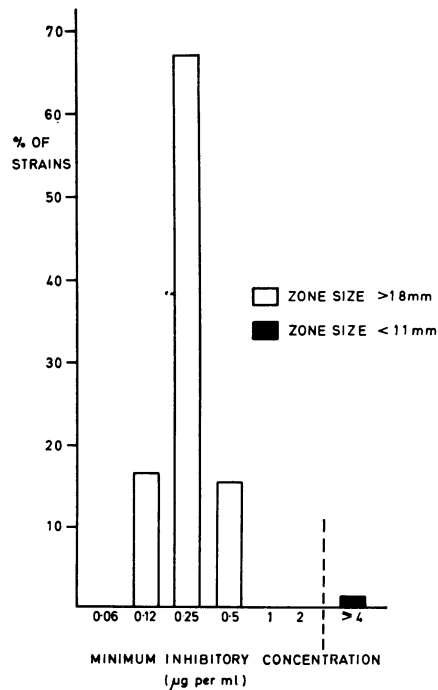


Fig. 2 Trimethoprim sensitivity, MICs, and zone sizes

MIC	No. of Strains Tested	Mean Zone Diameter <sup>1</sup>	Range of Zone Variation <sup>1</sup>
3.2	2	29	(28-30)
6.4	34	26	(24-30)
13	96	24	(18-30)
26	70	22	(18-26)
51	18	21	(18-24)
102	8	10	(6-14) <sup>2</sup>
205	9	7	(6-14) <sup>3</sup>
410	12	6	6
820	7	6	6
1640	11	6	6
3280	5	6	6
Total	272		

Table I *Sulphamethoxazole sensitivity: minimum inhibitory concentrations and zone diameters in disk test*

<sup>1</sup>Disk diameter 6 mm.

<sup>2</sup>Five of eight strains grew up to the disk edge.

<sup>3</sup>Six of nine strains grew up to the disk edge.

diameter (Table II). Of the three resistant strains two (with MICs of 8 and 16 µg per ml respectively) grew to within 2.5 mm from the edge of the disk and one (MIC 8 µg per ml) gave a zone of total diameter of 11 mm.

MIC	No. of Strains Tested	Mean Zone Diameter <sup>1</sup>	Range of Zone Variation
0.125	45	23	(20-28)
0.25	182	24	(18-29)
0.5	42	22	(18-27)
1.00	—	—	—
2.00	—	—	—
4.00	3	8	(6-11) <sup>2</sup>
Total	272		

Table II *Trimethoprim sensitivity: minimum inhibitory concentrations and zone diameters in disk test*

<sup>1</sup>Disk diameter 6 mm.

<sup>2</sup>Two of the three strains grew up to the disk edge.

#### FREQUENCY OF RESISTANCE

We therefore took a zone diameter of less than 18 mm as an indication of resistance to each of the drugs, and examined the remaining 405 cultures. Applying this criterion to the 675 strains, 18.5% were resistant to sulphamethoxazole and 1.6% to trimethoprim. All

the trimethoprim-resistant strains were also sulphamethoxazole resistant (Table III).

The strains had also been tested for resistance to 11 antibiotics, and it was apparent that the relation of trimethoprim and of sulphamethoxazole resistance to antibiotic resistance was somewhat different. Only 4% of the 134 strains that were sensitive to all antibiotics, and only 6% of the 376 strains that were resistant only to penicillin were sulphonamide resistant and in neither group were any resistant to trimethoprim. Among the 165 strains that were resistant to streptomycin, tetracycline, chloramphenicol, erythromycin, oleandomycin, or novobiocin or to any combination of these, with or without penicillin resistance, 60% were resistant to sulphamethoxazole and 7% to trimethoprim. Thus sulphamethoxazole resistance is in the main and trimethoprim resistance is entirely to be found among 'hospital' multiple-resistant staphylococci.

Table IV shows the frequency of sulphonamide and trimethoprim resistance among cultures from each of the seven hospitals. The percentage of sulphonamide-resistant strains ranged between 13 and 30% and one or more trimethoprim-resistant strains were detected in the collection of each of six of the seven hospitals.

Hospital	No. of Strains Tested	Percentage of Strains Resistant to	
		Sulphamethoxazole	Trimethoprim
A	100	15	1.0
B	100	20	1.0
C	85	13	1.2
D	98	30	3.0
E	98	22	1.0
F	99	14	4.0
G	95	17	0
Total	675	19	1.6

Table IV *Frequency of sulphamethoxazole and trimethoprim resistance of Staph. aureus strains from seven hospitals*

Table V gives further information about the 11 trimethoprim-resistant cultures. The minimum inhibitory concentration of trimethoprim ranged from

Resistance to Other Antibiotics	No. of Strains Tested	Percentage of Strains Sensitive or Resistant to Su and TM		
		Su Sensitive TM Sensitive	Su Resistant TM Sensitive	Su Resistant TM Resistant
Sensitive to all antibiotics	134	96	4	0
Resistant to penicillin only	376	94	6	0
Resistant to other antibiotics <sup>1</sup>	165	40	53	7
Total	675	81.5	17.0	1.6

Table III *Relation of sulphamethoxazole (Su) and trimethoprim (TM) sensitivity to antibiotic sensitivity*

<sup>1</sup>To streptomycin, tetracycline, chloramphenicol, erythromycin, novobiocin, oleandomycin, or any combination of these with or without resistance to penicillin.

Strain No.	Resistance Pattern	Hospital	Minimum Inhibitory Concentration ( $\mu\text{g per ml}$ )			Phage Typing Pattern
			TM	TM + Su	Su	
1	P S T E F B N Hg	A	8	4 + 80	1640	84/85
2	P S T E O I B N Hg	B	8	2 + 40	1640	85
3	P S T E O I B N Hg	C	16	2 + 40	1640	NT
4	P S T E O I B N Hg	D	64	8 + 160	3280	85
5	P S T E B N Hg	D	16	4 + 80	1640	85
6	P S T Hg	D	128	8 + 160	1640	NT
7	S T	E	16	4 + 80	820	NT
8	P S T E O I L B N Hg	F	32	8 + 160	1640	84
9	P S T E O I L B N Hg	F	16	8 + 160	1640	84
10	P S T E O I L B N Hg	F	8	4 + 80	1640	84
11	P S T E O I L B N Hg	F	32	8 + 160	1640	84

Table V Characters of strains resistant to trimethoprim and sulphonamide

P = penicillin  
Ol = oleandomycin  
N = neomycin

S = streptomycin  
L = lincomycin  
TM = trimethoprim

T = tetracycline  
F = fucidin  
Su = sulphonamide

E = erythromycin  
B = bacitracin  
NT = untypable

8 to 128  $\mu\text{g per ml}$ . Their resistance to sulphamethoxazole was distinctly greater than that of the average trimethoprim-sensitive strains. Ten of 11 trimethoprim-resistant strains, but only 16 of 49 trimethoprim-sensitive, sulphamethoxazole-resistant strains, grew in the presence of 1640  $\mu\text{g per ml}$  or more of sulphamethoxazole. All were multiple-antibiotic resistant. All but one were resistant to penicillin and all the penicillin resistant strains were mercury resistant. The number of strains resistant to other antibiotics were: streptomycin 11, tetracycline 11, erythromycin nine, oleandomycin, neomycin, and bacitracin eight, lincomycin four, and fucidin one. Eight of the 11 strains had the phage-typing pattern 84/85, 84, or 85, and three were untypable. None of the cultures hydrolysed Tween 80.

The trimethoprim-resistant cultures from hospital F were identical in phage-typing pattern and in antibiogram; two of the cultures of hospital D had a similar phage typing pattern but were different in antibiotic-resistance pattern, and the third culture was distinct.

The MICs of a 1:20 mixture of the two drugs for the 11 trimethoprim-resistant strains, although lower than that of either drug alone, are still too high for the combination to be of therapeutic value (Table V). The peak plasma level after a 100 mg of trimethoprim is 0.9 to 1.2  $\mu\text{g per ml}$ , of which 42-46% is protein bound. After a 2 g dose of sulphonamide it is 100  $\mu\text{g}$ , of which up to 98% can be protein bound (Garrod and O'Grady, 1971).

## Discussion

The resistance of *Staphylococcus aureus* to sulphamethoxazole and trimethoprim does not appear to have been reported before. Resistance to sulphonamides alone has been recognized for many years, though few recent surveys of its frequency have been

reported. Goldie, Alder, and Gillespie (1971) examined a small sample of strains isolated in 1969 and 1970, and reported sulphonamide resistance in 5% of strains from patients outside hospital and 40% of strains from hospital patients. They did not observe resistance to trimethoprim. It is not known whether trimethoprim-resistant strains of staphylococci existed naturally before the drug came into use, or whether the resistance has arisen spontaneously and been selected since. The fact that it has now been found only in multiple-antibiotic-resistant strains of types known to be endemic in hospitals suggests the latter. The frequent use of trimethoprim in combination with sulphamethoxazole for the treatment of infections of the urinary and respiratory tracts in hospital patients has resulted in the exposure to selection by trimethoprim of large numbers of *Staph. aureus* strains that are already sulphonamide resistant. Trimethoprim-resistant variants of coliform bacilli are easily selected *in vitro* (Darrell, Garrod, and Waterworth, 1968) and strains resistant to both drugs have been isolated from patients after combined therapy (Lacey, Gillespie, Bruton, and Lewis, 1972).

Most of our trimethoprim-resistant staphylococci were lysed by phage 84 or phage 85, or by both phages. They thus belong to a group of closely related strains that first became endemic in British hospitals in 1961 (Jevons and Parker, 1964; Jevons, John, and Parker, 1966). For several years they were almost the only neomycin-resistant staphylococci, and their spread was probably associated with the extensive use of this antibiotic. It is unfortunate that trimethoprim resistance has now appeared in similar strains that are already resistant to many other antimicrobial agents. In addition to restricting further the range of drugs that can be used for the treatment of infection with these strains, this addi-

tional resistance to a widely used drug may provide another selective advantage in hospitals for a highly undesirable staphylococcus. All the trimethoprim-resistant strains we have seen so far are methicillin sensitive, though some methicillin-resistant 84/85 strains were seen in earlier years.

It is clear that combined therapy with sulphamethoxazole and trimethoprim is unlikely to prevent the appearance of trimethoprim resistance in organisms, such as *Staph. aureus* and the enterobacteria, in which sulphonamide resistance is already common. We agree with Goldie and his colleagues (1971) that a reduction of the total exposure of the hospital population to these drugs is the only measure likely to prevent a further increase in the frequency of resistant strains.

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