THE ANTIBIOTIC RESISTANCE PATTERN OF 192 STRAINS OF STAPHYLOCOCCUS AUREUS

By Sng Ewe Hui and Lim Ah Loo

(Department of Pathology, Singapore)

It is not uncommon nowadays to isolate Staph. aureus resistant to a number of antibiotics. These strains are largely confined to a few phage types which prevail in the hospital environment. The extent to which they may be found in different hospitals will vary. To assess the local situation, a prospective study on the resistance pattern of 192 strains of Staph. aureus was undertaken. This paper presents the findings.

MATERIALS AND METHODS

192 strains of Staph. aureus were isolated by the bacteriology laboratory from clinical materials over a period of three weeks. No two of the strains were from the same patient. Identification was carried out by colonial morphology, microscopy and the tube coagulase test.

Strains were regarded as hospital acquired if isolated from lesions diagnosed after admission (post-operative wounds, etc.) or from specimens taken 3 or more days after admission from non-lesion sites such as skin, etc. Non-hospital acquired strains were those isolated from lesions diagnosed at the time of admission (abscesses, etc.), or from specimens taken less than 3 days after admission from non-lesion sites (skin, etc.).

Antibiotic sensitivity was determined by using commercial discs at the following concentrations: penicillin 2 u.; tetracycline 25 µg.; streptomycin 10 µg.; erythromycin 5 µg. gentamicin 10 µg.; cloxacillin 5 µg.; cephaloridine 30 µg.; sodium fusidate 10 µg.; lincocin 10 µg. and leucomycin 5 µg. The following moist discs were prepared according to the method of Cruickshank, 1965: chloramphenicol 10 µg.; kanamycin 10 µg.; and neomycin 10 µg.

Blood nutrient agar plates were flooded with 1/100 dilutions of the overnight broth cultures. The plates were dried for 1/2 hour, and the discs applied. After 18 hours incubation at 37° C, the inhibition zone diameters were measured. Strains having diameters 15 mm. or less were regarded as resistant. A control using Staph. aureus oxford was included in each batch of tests.

SOURCES OF STAPHYLOCOCCUS AUREUS

The most common sites (Table I) from which hospital acquired Staph. aureus were isolated were post-operative wounds (46%), and the respiratory tract (19%). On the other hand the skin (22%), abscesses (37%), and the respiratory tract (20%) accounted for most of the non-hospital acquired strains. It is clear that the important sources of Staph. aureus were abscesses and wounds for patients with Staph. aureus lesions, and the skin and respiratory tract for carriers.

The surgical units (Table II) were responsible for almost half (48%) the number of isolates. This was due to the high incidence of abscesses and wounds found these units. Almost all the isolates from the obstetric and gynaecological cases were hospital acquired, contrasting with the higher incidence of non-hospital acquired strains from the paediatric units. The former was due to post-delivery and post-operative infections, whilst the latter consisted largely of staphylococcal skin infections.

ANTIBIOTIC RESISTANCE

When the strains were arranged according to the sizes of the inhibition zone diameters (Fig. 1), it was apparent that the strains were mostly distributed in a bimodal manner with each antibiotic. One cluster (giving larger diameters) represented the sensitive strains, whilst the other (giving smaller diameters) the resistant ones. Hospital strains, however, did not show a significant cluster representing penicillin sensitivity.

Hospital strains (Table III) were more resistant to the five common antibiotics as compared to the non-hospital ones. More than two-thirds of the former were resistant to either penicillin, tetracycline or streptomycin, whilst less than one quarter of the latter were resistant to the antibiotics other than penicillin. The greatest differences in resistance between hospital and non-hospital strains were observed with tetracycline, streptomycin and erythromycin.

TABLE I

SITES FROM WHICH STAPH. AUREUS ISOLATED

Sites	Hospital	Non-hospital	Total
Sores, ulcers	7 (10%)	27 (22%)	34 (18%)
Abscesses	6 (9%)	45 (37%)	51 (27%)
Wounds	32 (46%)	11 (9%)	43 (22%)
Eyes, ears	2 (3%)	7 (6%)	9 (5%)
Respiratory tract	13 (19%)	24 (20%)	37 (19%)
Effusions	3 (4%)	5 (4%)	8 (4%)
Vaginal swabs	7 (10%)	3 (2%)	10 (5%)
TOTAL	70	122	192

TABLE II

TYPES OF CASES

Types of Cases	Hospital	Non-hospital	Total
Medical	16 (23%)	25 (20%)	41 (21%)
Paediatric	6 (9%)	36 (30%)	42 (22%)
Surgical	33 (47%)	59 (48%)	92 (48%)
Obstet/Gynaecologic	15 (21%)	2 (2%)	17 (9%)
TOTAL	70	122	192

TABLE III

STAPH. AUREUS RESISTANCE TO ANTIBIOTICS

Antibiotics	Hospital	Non-hospital	Total
Penicillin	61 (87%)	74 (61%)	135 (70%)
Tetracycline	54 (77%)	29 (24%)	83 (43%)
Streptomycin	48 (69%)	15 (12%)	63 (33%)
Erythromycin	31 (44%)	8 (7%)	39 (21%)
Chloramphenicol	13 (19%)	7 (6%)	20 (11%)

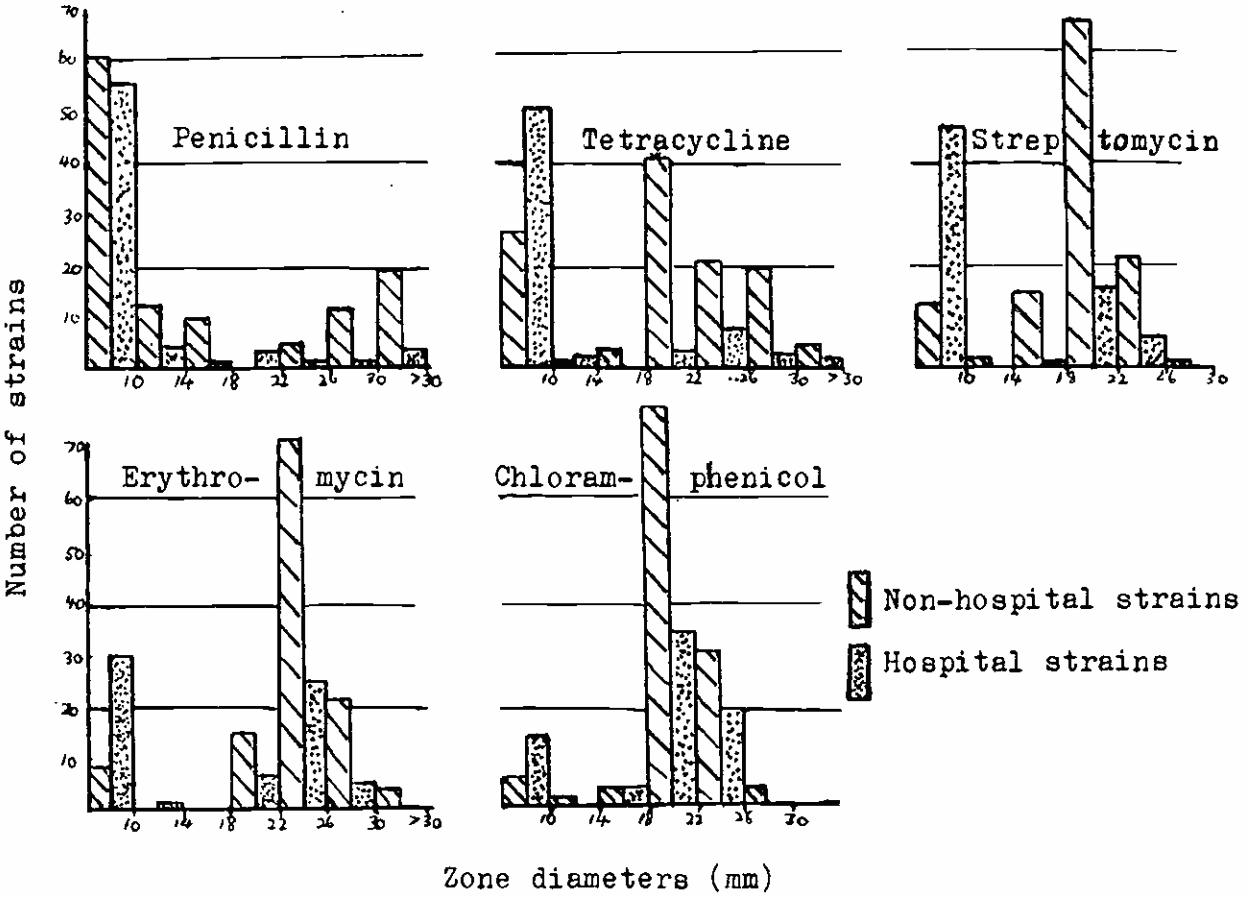

Fig. 1. Distribution of Zone diameters.

CROSS-RESISTANCE BETWEEN ANTIBIOTICS

Cross-resistance between the five antibiotics was frequently found (Table IV). Most of the strains resistant to erythromycin or chloramphenicol were also resistant to penicillin, tetracycline or streptomycin. Those resistant to tetracycline or streptomycin were also almost always penicillin-resistant.

On the whole penicillin resistance was least often associated with resistance to another antibiotic. On the other hand, erythromycin resistance was most often associated with resistance to one of the other antibiotics.

The aminoglycosides often show cross-resistance with one another. Table V shows that 26% of streptomycin-resistant strains were also kanamycin and neomycin resistant. All strains resistant to the latter two drugs were streptomycin-resistant. Thus the cross-resistance was one way—from kanamycin or neomycin to streptomycin. The cross-resistance between kanamycin and neomycin, however, was 100%.

Gentamicin was the most effective of the aminoglycosides, as only one strain was resistant to it, and this strain was also resistant to the other three drugs.

MULTIPLE ANTIBIOTIC RESISTANCE

Triple resistance was found in a number of strains (Fig. 2). 7-8% of all the strains were resistant to chloramphenicol and two other antibiotics. Resistance to erythromycin and two other antibiotics, other than chloramphenicol, was shown by 19-20% of the strains. Almost 1/3 (31%) of all strains were resistant to penicillin, tetracycline and streptomycin.

37 strains were resistant to penicillin, tetracycline, streptomycin and erythromycin, whilst 13 were resistant to penicillin, tetracycline,

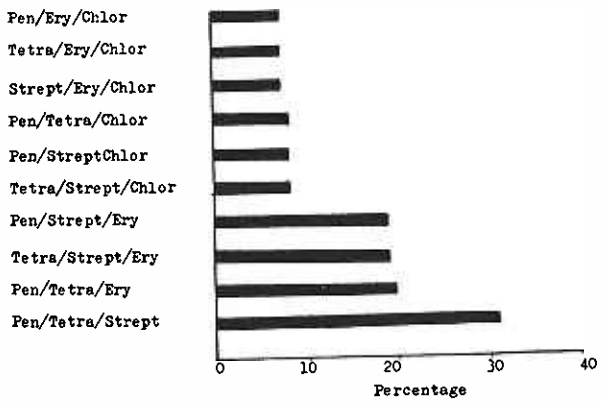

Fig. 2. Percentage Staph. aureus having triple resistance.

TABLE IV

CROSS-RESISTANCE BETWEEN DIFFERENT ANTIBIOTICS

Strains Resistant to		Cross Resistance to				
		Pen.	Tetra.	Strept.	Eryth.	Chlor.
Penicillin	135	—	73 (54%)	62 (46%)	39 (29%)	17 (13%)
Tetracycline	83	73 (87%)	—	59 (71%)	38 (46%)	17 (22%)
Streptomycin	63	62 (98%)	59 (93%)	—	37 (59%)	15 (24%)
Erythromycin	39	39 (100%)	38 (97%)	37 (95%)	—	13 (33%)
Chloramphenicol	20	17 (85%)	17 (85%)	15 (75%)	13 (65%)	—

TABLE V

CROSS-RESISTANCE BETWEEN AMINOGLYCOSIDES

Strains Resistant to		Cross Resistance to			
		Strept.	Kana.	Neo.	Genta.
Streptomycin	63	—	16 (26%)	16 (26%)	1 (2%)
Kanamycin	16	16 (100%)	—	16 (100%)	1 (6%)
Neomycin	16	16 (100%)	16 (100%)	—	1 (6%)
Gentamycin	1	1 (100%)	1 (100%)	1 (100%)	—

TABLE VI

MULTIPLE RESISTANCE TO FOUR ANTIBIOTICS

Pen./Tetra./Strept./Eryth. - -	37
Pen./Tetra./Strept./Chlor. - -	13

streptomycin and chloramphenicol (Table VI). This meant that 97% of the erythromycin-resistant strains and 65% of the chloramphenicol-resistant strains were resistant to the other three drugs.

DISTRIBUTION OF RESISTANT STRAINS

When the patterns of antibiotic resistance of strains acquired inside and outside the hospitals were compared (Fig. 3), a striking contrast was seen. 76% of the hospital strains were resistant to at least two of the antibiotics; multiple resistance to four drugs being the most common (33%).

Most of the non-hospital strains were either sensitive to all the drugs or resistant to one drug, and only 21% were resistant to more than one drug.

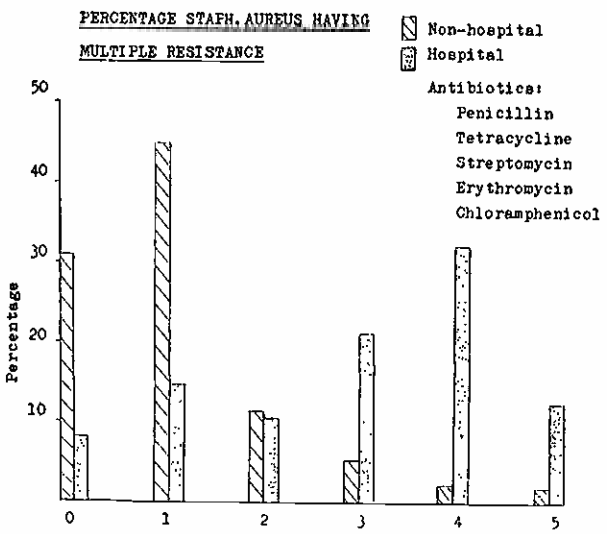

Fig. 3. Resistance to number of antibiotics.

OTHER ANTIBIOTICS

Of the 192 strains only one was resistant to cloxacillin, cephaloridine, sodium fusidate and lincomycin, whilst none was resistant to leucomycin. The singular strain resistant to the first four drugs was isolated from the chest wound of a patient with pulmonary tuberculous. The colonial morphology was 'gonidial' and two consecutive subcultures did not show reversion to its parent form. It is possible that had the test for

cloxacillin sensitivity been done with 5% sodium chloride incorporated in the medium, and the incubation at 30° C for 48 hours, more resistant strains might have been found.

DISCUSSION

Staph. aureus is a common cause of bacterial infections. The important sites of Staph. aureus infections were the wounds, abscesses, respiratory tract and skin. Attempts at controlling cross infections will need to take into account these sources of Staph. aureus.

The highest incidence of Staph. aureus came from the surgical units, where abscesses and various types of wound infections prevailed. There is great need for the prevention of cross infections in these wards.

As compared to the non-hospital strains, the hospital strains showed increased resistance to all the five common antibiotics. Similar high levels of resistance to these antibiotics shown by hospital strains have been reported by Spink (1956), and Jeljaszewicz *et al* (1967); but the non-hospital strains of the latter group of workers were considerably more resistant than those in our series. The high percentage of hospital resistant strains has arisen as a result of selective elimination of strains sensitive to the drugs, and in our study, no significant cluster of penicillin-sensitive hospital strains was noted.

Amongst anti-bacterial agents cross-resistance between structurally related drugs like the tetracyclines or sulphonamides is a common occurence. The aminoglycosides showed a similar relationship, but it was largely one way. Strains resistant to kanamycin, neomycin or gentamicin were also streptomycin-resistant, but not vice versa. The effectiveness of gentamicin confirms the findings of Barber and Waterworth (1966), who found it to be four times more active than kanamycin against Staph. aureus. They also found there was 100% cross-resistance between kanamycin and neomycin. In view of its effectiveness, gentamicin will likely prove itself useful in the treatment of certain types of staphylococcal infections.

The five common antibiotics tested bear no structural similarity to each other. Yet cross-resistance frequently occured. Almost all erythromycin-resistant strains were also resistant to either penicillin, tetracycline or streptomycin. The same was true with chloramphenicol, but to a lesser extent. The nature of some of these associated resistances has been shown to be due to the presence of transferable extrachromosomal particles of genetic material (Novick and Richmond, 1965). These workers found penicillinase formation and erythromycin resistance to be governed by such a particle. Evans and Waterworth, 1966, have also found a similar phenomenon with penicillinase formation and fusidic acid resistance, and tetracycline, kanamycin and fusidic acid resistance. The clinical significance of these findings, however, is still uncertain.

In view of the high degree of cross-resistance between the antibiotics, it is not surprising that one third of the strains showed resistance to penicillin, tetracycline and streptomycin. Furthermore, 97% of the erythromycin-resistant, and 65% of the chloramphenicol-resistant strains were insensitive to the three antibiotics.

The selective nature of the hospital environment on Staph. aureus is borne out by the difference in the patterns of antibiotic resistance of strains acquired inside and outside the hospitals. Three quarters of hospital strains were resistant to two or more drugs, whilst one fifth of those acquired outside showed the same resistance. The frequent occurence of such multiple drug resistant strains in hospitals has led to the suggestion (Barber, 1966) that for severe hospital acquired Staph. aureus infections treatment should commence with a penicillinase-resistant penicillin, until subsequent investigations indicate a change. However, with the emergence of methicillin and cephalosporin resistant strains, it has been advocated that the synergistic combination of a penicillinase-resistant penicillin or a cephalosporin with an aminoglycoside may prove useful (Editorial, B. M. J., 1967).

Fusidic acid, lincomycin and leucomycin may give rise to resistant strains in vitrio, and their clinical usefulness will need more studies.

SUMMARY

The antibiotic resistance of 192 strains of Staph. aureus was determined. Hospital strains were more resistant than non-hospital strains against penicillin, tetracycline, streptomycin, erythromycin and chloramphenicol. Cross-resistance was a common occurence, and 76% of the hospital strains were resistant to two or more of the antibiotics.

REFERENCES

1. Barber, M. (1966): "Antibacterial activity of antibiotics' in The Therapeutic Use of Antibiotics in Hospital Practice—Proceedings of a Symposium." Ridley, M. and Phillips, I., Eds. Livingston, London, p. 28.

2. Barber, M. and Waterworth, P. M. (1966): "Activity of Gentamicin against Pseudomonas and hospital Staphylococci." Brit. Med. J., 1, 203.

3. Cruickshank, R. (1965): "Medical Microbiology." 11th Ed., Livingston, London, p. 894.

4. Editorial, (1967): "Treatment of Staphylococcal Endocarditis." Brit. Med. J., 1, 515.

5. Evans, R. J. and Waterworth, P. M. (1966): "Naturally-occuring Fusidic Acid Resistance in Staphylococci and its linkage to other Resistances." J. Clin. Path., 19, 555.

6. Jeljaszewicz, J. *et al* (1967): "Comparison of the Antibiotic Resistance of Staphylococcus aureus strains isolated in Poland in the years 1960-1963 and 1964." Epid. Rev., 21, 101.

7. Novick, R. S. and Richmond, M. H. (1965): "Nature and Interactions of the Genetic Elements governing Penicillinase Synthesis in Staphylococcus aureus." J. Bact., 90, 467.

8. Spink, W. W. (1956): "The Clinical Problem of Antimicrobial Resistant Staphylococci." Ann. N.Y. Acad. Sci., 65, 175.