A very low level of endemicity of methicillin-resistant strains was observed in some hospitals. This was explained in part by the frequency of undetected symptomless infections, but there was also evidence that neither the disc sensitivity test, nor the tube sensitivity test as it is usually performed, was sufficiently sensitive to detect all resistant organisms.

REFERENCES

METHICILLIN-RESISTANT STAPHYLOCOCCI AND HOSPITAL INFECTION

MARY BARBER
Department of Bacteriology, Postgraduate Medical School of London, London, W.12

Methicillin-resistant strains of *Staph. aureus* have now been isolated from patients in hospitals in Britain (Jevons, 1961; Barber, 1961; Knox and Smith, 1961), France (Chabbert and Baudens, 1962), Turkey (Cetin and Ang, 1962), Denmark (Eriksen and Erichsen, 1963) and Poland (Borowski, Kamienska and Rutecka, 1964). The resistance appears to be naturally occurring since in most cases the strains have been isolated from patients who have never had any of the penicillinase-resistant penicillins, and those isolated in Poland came from a hospital where the penicillinase-resistant penicillins have never been used.

In spite of the diversity of source all the recorded strains show a similar, rather unusual, type of resistance. In addition all show high penicillinase-producing activity, and multiple resistance to other antibiotics and nearly all belong to one of only a few bacteriophage types of Group III. In all cases strains resistant to methicillin show cross resistance with all the other penicillinase-resistant penicillins.


Type of Resistance

Methicillin-resistant staphylococci isolated in vitro and in clinical practice differ in the type of resistance, although in neither case do the strains show an increased capacity to inhibit methicillin. In vitro, when methicillin-sensitive strains are passaged in medium containing methicillin they give rise to cultures which show uniform increased tolerance to the antibiotic. With penicillinase-producing organisms the resistant mutants in other respects resemble the parent cultures and the resistance is usually stable for many generations in the absence of antibiotic. With penicillin-sensitive strains, however, the resistant mutants tend to become poorly growing and relatively avirulent, resembling cultures of *Staph. aureus* rendered tolerant to benzyl penicillin in vitro (Barber, 1961).

On the other hand, naturally occurring methicillin-resistant strains, when tested in the usual laboratory media, grow quite abnormally in the presence of methicillin, and show a gross difference in sensitivity according to the
size of inoculum. This is not simply due to a difference in sensitivity of the cell population. Such a difference may exist, but all, or nearly all, the cells of these cultures show some increase in resistance to methicillin, although the resistance is only partial. On nutrient agar with a normal salt content, and containing methicillin, growth of these strains after 18-24 hours tends to be confined to the site of heavy inoculum and discrete colonies away from this site only make their appearance after prolonged incubation. These appearances are seen when the concentration of methicillin is similar to that which just inhibits sensitive staphylococci, but the strains continue to grow in a similar way as the concentration of methicillin is increased up to levels of from 50-250 μg./ml. Gram-stained films of the growth in the presence of methicillin show cells which vary in size and staining, with many swollen forms. The picture resembles that seen when penicillin-sensitive staphylococci are grown in the presence of sub-inhibitory concentrations of benzyl penicillin, but of course in the latter case only at a concentration close to that which completely inhibits growth.

Thus with the naturally occurring methicillin-resistant strains growth occurs, but is abnormal, over a wide range of methicillin concentrations, whereas with methicillin sensitive organisms or strains rendered resistant in vitro there is a sharp end point. These findings are consistent with the view that methicillin partially inhibits the cell wall synthesis of the naturally occurring methicillin resistant staphylococci at a concentration not much greater than the minimum concentration inhibiting methicillin-sensitive strains.

Further support for this view comes from the finding that if the cells of these strains are protected from lysis by increasing the osmotic pressure of the medium normal staphylococcal growth occurs in the presence of methicillin. This has been shown by Barber (1964) who studied the growth of naturally occurring methicillin-resistant strains of Staph. aureus in the presence of methicillin in laboratory media with and without excess sodium chloride or ammonium sulphate. She found that in medium containing 5% sodium chloride or 7.5% ammonium sulphate the strains grew like typical staphylococci in the presence of methicillin. In sensitivity tests using these high salt-containing media the strains showed a relatively sharp end point at from 50-250 μg./ml. methicillin.

**Bacteriophage Types**

Jevons et al. (1963) studied 102 methicillin-resistant strains sent to the Central Public Health Laboratory, Colindale, from 23 different laboratories in Britain and elsewhere. Only nine different phage patterns were found among the 102 strains and 80% of the strains were of one of the two types 75/77+ or 7/47/53/54/75/77+. All the cultures from Britain belonged to Group III, but 11 strains from Switzerland were type 29 (Group I). Strains studied in other laboratories in Britain (Barber, 1964), France (Chabbert and Baudens, 1962), Denmark (Eriksen and Erichsen, 1963) and Poland (Borowski, Kamienska and Rutecka, 1964) have been found to be of similar phage types.

**Associated Resistance to Other Antibiotics**

All the methicillin-resistant strains isolated from the clinical sources referred to in the first paragraph of this communication resemble hospital epidemic staphylococci in that they are resistant to penicillin, streptomycin and tetracycline and, when tests have been made, also to mercury. High penicillinase activity has also been shown recently to be a characteristic feature of hospital epidemic staphylococci (Richmond, Parker, Jevons and John, 1963) and once again it has been shown that naturally occurring methicillin-resistant strains are high penicillinase producers.

The reason for this correlation is not obvious. As already pointed out there is no direct correlation between the use of methicillin in individuals or institutions and the emergence of methicillin-resistant strains in hospitals. It cannot be concluded, therefore, that, as was the case with streptomycin and tetracycline, methicillin-resistant variants of the prevalent hospital staphylococci are being bred out by passage of these strains in patients treated with the antibiotic. We are left with the assumption that the multiple-resistant staphylococci so prevalent in hospitals today are, for some reason, liable to show a form of methicillin-resistance which is not seen among antibiotic-sensitive strains. It may be that in selecting out drug-resistant staphylococci in hospitals strains of high mutability have also been selected or possibly some strains of staphylococci carry cytoplasmic agents such as the resistance transfer factors of coliform bacilli which transmit multiple drug-resistance (see Watanabe, 1963).
Virulence

Many of the multiple resistant strains of Staph. aureus prevalent in hospitals appear to be of high virulence, and indeed as Williams (1959) has pointed out, all staphylococci responsible for epidemics in hospitals today are multiple drug-resistant strains. Moreover, as shown by Barber et al. (1960) these strains are responsible for the majority of severe and generalized infections in hospital. This does not of course mean that all drug-resistant staphylococci are virulent or that there is any direct association between virulence and drug-resistance, but it stems from the way in which drug-resistant strains have been selected in hospital. They are those strains which have survived in the tissues of patients and have been passed down from patient to patient.

As already pointed out the naturally occurring methicillin-resistant strains of Staph. aureus isolated in clinical practice share most of the properties of these hospital staphylococci, but their virulence and clinical significance are disputed. An analogy has been drawn between methicillin-resistant staphylococci and the penicillin-tolerant strains of Staph. aureus isolated by passage in benzylpenicillin in vitro and it has been suggested that the former, like the latter, will not be a clinical problem (Rolinson, 1960). The analogy, however, is not very close. Although naturally occurring methicillin-resistant strains of Staph. aureus grow abnormally in the presence of methicillin, in the absence of the antibiotic they show all the properties of typical strains of Staph. aureus including mouse virulence (Harding, 1963), whereas penicillin-tolerant staphylococci grow poorly and appear to be of low virulence in the presence or absence of penicillin.

Quite apart from theoretical speculation, evidence is now accumulating to show that some at least of the naturally occurring methicillin-resistant staphylococci are highly virulent in hospitals. Stewart and Holt (1963) record such a strain which infected no less than 37 children in seven wards of a hospital over a period of 12 months, and in one patient was responsible for fatal septicemia. Ayliffe and Barber (1963) also observed methicillin-resistant strains of Staph. aureus which caused multiple infections in a surgical ward, some of which were severe.

Once again it must not be assumed that all methicillin-resistant staphylococci are of high virulence. Infections with such strains are often trivial and the strain reported by Borowsk i et al. (1964) although it colonized the noses of about 50% of the mothers and babies in a maternity unit, did not cause any septicaemia. The severity of staphylococcal infection, however, depends as much, if not more, on the host as on the staphylococcus, so that even a particular strain only causes slight or negligible infection in healthy babies it cannot be concluded that it might not cause severe infection in more susceptible individuals. Harding (1963) records two patients infected with a single methicillin-resistant strain of Staph. aureus and in one case the infection was of a severe generalized nature whereas in the other there was only a superficial infection of an operation wound. In the outbreaks of infections due to single strains recorded by Stewart and Holt (1963) and Ayliffe and Barber (1964) the severity of infection in different individuals was also very variable.

Accepting the fact that some strains of methicillin-resistant Staph. aureus are fully virulent, in view of their abnormal growth in the presence of methicillin, it is reasonable to ask whether infections with such strains would respond to methicillin treatment. Information on this head is scanty, but the case recorded by Harding (1963) failed to respond to methicillin even in a dose of 2 g. four hourly and Ayliffe and Barber (1964) record a case which failed to respond to oral treatment with 500 mg cloxacillin six hourly. The latter investigators also found that mice infected with a methicillin-resistant strain of Staph. aureus responded less well to treatment with methicillin or cloxacillin than did mice infected with a methicillin-sensitive strain.

Conclusion

Methicillin-resistant strains have been isolated from patients in many hospitals, but at present the phenomenon has only been seen with strains of a few phage-types, which appear to be similar all over the world, and with all strains resistance has been only partial. The strains appear to be fully virulent in the absence of methicillin and in a few recorded instances infections by them respond poorly to methicillin and cloxacillin in the usual doses. At present the incidence of these strains does not represent a major problem and if the penicillinase-resistant penicillins continue to be used with discretion it may not become one.
Many variables affect the outcome of the encounter between bacteria and host. In recent years it has become quite fashionable to try to influence this interaction by means of various chemotherapeutic agents. With the advent of numerous semi-synthetic penicillins there is increasing need to make estimates or perhaps at least “best guesses” as to which of several compounds looks more promising for in vitro testing and to estimate under which situation one compound might be better than another. Such a guess is fraught with numerous chances for error but the desire to make it does give a convenient excuse for discussing some of the puzzling laboratory problems which affect the encounter between bacteria and antibiotic. Several groups of workers have called attention to the importance of variations in relative sensitivity of test organisms in comparing the activities of two antibiotics. Our attention was recently called to this fact when comparing ampicillin and penicillin G against both Esch. coli and Proteus mirabilis. Ampicillin generally had one-eighth the M.I.C. of penicillin G against Esch. coli but only half the M.I.C. against most strains of Proteus mirabilis. Another factor which affects the apparent “minimum inhibitory concentration” is the influence of serum binding as has been pointed out earlier in the conference. The factors which have caught our attention, however, have been of two varieties. One is the influence of mutants in the population of a given bacterial culture with increased resistance to the antibiotic. The other is the influence of factors affecting the kinetics of bactericidal activity. The experiments to be reported here were performed with the acid salt ampicillin and a strain of Esch. coli originally isolated from a patient with asymptomatic bacteriuria.

Previous experiments had indicated that organisms which had regrown after exposure to relatively low concentrations of ampicillin were on occasion mutants with increased ability to inactivate ampicillin compared with the parent strain (Seligman and Hewitt, 1963). Such destruction was presumably due to a penicillinase but its nature has not yet been investigated.

More recently, by alteration in test procedure, it has become apparent that there is an entirely different class of mutants present which have increased inherent resistance of the individual organisms to ampicillin. These mutants were isolated by plating out various concentrations of an overnight culture onto plates containing increasing concentrations of ampicillin.

At a critical or threshold concentration, around 5 ug./ml. at pH 7.3, a marked fall in colony count occurred. Figure 1 shows the typical colonial morphology of a non-mucoid Esch. coli which was plated out at 10^6 dilution of an overnight culture. Figure 2 shows the varying colonial morphology of a 10^2 dilution of the same culture on plates which contained 6 ug./ml. of ampicillin. Marked variation in colonial size is evident. The larger colonies have altered colonial morphology with depressed...