IN-VITRO SUSCEPTIBILITY OF *MYCOBACTERIUM FORTUITUM* AND RELATED STRAINS TO CEPHALOSPORINS

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MYCOBACTERIUM FORTUITUM, a rapidly growing mycobacterium belonging to Runyon's group IV, is often considered to be a saprophytic organism. It has, however, been well documented as a cause of human disease, and more than 20 reports of cutaneous lesions, abscesses, pulmonary and corneal infections are quoted in a recent review (Hand and Sanford, 1970). Biochemical and antigenic studies (Stanford and Beck, 1969; Nakayama, Nakayama and Takeya, 1970; Tsukamura, 1970) have established the existence of the closely related species *M. abscessus* (*M. runyonii* or *M. friedmannii*) and *M. borstelense*, which can produce disease in man identical to that produced by *M. fortuitum* (Hand and Sanford). All three species are resistant to the usual antimycobacterial agents. Isolated reports have appeared of their sensitivity to antibiotics such as tetracyclines (Brosbe *et al.*, 1964), erythromycin (Molavi and Weinstein, 1971*a*) and rifampin (Molavi and Weinstein, 1971*b*) but little is known of the effect of the numerous other antibacterial drugs on these three species. This paper reports on the susceptibility of *M. fortuitum*, *M. abscessus* and *M. borstelense* to cephaloridine and cephalothin.

MATERIALS AND METHODS

The 36 strains of rapidly growing mycobacteria studied were as follows: *M. fortuitum* (ATCC no. 9820); *M. fortuitum* (TMC [Trudeau Institute] no. 1529); *M. abscessus* (TMC no. 5129); *M. borstelense* (TMC no. 1524); six strains of *M. borstelense* and 12 of *M. abscessus* received from Dr J. L. Stanford, London; five strains of *M. borstelense* received from Dr Freerksen, Borstel, Germany. Seven strains identified as *M. fortuitum* and one as *M. abscessus* were isolated from clinical material in our laboratory or received from other laboratories. The strains isolated from clinical material were identified according to arylsulphatase activity, nitrate reduction, growth and acid production on McConkey agar, growth at 37°C and at 30°C.

The sensitivity tests were performed on Dubos Oleic Agar Base (Difco) enriched with Dubos Medium Albumin (Difco). Freshly prepared two-fold dilutions of penicillin G, methicillin, ampicillin, carbenicillin, cephaloridine, and cephalothin were added to the molten medium, which was then poured into petri dishes. Subcultures of each strain were made in Dubos Broth Medium (Difco) enriched with Dubos Medium Albumin (Difco). After incubation for 3-5 days at 37°C, each culture was diluted in sterile distilled water to a turbidity approximating that of the McFarland 1 standard (1 mg of bacterial mass per ml), and 0.15 ml of a 1 in 1000 dilution of this suspension was spread with a glass rod over the surface of the antibiotic-containing plates. Antibiotic-free plates were inoculated with 0.15 ml of 1 in 100, 1 in 1000 and 1 in 10,000 dilutions of the McFarland 1 standard

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suspension to serve as controls of the density of the inoculum. The plates were incubated at 37°C in sealed metal containers. Results were read at 3 and 6 days. The lowest concentration of the antibiotic completely inhibiting at least 99 per cent. of the growth was considered to be the minimum inhibitory concentration (MIC). With each strain, the sensitivity tests for each antibiotic were repeated three to five times.

FIGURE.—Minimum inhibitory concentrations of cephaloridine and cephalothin for *Mycobacterium fortuitum*, *M. abscessus* and *M. borstelense*.

Disk-sensitivity tests with 30 µg-cephaloridine and -cephalothin disks (BBL Laboratories, Maryland, USA) were performed on Dubos Oleic Agar Base (Difco) enriched with Dubos Medium Albumin (Difco). The inoculum was 0.15 ml of a 1 in 1000 dilution of the McFarland no. 1 suspension. Results were read at 3 and 6 days and the diameter of the zones of inhibition was measured.

RESULTS

The sensitivity patterns, which were constant and reproducible on repetition, are shown in the figure. The ten strains of *M. fortuitum* were all sensitive to cephaloridine but, with one exception, all highly resistant to cephalothin

(mean MIC: cephaloridine 20 μg per ml; cephalothin 800 μg per ml). The MIC of cephalothin for the exceptional strain was 64 μg per ml. The strains of *M. abscessus* and *M. borstelense* were resistant to cephalothin. All of the *M. abscessus* strains, and all but two strains of *M. borstelense* were also resistant to cephaloridine. The two exceptions—Stanford's strains no. 78 (NCTC946) and no. 482—were also slightly more sensitive to cephalothin than were the other strains of *M. borstelense*.

The strains of *M. fortuitum* were all highly resistant to penicillin G (mean MIC 100 μg per ml), methicillin (mean MIC 512 μg per ml) and carbenicillin (mean MIC 1024 μg per ml) but more sensitive to ampicillin (mean MIC 30 μg per ml).

With the cephaloridine disk-sensitivity test, strains of *M. fortuitum* always showed a clear zone of inhibition at least 20 mm in diameter. There was no inhibition of the strains of *M. abscessus* or of *M. borstelense*, except for the two more sensitive strains of the latter species. The cephalothin disks failed to produce inhibition of any of the strains.

DISCUSSION

There is little information available on the activity of the β -lactam group of antibiotics on mycobacteria. Hawkins and McClean (1966) showed that the growth of strain H37RV was inhibited by 50 units penicillin per ml in a synthetic medium, or by 200 units per ml in Dubos Broth Base enriched with oleic acid-albumin-dextrose complex. Against *M. kansasii* and *M. fortuitum* the drug was ineffective even at a concentration of 200 units per ml. Vaichulis and Vicher (1962) also found *M. fortuitum* to be resistant to 200 units of penicillin per ml of Dubos Tween Albumin Broth.

We found that, in Dubos Agar supplemented with albumin, the strains of *M. fortuitum* were highly resistant to all the penicillins examined, but slightly less resistant to penicillin G and ampicillin than to methicillin or carbenicillin. The difference between the MIC of the two cephalosporins for the strains of *M. fortuitum* is more important. Cephaloridine is in general more active than cephalothin against cephalosporin-sensitive strains of bacteria, but the differences are nowhere nearly as great as those seen in this study with *M. fortuitum*.

Cephaloridine may penetrate the cell more easily than does cephalothin, or *M. fortuitum* may produce an enzyme with greater activity on cephalothin than on cephaloridine. Mycobacteria are known to produce β -lactamases (Dufour, 1966), and Kasik (1968) found that the enzyme from a single strain of *M. fortuitum* hydrolysed cephalothin slightly more rapidly than cephaloridine. Preliminary studies in our laboratory showed that in the presence of actively growing *M. fortuitum*, 500 μg per ml of cephalothin is completely inactivated in 48 hr, whereas under the same circumstances cephaloridine retains all its activity.

The greater resistance of *M. abscessus* and *M. borstelense* than of *M. fortuitum* to cephaloridine may reflect an underlying difference between these otherwise closely related strains of rapidly growing mycobacteria. This is not an isolated phenomenon, because *M. fortuitum* is sensitive to 25 μg or less of

ethambutol per ml whilst *M. abscessus* and *M. borstelense* are resistant (Portaels and Pattyn, 1970); and *M. fortuitum* is highly resistant to erythromycin whilst *M. borstelense* is sensitive to 0.2 µg per ml (Molavi and Weinstein, 1971a).

One of the two cephaloridine-sensitive strains of *M. borstelense* (NCTC no. 946) was classified as *M. fortuitum* by Gordon and Smith (1955) but was reclassified as *M. friedmannii* (*borstelense*) by Stanford and Beck (1969). The precise taxonomy of these strains is uncertain, and it is possible that, when evidence of genetic relationships becomes available, many strains will be renamed. At the moment it seems that sensitivity to 50 µg per ml cephaloridine (or a zone of inhibition at least 20 mm in diameter around a 30-µg disk) may be an additional criterion for differentiating between *M. fortuitum* and the other strains.

These results also suggest a possible therapeutic application for cephaloridine. *M. fortuitum* infections are notoriously difficult to treat, because they are resistant to isoniazid, PAS, streptomycin, rifampicin and ethambutol. Slight sensitivity to gentamicin and kanamycin has been reported (Nicholson and Sevier, 1971). Serum levels in excess of the MICs that we have demonstrated can be obtained by standard dosage with cephaloridine (Griffith and Black, 1971). The nephrotoxicity of this drug seems to have been exaggerated (Dillon and Postlethwait, 1971), and systemic treatment of severe infection due to *M. fortuitum* may be justified. Animal models for producing ocular mycobacterial infections have been described (Bulmer and Sexton, 1971) and we are conducting in-vivo trials to see whether the local application of cephaloridine is likely to have a place in the treatment of the more common ocular infection with *M. fortuitum*.

SUMMARY

The sensitivity of ten strains of *Mycobacterium fortuitum*, 14 of *M. abscessus*, and 12 of *M. borstelense* to two cephalosporins was examined. *M. fortuitum* strains were resistant to cephalothin but sensitive to cephaloridine. The *M. abscessus* and *M. borstelense* strains were all highly resistant to cephalothin. All the *M. abscessus* strains and all but two strains of *M. borstelense* were resistant to cephaloridine. The use of cephaloridine resistance as a taxonomic aid is suggested. Cephaloridine may be useful in the treatment of infections with *M. fortuitum*.

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