DISC TEST FOR DIFFERENTIATION OF ENTEROCOCCI

VICTOR LORIAN AND URIEL ZANON

Central Laboratory of Tuberculosis, Cajú, Rio de Janeiro, Brazil

Received for publication 3 June 1963

Disc tests are coming into more and more frequent use because of their simplicity of execution. Levinson and Frank (J. Bacteriol. **69**:284, 1955) introduced a disc test with bacitracin to characterize group A streptococci, which are particularly sensitive to this antibiotic. Bowen et al. (J. Lab. Clin. Med. **49**:641, 1957) proposed a disc test with Optochin (ethylhydrocupreine hydrochloride) to differentiate pneunococci from alpha-hemolytic streptococci.

Staphylococci resistant to penicillin G (owing to production of penicillinase) are, with the exception of very rare strains, sensitive to oxacillin (5-methyl-3-phenyl-4-isoxazolyl penicillin), whereas the majority of enterococci are resistant to penicillin G by a mechanism other than the production of penicillinase (Chain and Duthie, cited by Florey, *Antibiotics*, p. 105. Oxford University Press, 1949), and are also resistant to oxacillin, since this is also a penicillin.

In this paper, we present a disc test to differentiate between staphylococci and enterococci, based on the action exerted on them by oxacillin.

A total of 100 strains of staphylococci resistant to penicillin G (5 units per disc) were isolated from routine cultures of various pathological materials and identified by morphological characteristics, by growth in Mannitol Salt Agar (Difco), and by production of catalase and coagulase.

A total of 40 strains of enterococci were isolated from routine cultures of various materials, particularly urine, and identified by morphological characteristics, by growth in Enterococcus Confirmatory Agar (BBL) and Enterococcus Confirmatory Broth (BBL), by absence of catalase, and by resistance to a temperature of 60 C for 30 min. These samples were chosen from among those most sensitive to common antibiotics.

We used antibiotic discs (7 mm in diameter) of filter paper on which we placed, with a Microtiter pipette (kindly supplied by Cooke Engineering Co.), 0.025 ml of a solution of penicillin or oxacillin, so as to achieve 2 μg and 5 μg per disc.

The culture medium was Tryptose Agar base.

Activity of the penicillin and oxacillin discs was controlled by seeding *Staphylococcus aureus* ATCC 6538 on plates of the same medium.

The diameters of the zones of inhibition shown by the culture of *S. aureus* ATCC 6538 were as follows. On discs with 2 μg of penicillin G, the zone was 39 mm in diameter; with 5 μg, the zone was 42 mm. On discs with oxacillin, 2 μg produced a zone 31 mm in diameter, and 5 μg produced a zone 37 mm in diameter.

The staphylococci tested with the oxacillin discs showed zones of inhibition between 17 and 30 mm for the 2-μg discs, and between 20 and 37 mm for the 5-μg discs. Among the 40 strains of enterococci, 1 strain was slightly sensitive to the 2-μg penicillin G disc (9-mm zone), and 5 others showed moderate sensitivity to the 5-μg disc (12-mm zone) of the same antibiotic. No sample of enterococcus was sensitive to the 2-μg or 5-μg oxacillin discs.

Since the staphylococci were sensitive to the 5-μg oxacillin discs, and since all the enterococci tested were resistant to the same concentration, we consider this fact useful as a test to differentiate between staphylococci and enterococci.