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NOTES

Simple Method for Detection of Penicillinase-Producing *Neisseria gonorrhoeae*

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A simple, reliable, and economical method for detection of penicillinase-producing *Neisseria gonorrhoeae* using a penicillin disk and a penicillin-sensitive organism is described.

The recent isolation of penicillinase-producing *Neisseria gonorrhoeae* (1, 6) has necessitated testing for this property in current isolates, particularly those isolated from "treatment failures" or traced to contacts in the Far East. A variety of different assays for the detection of penicillinase production are available (3). These assays are dependent upon the lowering of pH by the formation of penicilloic acid (4, 6, 9, 10), upon the hydrolysis of the lactam bond of a cephalosporin substrate (7), or direct inhibition of penicillin activity (5).

However, in small, low-volume laboratories these tests have presented difficulties related principally to storage of materials and maintenance of control organisms.

Described herein is a simple adaptation of a direct method utilizing a penicillin sensitivity disk and a penicillin-sensitive organism.

A penicillin-sensitive organism, preferably *Staphylococcus aureus,* was streaked upon a plate in a manner similar to that used for antibiotic sensitivity testing by the Bauer-Kirby method (2). A penicillin disk (10 U) was placed on the lawn of organisms. A heavy inoculum of test organisms was streaked outward from the disk. Strains producing penicillinase distorted the zone of inhibition (Fig. 1). A penicillinase-producing (not necessarily gonococci) and non-penicillinase-producing organism were also streaked as controls. Penicillinase-producing gonococci as well as other penicillinase-producing organisms, such as *Haemophilus influenzae, Escherichia coli, Serratia marcescens,* and *S. aureus,* could be detected.

Furthermore, by streaking various dilutions of commercially available penicillinase preparations, the amount of penicillinase produced by the unknown organisms could be quantitated (Fig. 2).

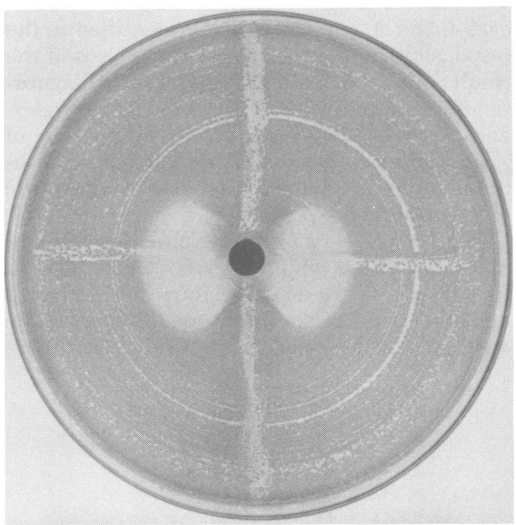

FIG. 1. *Penicillinase-producing organisms distort the zone of inhibition produced by a 10-U penicillin disk (Difco Laboratories, Detroit, Mich.) on a lawn of S. aureus (ATCC 25923).*

Comparison of this technique with other methods (4, 6, 9, 10) has given identical results. Two out of 120 strains of *N. gonorrhoeae,* 9 of 11 strains of *H. influenzae,* 4 of 8 strains of *E. coli,* 1 of 5 strains of *S. aureus,* and 1 strain of *S. marcescens* were positive for beta-lactamase production. Two strains of *Klebsiella pneumoniae,* three strains of streptococci, one *Staphylococcus epidermidis* strain, four pseudomonads, two strains of *Enterobacter cloacae,* and one strain of *Acinetobacter lwoffi* were negative.

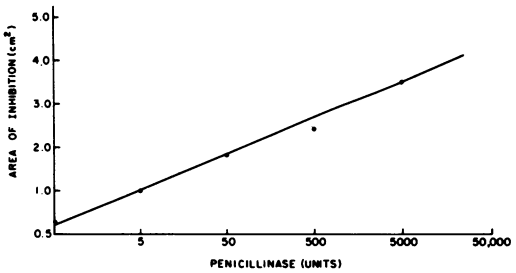

FIG. 2. *Tenfold dilutions of penicillinase (BBL, Cockeysville, Md.; Difco) were streaked on the lawn of organisms, using a quantitated pipette. A slight depression was made in the agar to assure an even distribution of penicillinase.*

This simple technique has the advantage of utilizing a standard antibiotic sensitivity testing method and readily available penicillin disks. Maintaining sensitive staphylococci on a slant or plate over a long period of time is quite easy. Preliminary results can be obtained in 6 h, and this technique is quite sensitive and specific for penicillinase production. Also, non-beta-lactamase-mediated resistance can be discerned when an unknown organism grows in the presence of penicillin but does not distort the zone of inhibition of the test organism.

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