Rapid Penicillinase Paper Strip Test for Detection of Beta-Lactamase-Producing Haemophilus influenzae and Neisseria gonorrhoeae

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A modified 1-min iodometric paper strip test for penicillinase activity was developed for detection of beta-lactamase-producing isolates of Haemophilus influenzae and Neisseria gonorrhoeae. The test is simple to perform and uses reagent-impregnated strips that may be stored for 1 year or more prior to use.

The emergence of ampicillin resistance in Haemophilus influenzae has caused considerable interest in rapid, reliable techniques for recognition of resistant isolates. Several new media formulations and procedures have been described for performance of reliable antimicrobial drug susceptibility tests (3, 4, 5, 9). However, all of these have been modifications of conventional susceptibility test methods that require an additional 18- to 24-h period for completion after the organism is isolated on primary growth media. More recently, penicillinase-producing strains of Neisseria gonorrhoeae have been isolated from at least 11 different countries, including the United States (Morbidity Mortality Weekly Rep. vol. 26, no. 5, p. 29, 4 February 1977). Like the ampicillin-resistant Haemophilus isolates, penicillin-resistant strains of gonococci may occur sporadically in widely separated geographic areas in the future.

Several rapid penicillinase tests have recently been described which allow recognition of beta-lactamase-producing (and thus penicillin- and ampicillin-resistant) bacteria as soon as growth is apparent on initial media (1, 2, 7, 8). The present report describes an even more rapid, simplified procedure for detection of penicillinase activity in H. influenzae and N. gonorrhoeae that is based on a method originally described by Shaefler (Abstr. Annu. Meet. Am. Soc. Microbiol. 1972, M209, p. 114).

Strips of Whatman no. 3 filter paper (approximately 5 by 1 cm) are immersed in a solution of 0.2% soluble starch (BBL) and 1% penicillin (potassium penicillin G for injection). The strips are then allowed to dry at room temperature for approximately 2 h on a perforated metal rack. Dried strips are then stored at \(-20^\circ\)C, which ensures their stability for at least 1 year.

To perform the test, a strip is removed from the freezer and placed in a disposable plastic petri dish. The strip is thoroughly moistened with either Gram or Lugol iodine, producing a deep purple color, and excess liquid is then poured off. Approximately 10 colonies of the primary growth of the test organism on chocolate agar or Thayer-Martin medium are then applied to the center of the moistened strip by rubbing it with an inoculating loop in a circular fashion, describing a circle approximately 5 mm in diameter. For purposes of quality control, stock strains of H. influenzae or N. gonorrhoeae showing both ampicillin (or penicillin) resistance and susceptibility are likewise immediately applied to either end of the paper strip. If penicillinase is produced, the deep purple color of the strip becomes white within 1 min in the area where the organisms were applied. If penicillinase is not produced, the strip remains purple or becomes slightly yellow at the immediate site of inoculation. A completed test is considered valid only if the resistant control produces a large white zone, and the susceptible control strain does not.

The mechanism of the test involves a preliminary positive starch test as evidenced by the strip turning purple when iodine solution is added. The test area of the strip becomes white if penicilloic acid produced by the action of penicillinase converts the iodine to idioide, which is then no longer available to form the purple starch-iodide complex.

Parallel agar dilution ampicillin susceptibility tests (3) and penicillinase tests performed on a group of 100 isolates (68 susceptible, 32 resistant) of H. influenzae showed agreement be-
tween the two procedures in 99 of 100 instances. One strain had an ampicillin minimal inhibitory concentration (MIC) of 8 µg/ml, but consistently gave negative penicillinase tests by the present method and two previously described techniques (1, 7). This strain would seem to be unique in possessing a resistance mechanism other than penicillinase production, and it bears further investigation.

Twenty-seven strains of *N. gonorrhoeae* were examined for penicillinase production and susceptibility to penicillin by an agar dilution procedure that uses Mueller-Hinton agar supplemented with 1% IsoVitaleX. Seventeen isolates were penicillinase test negative and had penicillin MICs of ≤0.5 U/ml, whereas 10 isolates with MICs of ≥2 U/ml yielded positive penicillinase tests.

The current policy in our hospital laboratory is to perform rapid penicillinase tests on all clinically significant *Haemophilus* isolates and all isolates of *N. gonorrhoeae*. These are followed by confirmatory conventional susceptibility testing of *H. influenzae* with ampicillin and chloramphenicol (3). This assures that ampicillin-resistant, but non-penicillinase-producing, strains, as well as the recently described chloramphenicol-resistant strains of *Haemophilus*, (6) may be detected. Likewise, any penicillinase-producing isolate of *N. gonorrhoeae* is verified by a conventional agar dilution susceptibility test. The method that we have described is a simpler, more rapid modification of the iodometric technique for detection of penicillinase activity. We have found it to be superior to previously described methods with respect to technical simplicity, time required, and stability of reagents.

LITERATURE CITED