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Simple Test for Identifying Penicillinase-producing Staphylococci

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The resistance of staphylococci to penicillin depends primarily on the ability of the organism to produce penicillinase (β-lactamase). In some strains of potentially penicillin-resistant staphylococci, demonstration in vitro of resistance (i.e., of penicillinase production) may require not only prior induction of synthesis of the enzyme but also a highly sensitive method for detecting its presence. This report concerns a procedure for induction and demonstration of penicillinase that is simple, reliable, sensitive, and suitable for routine use in clinical bacteriology laboratories.

The test is an adaptation of the *N*-phenyl-1-naphthylamine-azo-*o*-carboxybenzene (PNCB) test of Novick (2, 3). PNCB is an acid-base indicator that is water-soluble and orange-yellow when basic, and water-insoluble and purple when acid. The test employs a methicillin sensitivity-test disc for induction, and is performed directly on staphylococcal colonies in situ. It may be referred to as the methicillin-induced PNCB test (MI-PNCB test).

To perform the test, the surface of a Mueller-Hinton plate (Difco, *p*H 7.4) is seeded with the staphylococcus culture to be tested, as in the disc-agar diffusion method of antibiotic sensitivity testing. A 5-μg methicillin disc (BBL) is pressed gently on the seeded surface, and the plate is then incubated overnight at 37 C. In the diffusion gradient of methicillin emanating from the 5-μg disc, at some point or points beyond the zone of inhibition, methicillin is present in concentrations ideal for maximum induction.

After incubation overnight, the plates are opened and dried at 37 C for about 1 hr. Next, the agar surface, especially the area containing organisms proximal to the zone of inhibition, is flooded with about 1.5 ml of 0.25% (w/v) stock solution of PNCB (K & K Laboratories, Inc., Plainview, N.Y.) in *N*,*N*-dimethyl formamide (Fisher Scientific Co., Pittsburgh, Pa.) with 6% (v/v) 1 N NaOH. The plates are then placed in a hood for drying for about 45 min. After drying, the areas stained by the PNCB indicator are flooded with 1.5 ml of a refrigerated stock solution of 10% aqueous benzylpenicillin.

If penicillinase is present, hydrolysis of benzylpenicillin to penicilloic acid rapidly occurs. The production of another —COOH group per molecule of penicillin changes the PNCB indicator from basic to the acidic, water-insoluble, purple compound. In such cases, not only does an immediate, purple precipitate occur over the colonies surrounding the zone of inhibition, but also the intensity of staining decreases as the radial distance from the disc increases (Fig. 1 and 2). If penicillinase is not present and benzylpenicillin is essentially unaltered, no change in the orange-yellow acid-base indicator is observed. The staphylococcus in question is considered a nonproducer of penicillinase if no change is observed after 45 min.

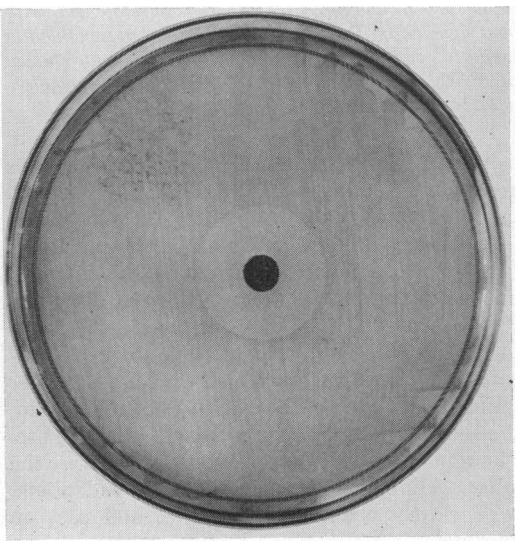

FIG. 1. *Mueller-Hinton plate containing penicillinase-producing S. aureus after staining with PNCB indicator but before development with benzylpenicillin. Note inhibition zone of 5-μg methicillin disc.*

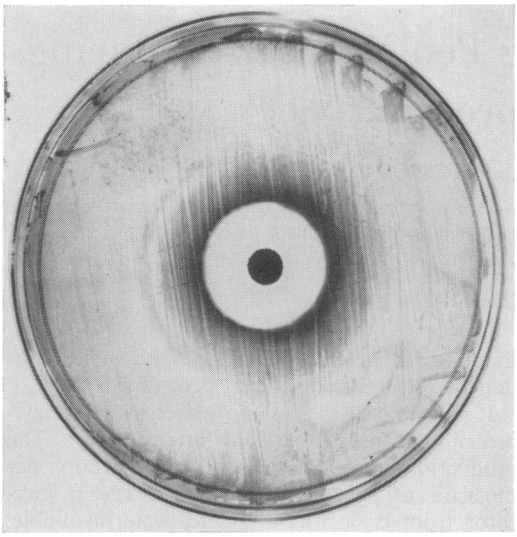

FIG. 2. *Mueller-Hinton plate containing penicillinase-producing S. aureus 15 min after development with benzylpenicillin. Dark areas (purple) around methicillin inhibition zone represent precipitation and color change of PNCB indicator due to formation of penicilloic acid.*

Two hundred ten cultures of *Staphylococcus aureus* and *S. epidermidis* were tested for penicillinase activity by the MI-PNCB test and by the iodometric procedure (1; Table 1). Of 76 cultures in which penicillinase could be demonstrated by an iodometric test, all were also positive by the PNCB test. Of 134 strains in which the iodometric test failed to detect penicillinase, 20 cultures were positive by the MI-PNCB test. After induction of seven of these cultures by methicillin, the iodometric test was positive for penicillinase. The remaining 13 cultures were not tested by the latter technique after induction. All end points were clearly positive or negative, and easy to interpret.

These data indicate the usefulness of the methicillin-induction portion of the procedure, and the relative sensitivity of the PNCB test. These qualities, plus the ease of performance and interpretation, the economy, and the simplicity of the method recommend the test as a routine procedure for determining the penicillin resistance of staphylococci in clinical bacteriology laboratories.

TABLE 1. *Comparison of methicillin-induced PNCB test with iodometric test for detecting penicillinase production by staphylococci*

Determination	Penicillinase present by methicillin-induced PNCB test		Penicillinase absent by methicillin-induced PNCB test	
	No. of *S. aureus* isolates	No. of *S. albus* isolates	No. of *S. aureus* isolates	No. of *S. albus* isolates
Penicillinase present by iodometric test	31	45	0	0
Penicillinase absent by iodometric test	4[a]	16[b]	62	52
Total no. of isolates tested	35	61	62	52

[a] After methicillin induction, one isolate produced penicillinase as measured by iodometric test. Remaining isolates not induced.

[b] After methicillin induction, six isolates produced penicillinase as measured by iodometric test. Remaining isolates not induced.

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