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Rapid Detection in Spinal Fluid of Beta-Lactamase Produced by Ampicillin-Resistant *Haemophilus influenzae*

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The chromogenic cephalosporin nitrocefin was used to detect the presence of beta-lactamase in cerebrospinal fluid of patients suffering from ampicillin-resistant *Haemophilus influenzae* meningitis. Five samples of spinal fluid containing ampicillin-resistant isolates were studied, and all had beta-lactamase activity. When samples of spinal fluid containing 33 ampicillin-sensitive isolates and 234 sterile specimens were tested, no beta-lactamase activity was detected.

In recent years, efforts have been made to rapidly detect ampicillin resistance of *Haemophilus influenzae*, especially that of *H. influenzae* isolated from cerebrospinal fluid (CSF). Most of these methods require that the causative agent be isolated on semisolid media before the test is performed (2, 5, 7). Recently, there has been a report of a method for the direct detection of beta-lactamase in body fluids (9). This procedure requires radioisotopes and radioisotopic measurement equipment.

The method reported here, although limited to CSF, is rapid and inexpensive and may be performed at the clinical microbiology bench with no special equipment.

Specimens of CSF were obtained from patients by established procedures. The specimens were centrifuged at 1,100 relative centrifugal force for 15 min. The pellet containing the cells was separated from the supernatant. The pellet was subsequently examined by Gram stain, plated on microbiological growth media, and incubated at 35°C in 6 to 10% CO_2 for 18 h.

After the pellet was plated, the pellet and supernatant were tested separately for the presence of beta-lactamase activity as described below.

Isolates recovered after incubation were tested for beta-lactamase production followed by a modified Bauer-Kirby susceptibility method (5), and resistant strains (which were all beta-lactamase-producing strains) were also tested to determine the minimum inhibitory concentrations (MICs) of ampicillin for these strains.

A slight modification of the chromogenic cephalosporin test was used to detect beta-lactamase activity in CSF (8). One to two drops of nitrocefin (chromogenic cephalosporin compound 87/312; Glaxo Group Research Ltd., Middlesex, England) was added directly to the CSF supernatant, and one drop was added to the pellet. The mixture was then incubated at room temperature for 1 h. A red color, which usually deepens to a deep burgundy, indicates a positive reaction.

A working solution of nitrocefin was prepared by the addition of 0.5 ml of dimethyl sulfoxide to 5 mg of solid. Immediately after the compound had dissolved, 9.5 ml of 0.1 M phosphate buffer (pH 7.0) was added and shaken well to mix. The solution may be stored in the dark in the refrigerator (5°C) for up to 14 days.

Bauer-Kirby susceptibilities were performed with Mueller-Hinton agar containing 5% chocolatized rabbit blood and 1% IsoVitaleX (BBL Microbiology Systems) (1, 5).

A modified microdilution susceptibility method, with Levinthal broth supplemented with 5% Fildes as the test medium, was used to test the MICs of ampicillin for beta-lactamase producers (3).

A total of 272 CSF specimens were tested for beta-lactamase activity (Table 1). We recorded no positive reactions in any specimen other than those that grew an ampicillin-resistant strain of *H. influenzae*.

Four CSF specimens were positive for beta-lactamase within 10 to 30 min, and the microorganisms demonstrated no zone of inhibition to ampicillin when tested by the disk diffusion method. Ampicillin MICs for these isolates were 3.1, 6.25, 12.5, and 50 μg/ml.

In one case, a CSF specimen was accidentally incubated overnight and was positive the next morning. This specimen was retested on a duplicate aliquot and required 7 h to become positive. The microorganism had a Bauer-Kirby zone size of 17 mm. Unfortunately, the organism was discarded, and an MIC determination could not be performed. Twelve sterile spinal fluid samples and two spinal fluid samples containing ampicillin-sensitive *H. influenzae* were incubated for 18 h and remained negative.

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TABLE 1. Summary of beta-lactamase tests on sterile and nonsterile CSF

No. tested	Culture result	Beta-lactamase	Disk diffusion ampicillin susceptibility	Ampicillin MIC (μg/ml)
1	*H. influenzae* b	Positive	Resistant	50
1	*H. influenzae* b	Positive	Resistant	12.5
1	*H. influenzae* b	Positive	Resistant	6.25
1	*H. influenzae* b	Positive	Resistant	3.1
1	*H. influenzae* b	Delayed positive[a]	Resistant[b]	ND[c]
26	*H. influenzae* b	Negative	Sensitive	
1	*H. influenzae* a	Negative	Sensitive	
3	*Streptococcus pneumoniae*	Negative	Sensitive	
2	*Streptococcus agalactiae*	Negative	Sensitive	
1	*Neisseria meningitidis*	Negative	Sensitive	
234	Sterile	Negative		

[a] See text.
[b] Zone size was 17 mm.
[c] ND, Not done.

With patients from whom ampicillin-sensitive microorganisms were cultured, we recorded CSF leukocyte counts ranging from 700 to 1,000/mm^3, protein ranging from 15 to 382 mg/100 ml, and glucose ranging from 0 to 55 mg/100 ml. There were no false-positive reactions. Grossly bloody taps were discarded because the color may mask a positive reaction. Large amounts of serum protein may produce a false-positive reaction.

We recorded no false-negative reactions, but we recognize that they are possible if the number of microorganisms is too small to produce detectable amounts of enzyme. There have been occasional reports of beta-lactamase nonproducing, ampicillin-resistant *H. influenzae* (4, 6), but we recovered none of these strains during our study.

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