A Simple Disk Diffusion Test Using Cephalexin to Identify β-Lactamase-Negative, Ampicillin-Resistant Haemophilus influenzae

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Background: β-lactamase-negative (BLN), ampicillin-resistant (AR) strains of Haemophilus influenzae are now becoming prevalent in Japan. BLNAR strains are principally defined by the presence of specific mutation(s) in the ftsI gene, but are not distinguishable by the phenotypic ampicillin-susceptibility. In the study, we demonstrate the alternative, phenotypic test method to identify BLNAR strains, that is, the cephalexin disk diffusion test can discriminate between BLNAR and BLN, ampicillin-susceptible (BLNAS) strains of H. influenzae.

Methods: A total of one hundred forty clinical isolates of BLN H. influenzae were included. All the isolates were genetically defined through detection of TEM gene and partial sequencing the ftsI gene. The CLSI standard disk diffusion and broth microdilution tests were performed using Haemophilus test medium (HTM) and HTM broth, respectively. In disk diffusion test, visible inhibitory zone diameter was measured after a 24 hour-incubation.

Results: Of 140 isolates of BLN H. influenzae tested, 110 (79%) were genetically identified as being BLNAR strains and the remaining 30 were BLNAS. Of 110 BLNAR isolates, 23 were interpreted as being ampicillin-susceptible with MICs of <2 μg/ml, the results indicating 21% of false-susceptible occurrences. Also, disk diffusion tests demonstrated 16% of false-susceptible rate to ampicillin. Alternatively, a total of 34 cephems were tested to discriminate between BLNAR and BLNAS strains. Of the agents tested, cephalexin was the best. After a 24 hour-incubation, 106 (96%) of 110 BLNAR isolates did not produce any visible inhibitory zones, whereas all the BLNAS isolates produced significant inhibitory zones.

Conclusions: The CLSI standard disk diffusion test adjusted using cephalexin is an alternative, simple, and easy-to-perform phenotypic test method to detect BLNAR strains of H. influenzae in clinical microbiology laboratories.