A Simple Disk Diffusion Test to Identify $\beta$-Lactamase-Negative, Ampicillin-Resistant Haemophilus influenzae A pplication of Cephalexin, Cefsulodin and Cefaclor Disks

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Abstract: Currently, $\beta$-lactamase-negative (BLN) ampicillin-resistant (AR) strains of Haemophilus influenzae are prevalent in Japan. BLNAR strains are defined by the presence of specific mutation(s) in the $ftsI$ gene but are not phenotypically distinguishable by ampicillin (ABPC) susceptibility. In the present study, we showed that cephalexin (CEX), cefsulodin (CFS), and cefaclor (CCL) disk diffusion tests can be effectively used to identify BLNAR strains. A total of 169 clinical isolates of BLN H. influenzae, consisting of 113 of BLNAR and 56 of BLN, ampicillin-susceptible (AS), were included. All the isolates were genetically defined by detection of the TEM gene and partial sequencing of the $ftsI$ gene. The Clinical and Laboratory Standards Institute (CLSI) standard broth microdilution and disk diffusion tests for ABPC provided 20% and 19% false-susceptible rates, respectively. Alternatively, 34 cepham agents were tested using disk diffusion. Of the agents tested, CEX, CFS, and CCL disks could effectively discriminate between BLNAR and BLNAS isolates. All the BLNAS isolates showed visible growth inhibitory zones around CEX and CFS disks, but 108 (95.6%) and 106 (93.8%) BLNAR isolates did not. The results indicated 100% predictive values (PVs) for BLNAR and PVs for BLNAS were 91.8% for CEX and 88.9% for CFS. The CLSI-based interpretations for CCL ($\geq$20 mm) also highly correlated with BLNAR and BLNAS, PVs for BLNAR and for BLNAS being 100% and 93.3%, respectively.

With simplicity and discriminability of the test method, we recommend a CEX disk diffusion test in combination with a rapid $\beta$-lactamase test to identify BLNAR isolates in clinical laboratories.

Key words: $\beta$-lactamase-negative, ampicillin-resistant (BLN-ラクタマーゼ陰性, アンピシリン耐性), Haemophilus influenzae (ヘモフィルス・インフルエンザ菌), disk diffusion test (ディスク拡散法), cephalaxin (セファレキシン), phenotypic identification (表現型同定)