Multicenter-Evaluation of Optimal Cell Density to Determine Antimicrobial Susceptibility for Haemophilus influenzae by the Automated RAISUS System When the Early-Harvested Bacterial Cells Were Used.
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【Abstract】
The fully automated microbial system, RAISUS (Nissui Pharmaceutical, Tokyo, Japan) can provide antimicrobial susceptibility test results for the isolates of Haemophilus influenzae. It is known that viable cell concentrations (colony forming unit/ml) of H. influenzae significantly vary depending on the incubation period. For the rapid reporting of antimicrobial susceptibility test results, we evaluated optimal cell density when we prepared the cell suspension using the early-harvested (6 hour-incubation) cells for RAISUS. A total of 180 clinical isolates, comprising of 36 ampicillin-susceptible isolates, 111 β-lactamase-negative but ampicillin-resistant isolates and 33 β-lactamase-positive and amoxicillin/clavulanic acid-susceptible or -resistant isolates, were included. All the isolates were genetically defined according to the detection of TEM gene and specific mutation(s) in fts I gene. The isolates were incubated on chocolate agar plates for 6 hours, and then the cell suspensions were prepared and adjusted to 0.5, 0.25 and 0.125 McFarland standards through serially dilutions. The respective cell suspensions were tested by the RAISUS AST panels. The % agreements between RAISUS and Clinical and Laboratory Standards Institute standard microdilutions in ampicillin category interpretations were
66.7% (McFarland 0.5), 77.8% (McFarland 0.25) and 83.9% (McFarland 0.125). When the McFarland 0.125 cell suspensions were inoculated, the majority of discrepant interpretations were minor errors (15.0%) and the occurrence of major error was 3.4%. There was no very major error throughout the study. Essential agreement in MIC determinations (with or within ±1 doubling dilution) for 11 β-lactam antimicrobial agents tested improved to 95.2% by McFarland 0.125 when compared to 77.4% by McFarland 0.5. It was also demonstrated that the viable cell concentrations prepared from 6 hour-incubation cultures were 2.5 to 6.5 times higher than those from 22 hour-incubations. With these results, it can be concluded that the early-harvested cell suspension of *H. influenzae* is applicable to RAISUS antimicrobial susceptibility test with lower cell density (McFarland 0.125). With this adjustment, the antimicrobial susceptibility test for *H. influenzae* will be completed by RAISUS within 26 hours after primary isolation.

Key Words: Early-harvested bacterial cell (短時間培養細菌細胞), *Haemophilus influenzae* (インフルエンザ桿菌), ampicillin-resistance (アンピシリン耐性), antimicrobial susceptibility test (薬剤感受性試験), automated RAISUS System (ライサス自動細菌検査装置)

【要旨】
全自動細菌検査装置、RAISUS を用いた *H. influenzae* 薬剤感受性試験の検査所要時間の短縮を目的に、継代純培養時間を 6 時間に短縮し、接種菌液濃度を McFarland 0.125 濁度に設定することで CLSI 微量液体希釈法と高い互換性をもつ結果判定が可能であると結論された。この検査手順を採用することで、従来法と比べ 1 日早い結果報告が可能となる。しかし、β-ラクタマーゼ陰性、ABPC 耐性の BLNAR については、CLSI 勧告が検査原理とする微量液体希釈法および RAISUS での MIC 測定では、遺伝子型との一致率が低く、正確な同定には限界が認められた。