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.


Background: The fully automated microbial system, RAISUS (Nissui Pharmaceutical, Tokyo, Japan) can provide antimicrobial susceptibility test (AST) results for the isolates of Haemophilus influenzae. It is known that viable cell concentrations (CFU/ml) of H. influenzae significantly vary depending on the incubation period. For the rapid reporting of AST, we evaluated optimal cell density when we prepared the cell suspension using the early-harvested (6 hour-incubation) cells for RAISUS.

Method: A total of 180 clinical isolates, comprising of 36 ampicillin (AMP)-susceptible isolates, 111 β-lactamase (BL)-negative but AMP-resistant isolates and 33 BL-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 (McF.) standards through serially dilutions. The respective cell suspensions were tested by the RAISUS AST panels.

Results: The %agreements between RAISUS and CLSI standard microdilutions in AMP category interpretations were 66.7% (McF. 0.5), 77.8% (McF. 0.25) and 83.9% (McF. 0.125). When the McF. 0.125 cell suspensions were inoculated, the majority of discrepant interpretations were minor errors (15.0%) and the occurrence of major error was 1.1%. There was no very major error throughout the study. Essential agreement in MIC determinations (±1 doubling dilution) for 11 beta-lactam antimicrobial agents tested improved to 95.2% by McF. 0.125 when compared to 77.4% by McF. 0.5. It was also demonstrated that the viable cell concentrations (CFU/ml) prepared from 6 hour-incubation cultures were 2.5 to 6.5 times higher than those from 22 hour-incubations.

Conclusion: The early-harvested cell suspension of H. influenzae is applicable to RAISUS AST with lower cell density (McF. 0.125). With this adjustment, the AST results for H. influenzae will be completed by RAISUS within 26 hours after primary isolation.