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VALIDATION OF COMMERCIAL AUTOMATED BACTERIAL SUSPENSION PREPARATION AND PLATE STREAKING FOR ANTIBIOTIC DISK DIFFUSION SUSCEPTIBILITY TESTING

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BACKGROUND

- One of the most crucial responsibilities of clinical microbiology laboratories involves conducting precise and fast antimicrobial susceptibility testing (AST) on bacterial isolates, necessary to guide antibiotic therapy.
- Standardized disk diffusion, a manual AST method, consumes a significant amount of time and is error-prone.
- Total lab automation in microbiology should enable a lower workload, high traceability, and standardization in AST.
- Therefore, we examined the concordance at the categorical level between the manual reference method and a new automated approach for bacterial suspension preparation and plate streaking in AST.

OBJECTIVE

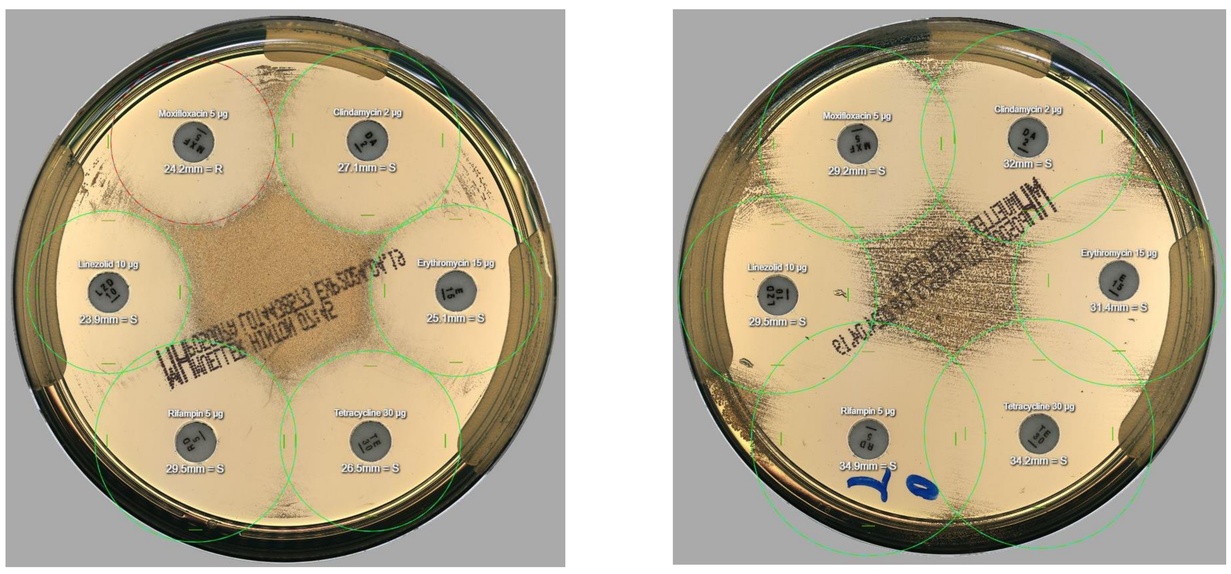
In this study, we validated the automated bacterial suspension preparation by Colibri® and plate streaking by WASP® for antibiotic disk diffusion susceptibility testing.

METHODS

- Fifty-three non-duplicate bacterial strains, derived from a variety of different bacterial species, encompassing key known resistance mechanisms and comprising both Gram-positive (N=16) and Gram-negative (N=37) strains, were tested.
- The strains were identified using matrix-assisted laser desorption/ionisation time of flight mass spectrophotometry, according to the manufacturer's instructions.
- Both the manual (reference) and the automated (Colibri® with WASP®) method for AST preparation and plate streaking, used the Radian® in-line carousel and expert system for antibiotic susceptibility interpretation.
- Regarding halo adjustments, we typically needed to make adjustments in fewer than 1% of cases.
- European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints (version 13.1) were used to interpret susceptibility results.
- The categorization of agreement follows the ISO-20776-2 standard, which includes the identification of minor (mES), major (MEs), and very major errors (VMEs).

RESULTS

- The overall categorical agreement between the two compared methods was 96.5% (635/658).
- We identified 2.6% (17/658) mES, 1.7% (5/658) MEs, and 0.3% (1/658) VMEs. The sole VME occurred during the examination of piperacillin/tazobactam in *Escherichia coli*. However, it is noteworthy that the zone diameter in the manual method measured was 19 mm, placing it within the "area of technical uncertainty".
- Overall, we tend to see more confluent growth when using the novel method.



A. B.

Figure 1. *Two images depicting disk diffusion of a clinical Staphylococcus aureus isolate on Mueller-Hinton agar were captured using WASPLAB®. Both images, labeled A and B, utilized the Radian in-Line Carousel® and the Radian Expert System® for antibiotic disk dispensing and halo interpretation. Plate streaking and suspension preparation were conducted by WASP® in image A, whereas they were performed manually in image B. In this case, we observe a discrepancy (ME) where moxifloxacin is classified as resistant according to the new method but susceptible according to the standard/reference method.*

Figure 2. *A picture of the Copan's Colibrí® we used in this validation, a device designed to automatize colony picking, and preparation of targets for the identification of bacteria and yeasts through MALDI-TOF technology, and bacterial suspensions for AST. The Colibrí® coupled with the Radian in-Line Carousel® and the Radian Expert System® for antibiotic disk dispensing and halo interpretation, provide a fully automated solution for antimicrobial disk diffusion susceptibility testing.*

CONCLUSION

The combination of Colibri® and WASP® appears to be a compelling automated tool for the automated preparation of bacterial suspensions and plate streaking in AST, with an accuracy that is equal to the reference method. Furthermore, it enables the optimization of hands-on time and standardization of (pre-) analytical procedures. At a later stage, the sample set will be augmented with additional species and resistance mechanisms to confirm the findings of this preliminary data set.