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Performance Evaluation for Piperacillin/Tazobactam in ESBL-Producing *E. coli* Strains (OXA-1 and TEM-1) in an Automated Rapid AST System Using the New FDA Breakpoints

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Background

E. coli isolates with the bla_{OXA-1} resistance mechanism yield higher minimum inhibitory concentration (MIC) values of piperacillin/tazobactam (PTZ). The MERINO trial found that infections caused by OXA-1-producers had higher 30-day mortality than other isolates. These isolates were associated with poor reliability in PTZ antimicrobial susceptibility testing (AST) from commercial panels and frequently yielded MIC values within the area of technical uncertainty category (ATU), possibly contributing to an increased mortality rate[2]. bla_{TEM-1} is one of the most common β-lactamase resistance mechanisms and can also increase PTZ MIC with values within ATU[3]. Due to these problems, IDSA, European and British guidelines have recently rejected the use of PTZ as a carbapenem-sparing agent in ESBL-producers infection.

ASTar is a rapid phenotypic AST device produced by Q-linea, shown in Figure 1. The ASTar system processes blood culture samples to generate data on MICs and susceptible, intermediate, or resistant (SIR) categories. ASTar provides a susceptibility report in approximately 6 hours. The aim of this study was to evaluate the performance of ASTar regarding PTZ MIC among whole genome sequence (WGS) characterised *E. coli* from a recent multicenter Spanish study. ASTar was evaluated using the latest standard for AST, ISO 20776-2:2022. In this standard, discrepancy resolution is performed for MIC results outside of EA (essential agreement). Discrepancies should be resolved with a triplicate test of the reference method and ASTar. ISO 20776-2:2022 requires a bias assessment with an acceptable bias of ±30%. This poster shows the preliminary results for this study.

Fig 1. ASTar Instrument.

Materials and methods

68 WGS-characterised *E. coli* isolates, were selected from the study site isolates and a targeted ST131 clone survey: 34 had $bla_{OXA-1,}$ and 34 had bla_{TEM-1}. 9 ml of human blood was spiked with isolates at the Q-linea laboratory into blood culture bottles (BD BACTEC Plus plastic Aerobic/F) and grown until signalling positive. An aliquot of positive blood culture was loaded into an ASTar instrument. The instrument automatically purified bacteria from the blood culture, determined the concentration of the isolated bacteria, and adjusted to a controlled inoculum before transferring the bacterial culture to the ASTar Disc (Piperacillin/tazobactam concentration range 0.25–256 mg/l). The results were compared with the modal MIC from the reference triplicate broth microdilution (BMD). Essential agreement (EA) and bias were calculated.

Results

18 (51%) OXA-1 producers and 30 (88%) TEM-1 producers were piperacillin/tazobactam susceptible, according to BMD. All MIC values for both ASTar results, and the reference method results were on-scale. Overall EA for the rapid automated system and BMD for PTZ was 94%: 88% for TEM-1 producers and 100% for OXA-1 producers, MIC comparisons for the OXA-1 isolates is shown in Figure 2, and in Figure 3 for TEM-1 isolates.

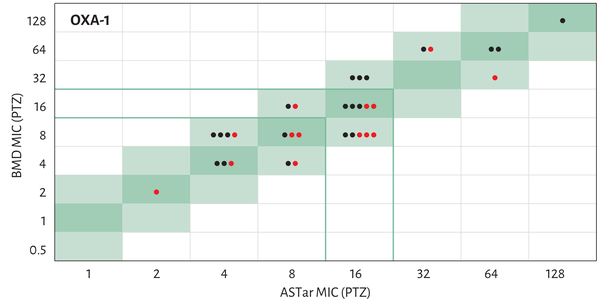

Fig 2. MIC comparison between ASTar and broth microdilution for OXA-1 producers. The shaded cells are in essential agreement with the reference method. The red points represent isolates that also contain the ESBL gene CTX-M. The highlighted column and row edges represent the FDA breakpoints; susceptible ≤ 8, intermediate = 16, resistant ≥ 32.

The overall categorical agreement was 81% with no Very major discrepancies, 2 (4%) Major discrepancies and 11 (16%) Minor discrepancies. This panel was challenging because the MIC values were close to the breakpoint, especially for OXA-1. The total bias for all results was 5.9% (8.8% for OXA-1 and 2.9% for TEM-1). Discrepancy resolution is continuing and will be completed as part of a future publication.

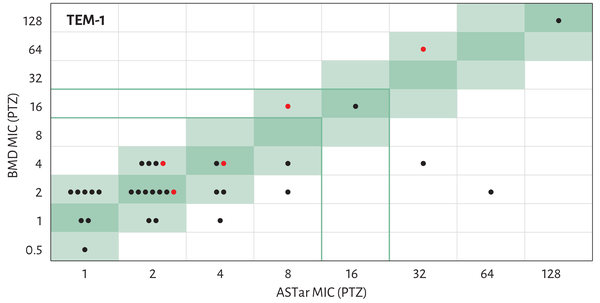

Fig 3. MIC comparison between ASTar and broth microdilution for TEM-1 producers. The shaded cells are in essential agreement with the reference method. The red points represent isolates that also contain the ESBL genes CMY and CTX-M. The highlighted column and row edges represent the FDA breakpoints; susceptible ≤ 8, intermediate = 16, resistant ≥ 32.

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Conclusion

The ASTar system can provide MIC of PTZ on the same working day of detecting a positive blood culture of *E coli*, with a good concordance with reference even in isolates that pose difficulties with other commercial devices. With this improvement in the accuracy of piperacillin/tazobactam sensitivity, the use of this combination in ESBL-producing infections could be re-evaluated.

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