Performance of a rapid phenotypic AST System for susceptibility testing of Gram-negative bacteraemia directly from positive blood culture bottles

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Background

Q-linea has developed the ASTar® System for automated AST direct from positive blood cultures. The instrument is shown in Figure 1. This study aimed to assess the performance of ASTar, which is newly available to the European market.

Fig 1. ASTar Instrument.

Materials and methods

The performance of ASTar was assessed against broth microdilution (BMD), as the reference method (Sensititre). Testing was performed across three sites, utilising 412 contrived blood samples and 74 clinical patient samples. The 23 tested antimicrobials and 14 gram-negative (G–) bacterial species (fastidious and non-fastidious) generated a total 222 antimicrobial-species combinations, and in addition cefoxitin was used as a screening agent for AmpC.

The AST consumable, in the form of a disc, contains dried antimicrobials in two-fold dilution steps. Bacteria are isolated followed by concentration determination and adjustment in Cation Adjusted Mueller-Hinton Broth (CAMHB) and in fastidious-supplemented CAMHB. The range and number of dilutions for each antimicrobial present in the ASTar Disc is shown in Table 3.

The 'ISO 20776-2:2007' definitions of S, I and R (susceptible, intermediate, and resistant) susceptibility testing categories do not match those of recent EUCAST clinical breakpoint updates[1,2]. VMDs and MDs can only be calculated when S and R categories are present. Therefore, calculations were made using two different strategies, shown in Table 1.

Table 1. Discrepancy calculation strategies.

Strategy 1	Strategy 2
For combinations lacking an "S" category, only minor discrepancies can be calculated.	For combinations lacking an "S" category the "I" category can be interpreted as "S". This way, MDs and VMDs can be calculated. This may result in higher VMD and MD percentages.

Reference

1. *ISO 20776-2 Clinical laboratory testing and in vitro diagnostic test systems — Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices — Part 2: Evaluation of performance of antimicrobial susc.*
2. European Committee on Antimicrobial Susceptibility Testing Breakpoint tables for interpretation of MICs and zone diameters Version 12.0. (2022). In *http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_5.0_Breakpoint_Table_01.pdf*

Conclusion

The ASTar System shows an overall EA and CA of more than 95% for a large set of different antimicrobials and for both fastidious and non-fastidious bacteria. Combined with rapid MIC results and high automation, ASTar has the potential to support both antimicrobial escalation as well as de-escalation.

Results

The accuracy data set comprised 8,650 data points, demonstrating an overall essential agreement (EA) of 95.8% and categorical agreement (CA) of 97.6%. EA and CA values for the antimicrobial are shown in Table 2.

In cases when isolates have MIC close to clinical breakpoints, or where there is no intermediate category, inaccurate results can cause a greater percentage of MDs and VMDs, even if the results are in Essential Agreement with the reference method. Few available resistant isolates may also increase VMDs. In both cases, the EA may give a better estimate of the AST performance. The number of isolates close to susceptible and resistant clinical breakpoints are shown in Table 2.

Regarding cefoxitin, ASTar measured a positive agreement of 93.2% and a negative agreement of 92.1% when compared to BMD, shown in Table 4.

Table 2. EA, CA performance characteristics data for each antimicrobial in the ASTar panel, with interpretation according to strategy 1. The number of on-scale isolates within ±1 dilution of clinical breakpoints (CBP), for susceptible isolates (S-CBP) and resistant isolates (R-CBP).

Antimicrobial	EA (%)	CA (%)	±1 from S-CBP (n)	±1 from R-CBP (n)	Total (n)
Ampicillin	96.7	98.3	79	21	241
Amoxicillin-clavulanic acid	95.5	93	158	87	357
Piperacillin-tazobactam	95.4	97.7	99	44	436
Cefazolin	96.5	91.6	0	117	286
Cefepime	97.3	98.6	17	12	452
Cefotaxime	95.3	98.9	32	7	443
Cefoxitin	NA	NA	233	86	325
Ceftazidime	97.5	97	81	22	399
Ceftazidime-avibactam	91.6	98.4	15	7	429
Ceftolozane-tazobactam	97.7	98.1	40	16	426
Ceftriaxone	96.6	99.1	25	4	444
Cefuroxime	95.9	96.9	0	106	294
Ertapenem	94.7	99.8	9	7	413
Meropenem	94.6	95.8	18	14	481
Aztreonam	98.6	98.6	19	19	427
Ciprofloxacin	96.4	96	36	29	447
Levofloxacin	98.1	96.6	52	21	475
Amikacin	92.2	98.7	103	38	448
Gentamicin	95.6	98.1	128	33	431
Tobramycin	94.9	99.3	163	60	451
Tigecycline	96.4	99.5	109	19	196
Colistin	94.4	100	31	6	251
Trimethoprim-sulfamethoxazole	95.3	96.9	15	6	423

Table 3. The dilution range and number of dilution points for each antimicrobial present in ASTar Disc panel.

Antimicrobial	Reportable range		0.008 0.015 0.03 0.06 0.125 0.25 0.5 1 2 4 8 16 32 64 128 256	Dilutions
Non-fastidious	(mg/L)			
Ampicillin	1	64		7
Amoxicillin-clavulanic acid[1]	1	32		6
Piperacillin-tazobactam[2]	0.25	256		11
Cefazolin	0.25	16		7
Cefepime	0.25	64		9
Cefotaxime	0.015	128		14
Cefoxitin (screen)	1	64		7
Ceftazidime	0.25	64		9
Ceftazidime-avibactam[3]	0.125	32		9
Ceftolozane-tazobactam[2]	0.125	32		9
Ceftriaxone	0.015	128		14
Cefuroxime	1	64		7
Ertapenem	0.015	16		11
Meropenem	0.06	64		11
Aztreonam	0.25	64		9
Ciprofloxacin	0.06	8		8
Levofloxacin	0.125	16		8
Amikacin	0.5	128		9
Gentamicin	0.25	32		8
Tobramycin	0.06	32		10
Tigecycline	0.03	16		10
Colistin	0.25	8		6
Trimethoprim-sulfamethoxazole[4]	0.06	8		8
Fastidious				
Ampicillin	0.03	4		8
Amoxicillin-clavulanic acid[1]	0.5	32		7
Cefotaxime	0.015	2		8
Ceftriaxone	0.03	2		7
Meropenem	0.015	8		10
Levofloxacin	0.03	8		9

[1] For susceptibility testing purposes, the concentration of clavulanic acid is fixed at 2 mg/L
[2] For susceptibility testing purposes, the concentration of tazobactam is fixed at 4 mg/L
[3] For susceptibility testing purposes, the concentration of avibactam is fixed at 4 mg/L
[4] Trimethoprim:sulfamethoxazole in the ratio 1:19

Table 4. Performance characteristics data for screening agent Cefoxitin. MIC >8 mg/L is interpreted as a positive screening test for AmpC, using BMD as a reference.

Antimicrobial agent	Positive agreement	Negative agreement
Cefoxitin	93.2%	92.1%

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