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Comparison of MICs in *Escherichia coli* isolates from human health surveillance with MICs obtained for the same isolates by broth microdilution

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Objectives: Human health surveillance and food safety monitoring systems use different antimicrobial susceptibility testing (AST) methods. In this study, we compared the MICs of *Escherichia coli* isolates provided by these methods.

Methods: *E. coli* isolates ($n = 120$) from human urine samples and their MICs were collected from six medical laboratories that used automated AST methods based on bacterial growth kinetic analyses. These isolates were retested using broth microdilution, which is used by the food safety monitoring system. The essential and categorical agreements (EA and CA), very major errors (VME), major errors (ME) and minor errors (mE) for these two methods were calculated for 11 antibiotics using broth microdilution as a reference. For statistical analysis, clinical breakpoints provided by EUCAST were used.

Results: Five study laboratories used VITEK®2 and one MicroScan (Walkaway Combo Panel). Out of 120 isolates, 118 isolates (98.3%) were confirmed as *E. coli*. The 99 *E. coli* isolates from five study laboratories that used VITEK®2 showed high proportions of EA and CA with full agreements for gentamicin, meropenem, imipenem and ertapenem. Additionally, 100% CA was also observed in cefepime. Few VME (0.5%), ME (1.9%) and mE (1.5%) were observed across all antibiotics. One VME for ceftazidime (7.1%) and 12 MEs for ampicillin (29.4%), cefotaxime (2.4%), ciprofloxacin (3.2%), tigecycline (1.5%) and trimethoprim (22.2%) were detected.

Conclusions: MICs from *E. coli* isolates produced by VITEK®2 were similar to those determined by broth microdilution. These results will be valuable for comparative analyses of resistance data from human health surveillance and food safety monitoring systems.

Introduction

Few efforts have been made to compare the results of routinely performed antimicrobial susceptibility testing (AST) in medical laboratories with broth microdilution as used for food safety monitoring in Germany and Europe. The direct comparison of MICs will facilitate reliable comparative analyses that are also robust when changes are made in the evaluation criteria or breakpoints over time.[1] The comparison needs to consider that MICs in the human, animal and food sectors are determined by different AST methods.[2–7] Better harmonization of surveillance and monitoring for antibiotic resistance in the human and animal sector is demanded by the German national antibiotic resistance strategy DART.[8] Therefore, comparison of AST results generated by different methods is crucial. The main objective was to study the comparability of

the MICs of *Escherichia coli* isolates determined by two different methods: automated AST systems used in German human health surveillance and the broth microdilution method used in German food safety monitoring. The agreement of the results from these two methods was calculated.

Materials and methods

One hundred and twenty randomly chosen *E. coli* isolates from urine samples were collected from six medical laboratories between March and May 2019 (20 isolates per participating laboratory). The medical laboratories participated regularly in the German *Antibiotika Resistenz Surveillance* (ARS) system[9] from 2014 to 2017 and provided their results as MICs. *E. coli* isolates were sent to the National Reference Laboratory for Antimicrobial Resistance (NRL-AR) using transport swabs (Amies Agar Gel Transport Swab, Thermo Scientific Oxoid TS0001A). They were non-selectively cultured on Columbia blood agar (Oxoid, Wesel, Germany). Following incubation at $37 \pm 2°C$ for 16–22 h, the purity of the isolates was assessed. Bacterial species were confirmed as *E. coli* using a MALDI-MS Biotyper (Bruker, Bremen, Germany). If the colony morphologies of an isolate differed after initial cultivation on blood agar, PFGE (XbaI, PulsNet) was conducted. AST was performed by lyophilized broth microdilution according to the CLSI guidelines (ISO 20776-1:2006 or CLSI M31-A3) using a standardized antibiotic panel [EUVSEC and EUVSEC2 scheme, TREK Diagnostic Systems/Thermo Fisher Scientific (lyophilized), Schwerte, Germany]. Essential agreement (EA) was stated if MICs determined by the automated AST systems and by broth microdilution showed no discrepancies. A discrepancy was observed if the MICs differed by more than one dilution step (Table S1, available as Supplementary data at *JAC-AMR* Online). For the measurement of categorical agreement (CA) and errors, MICs were interpreted using clinical breakpoints published by EUCAST (Version 9.0).[10] CA was the agreement between the two measurements concerning the resulting evaluation as susceptible, intermediate or resistant. A very major error (VME) was stated if the reference test result was 'resistant' while the result from automated AST systems was 'susceptible'. A major error (ME) was defined as reference test result 'susceptible' while the automated AST systems resulted in 'resistant'. A minor error (mE) was determined if the results of one method was 'intermediate' and in the other method it was either 'susceptible' or 'resistant'. All analyses were run in R (R 3.5.1; Rstudio 1.1.442).

Results

Five participating laboratories used VITEK®2 (bioMérieux, Nürtingen, Germany). One laboratory used the MicroScan (Walkaway Combo Panel, Beckmann Coulter, Germany). The use of three different AST cards for the VITEK®2 system was reported (GN AST N387, GN AST-N371 and GN AST N263). Since the data were coming mostly from VITEK®2, this study will focus on the results of VITEK®2 system. The results and analyses of MicroScan are documented separately in the Supplementary data (Table S2). One hundred presumptive *E. coli* isolates were obtained from the five participating medical laboratories (20 isolates/participating laboratory). Out of these, 99 isolates (99%) were confirmed as *E. coli*. One isolate was identified as *Klebsiella pneumoniae* and excluded from the analyses. Of the 99 *E. coli* isolates, 7 isolates exhibited two different colony morphologies with similar PFGE patterns (Figure S1). Both of the seven pairs of isolates were included in the analyses to study this potential source of variation (Table S3). In total, 106 isolates were included in the analysis. Table 1 highlights the results of agreements and errors. Full EA and CA

(100%) were observed for gentamicin, meropenem, imipenem and ertapenem. Additionally, 100% CA was detected in cefepime. One VME was detected for ceftazidime (1 VME/14 ceftazidime-resistant isolates, 7.1% and 1/199 all resistant isolates, 0.5%). Twelve MEs (12 MEs/623 all susceptible isolates, 1.9%) were detected for ampicillin (5/17 susceptible isolates, 29.4%), cefotaxime (2/83 susceptible isolates, 2.4%), ciprofloxacin (2/63 susceptible isolates, 3.2%), tigecycline (1/65 susceptible isolates, 1.5%) and trimethoprim (2/9 susceptible isolates, 22.2%). Eight mEs (8 mEs/530 tested isolates, 1.5%) were detected in cefotaxime (1/106 tested isolates, 0.9%), and ciprofloxacin (7/106 tested isolates, 6.6%). All mEs were observed with a difference of one dilution step.

Discussion

Good agreement was observed between the result of the automated AST systems and broth microdilution (Table 1). Our study results are in line with earlier studies that reported a high level of agreement between VITEK®2 test results and broth microdilution as the reference method for AST *E. coli* isolates.[11,12] Both studies found fewer VMEs and MEs than our study (Tables 1 and S4). In these studies, testing with the automated system was repeated if discrepancies occurred. Bobenchik *et al.* (2015)[12] reported the correction of 12 VMEs out of 13 VMEs from the initial testing for their study antibiotics and 9 of 24 MEs after repeated measurements. Only if the errors still occurred after repeating the measurements were these errors included in the analyses.[11,12] This repeated testing was not foreseen in our study as we wanted to compare routine results rather than results optimized by repeated testing. As part of routine diagnostics, AST will probably only be repeated if the results are contradictory (e.g. *E. coli* resistant to cefotaxime but susceptible to ampicillin). Therefore, surveillance data are not optimized as in the cited studies. The comparative interpretation of MICs was limited by different antibiotics included in the AST in the five participating laboratories (Table S5). Different concentration ranges of antibiotics were tested in the participating laboratories and NRL-AR (Tables S6 and S7). In the medical laboratories, the variability of antibiotic substances and their range of MICs is the consequence of the use of three different AST cards manufactured for slightly different purposes[13] that contain slightly different antibiotics[7] (Table S8). Two cards were manufactured for all Gram-negative bacteria. Another card is specifically manufactured for Gram-negative bacteria from urinary samples. In food safety monitoring, fixed EUVSEC panels established by the European Commission and harmonized across Europe are used for AST of *E. coli* and *Salmonella*.[6] These panels include antimicrobial agents that are relevant to human and veterinary medicine and are considered representative of the different antimicrobial families. Some of the frequently tested antibiotics for *E. coli* in the participating laboratories, e.g. piperacillin/tazobactam, are not included in the EUVSEC panels (Table S5).[14] A broader range of concentrations than in medical laboratories is tested in the monitoring of food safety to allow for further epidemiological analyses. This is however not the purpose of routine medical laboratories that primarily aim to guide therapy decisions. The difference of the ranges results in a limited comparability of the individual MICs with respect to EA. However, as all ranges included the clinical breakpoints provided by EUCAST, the CA could be fully analysed.

Table 1. EA, CA, VMEs, MEs and mEs for each antibiotic that was tested with VITEK®2 and included in the food safety resistance monitoring panel (EUVSEC)

Antibiotics	No. of tested isolates (*n*)	EA (%)	Ref based on EUCAST[10]			CA (%)	VME (%)	ME (%)	mE (%)
			S	I	R				
Ampicillin	106	101 (95.3)	17	ND[a]	89	101 (95.3)	0 (0)	5 (29.4)	ND[a]
Cefotaxime	106	102 (96.2)	83	0	23	103 (97.2)	0 (0)	2 (2.4)	1 (0.9)
Ceftazidime	84	82 (97.6)	70	ND[a]	14	83 (98.8)	1 (7.1)	0 (0)	ND[a]
Cefepime	41	40 (97.6)	30	0	11	41 (100)	0 (0)	0 (0)	0 (0)
Ciprofloxacin	106	104 (98.1)	63	4	39	97 (91.5)	0 (0)	2 (3.2)	7 (6.6)
Gentamicin	85	85 (100)	74	0	11	85 (100)	0 (0)	0 (0)	0 (0)
Meropenem	106	106 (100)	106	0	0	106 (100)	0 (NA)	0 (0)	0
Imipenem	65	65 (100)	65	0	0	65 (100)	0 (NA)	0 (0)	0 (0)
Ertapenem	41	41 (100)	41	ND[a]	0	41 (100)	0 (NA)	0 (0)	ND[a]
Tigecycline	65	64 (98.5)	65	ND[a]	0	64 (98.5)	0 (NA)	1 (1.5)	ND[a]
Trimethoprim	21	19 (90.5)	9	0	12	19(90.5)	0(NA)	2 (22.2)	0

Ref, reference AST (lyophilized broth microdilution); S, susceptible; I, intermediate; R, resistant.
[a]Not determined (ND): no breakpoints for 'intermediate' AST results.

Our study has a few limitations. The measurements for errors could not be repeated since VITEK®2 and broth microdilution were performed in different laboratories. Moreover, this study does not cover the complete current situation of AST testing in medical laboratories in Germany because of the limited number of participating laboratories (*n* = 6) and the exclusive testing of *E. coli*. *E. coli* was chosen because it represents a substantial part of the AST data in the ARS system[9] (21.6% out of all collected pathogens in 2018) and is likewise routinely tested in food safety monitoring where it is considered as an indicator of the antimicrobial resistance situation in the population.[15] We only wanted to include laboratories that routinely provide MIC values to the ARS system together with SIR results. One laboratory used the MicroScan for automated AST and was finally excluded from the analysis. However, we observed no obvious difference between the results for this laboratory and the other laboratories (Table S2). Further comparisons of routine results of other automated AST methods with broth microdilution also using a wider range of bacteria are therefore necessary.

Conclusions

To the best of our knowledge, this is the first study that compares MIC data, which are routinely generated by automated AST systems in medical laboratories, with the results of broth microdilution used in food chain monitoring. The study findings underline the overall comparability of the AST results from medical laboratories that are part of human health surveillance with the AST results from food safety monitoring.

Transparency declarations

None to declare.

Supplementary data

Figure S1 and Tables S1 to S8 are available as Supplementary data at *JAC-AMR* Online.

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