Evaluation of CDS disc test and Thermo Fisher Sensititre™ broth microdilution method against reference ISO broth micro dilution for Enterobacteriaceae species

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Introduction

Determination of accurate antimicrobial susceptibility testing (AST) by a clinical microbiology laboratory can have a significant impact on the appropriate selection of antibiotics and patient's response to treatment. Bacterial species possessing multiple drug resistance and species with minimum inhibitory concentrations (MICs) close to the breakpoint pose a challenge for any AST. Enterobacteriaceae species are being increasingly reported to have acquired various drug resistant mechanisms with limited available antibiotic options. These newer or emerging antimicrobial resistance require constant monitoring of the ability of the available AST methods to accurately determine resistance.

A number of Enterobacteriaceae isolates with known resistant mechanisms were sourced from the United Kingdom National External Quality Assurance Scheme (UKNEQAS). The reported MICs to a number of antibiotics were determined by the ISO 20776-1:2006 broth micro dilution (BMD) reference method. The performance of two AST methods, the Calibrated Dichotomous Sensitivity (CDS) test and Thermo Fisher Sensititre GNX2Fwas compared using these isolates.

Method

Isolates

Seventeen bacterial isolates belonging to the Enterobacteriaeceae family with established MICs which were part of the UKNEQAS distribution between Nov 2016 and Jan 2019.This included six multi drug resistant organisms(resistant to three or more classes) for which the resistance mechanism had been determined by molecular methods.

Antibiotics

Nine antibiotics common to CDS, Sensititre™ GNX2F and BMD methods were compared.

CDS Disc test

Antibiotic discs were applied to the surface of a Sensitest agar plate after inoculation with a standard CDS bacterial suspension. Susceptible strains were those with an annular radius of ≥ 6 mm for all antibiotics tested except aminoglycosides (≥ 4 mm).

Sensititre test

Thermo Fisher Sensititre™ broth microdilution was performed using GNX2F plate with all antibiotics available in a range of 4 microtitre wells except Ertapenem(5), Piperacillin- Tazobactam(5) and Ceftazidime(6).

MIC

The minimum inhibitory concentration (MIC) determined by ISO 20776-1:2006 BMD method performed at two independent UKNEQAS laboratories was considered the reference standard.

Calculation

Categorical agreement (CA) was defined as agreement in the interpretation of the CDS, Sensititre™ and BMD.

Essential agreement (EA) defined as the agreement within plus or minus one 2-fold dilution of the MIC determined by the Sensititre™ and BMD.

Very Major Errors (VMEs) - Test method MIC interpretation susceptible and the BMD MIC interpretation resistant.

Major Errors (MEs) - Test method MIC interpretation resistant and the BMD MIC interpretation susceptible.

Results

Drug	CDS			Sensititre™ GNX2F		
	% CA	% VMEs	% MEs	%CA	%VMEs	%MEs
Amikacin	100	0	0	94.11	0	5.89
Cefotaxime	100	0	0	94.11	0	5.89
Ceftazidime	94.11	0	5.89	94.11	0	5.89
Ciprofloxacin	100	0	0	100	0	0
Ertapenem	100	0	0	100	0	0
Gentamicin	100	0	0	100	0	0
Meropenem	94.11	0	5.89	100	0	0
Piperacillin-Tazobactam	94.11	0	5.89	100	0	0
Tobramycin	100	0	0	100	0	0

Summary and Conclusion

The CDS test and the Sensititre™ test methods had excellent categorical agreement with the BMD method for the antibiotics tested. This resulted in identical susceptibility category results with no VMEs. There was a low percentage of MEs with both test methods for isolates with MICs close to the breakpoints.The essential agreement between the BMD and the Sensititre™ could not be calculated due to the limited number of antibiotic microtitre wells in the standard Sensititre™ GNX2F plate.

The CDS test can be effectively used to qualitatively determine the susceptibility of an isolate and proved to be an efficient method for reporting the multi drug resistant organisms. The majority of the work in the clinical laboratory is performed using a qualitative technique, for which CDS test proved to be an efficient method. When a quantitative result (i.e, the MIC) is required, the Thermo Fisher Sensititre™ GNX2F plates can be used as a suitable alternative to the reference BMD standard. Considering that the reference BMD method cannot be routinely performed in clinical laboratories, the Sensititre™ GNX2F plates can be used as a suitable alternative with its pre-prepared panels and an opportunity for automation. However there maybe inflexibility of antibiotic selection in a standard Sensititre™ commercial plate.

References

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Website: https://ukneqasmicro.org.uk

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