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A Multisite Evaluation of the Sensititre® ESBL Confirmatory Test Plate (ESBL CTP) for the Confirmation of ESBL Producing Strains of *Enterobacteriaceae (Ent.)*

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ABSTRACT

Background: Increasing reports over the past two decades of *Enterococcus.* producing plasma-mediated extended spectrum ß-lactamases (ESBL) conferring resistance to cephalosporins, penicillins, and aztreonam has necessitated a need for laboratory methods to identify these strains. A phenotypic test described by NCCLS is the strategy commonly used by the clinical laboratory. This multi-lab study was conducted to determine the efficacy of the ESBL CTP (TREK) using the NCCLS methodology as the comparator.
Methods: The ESBL CTP consists of the NCCLS recommended cephalosporins and ranges with and without the ß-lactamase inhibitor, clavulanic acid, in a dried microtitre plate, allowing for ease of use and 24mo. shelf life at room temp. The ESBL CTP contains a fluorogenic substrate allowing it to be autoread on the Sensititre Autoreader or read manually. Three clinical sites tested known ESBL + isolates characterized by phenotypic and molecular methods. Overall 513 strains were evaluated: 186 *E. coli*, 120 were confirmed ESBL+ and 337 *Kleb.spp*, 283 were confirmed ESBL+.
Results: Statistical analysis was performed to determine Sensitivity and Specificity of the ESBL CTP. Overall the Sensitivity (ability to positively determine an ESBL+) was 97.3% and 98.3% for manual and autoread respectively. The Specificity (ability to negatively determine a ESBL-) was 98% and 99% for manual and autoread respectively. Of the 186 *E. coli* strains tested, the Sensitivity/Specificity was 97.5%/100% and 99%/100% for manual and autoread respectively. Of the 337 *Kleb. spp.* tested, the Sensitivity/Specificity was 97%/96% and 98%/98% for manual and autoread respectively.
Conclusions: This study suggests the ESBL CTP can be used with confidence by clinical laboratories for the detection of ESBL in *Enterococcus.*

PURPOSE

To evaluate the performance of the Sensititre ESBL Confirmatory Test Plate in a multi Laboratory comparison study using the NCCLS microdilution reference method for phenotypic identification of ESBL producing isolates (M7 – A6).

MATERIALS & METHODS

Sensititre ESBL Confirmatory Test Plate

ESBL Confirmatory Antimicrobials Tested	Range Tested µg/ml
Ceftazidime	0.06 – 128
Ceftazidime/ Clavulanic acid	0.06/4 – 128/4
Cefotaxime	0.06 – 128
Cefotaxime/ Clavulanic acid	0.06/4 – 128/4

Summary and Principals of Use:
The Sensititre susceptibility system is a micro version of the classic broth dilution method and can provide both qualitative and quantitative susceptibility results in a dried plate format. Each microdilution plate is dosed with antimicrobial agents at appropriate dilutions and then dried.

After inoculation, the plate is sealed with an adhesive seal, incubated at 34-36C for 18-24 hours and the contents of the wells examined for bacterial growth utilizing the Sensititre automated reading system or read manually.

The Sensititre AutoReader system utilizes fluorescence technology to read 18-24 hour test plates. The technology involves the detection of bacterial growth by monitoring the activity of specific surface enzyme produced by the test organism. Growth is determined by generating a fluorescent product from a non-fluorescent (fluorogenic) substrate. The non-fluorescent substrate is prepared by conjugating a fluorescent compound to the specific enzyme substrates with a bond, which prevents fluorescence. The fluorophore is then said to be quenched. The substrate can be added to the inoculum broth and dispensed into the test plates at the same time as the test organism or the plates can be prepared with the substrate already added to the plate (SIW, Substrate In Well). Enzymatic action of the bacterial surface enzymes on the specific substrates cleave this bond releasing the fluorophore, which is now capable of fluorescing. The amount of fluorescence detected is directly related to the activity of the bacterial surface enzyme and, therefore, to bacterial growth.

Comparator method:
The reference plate was tested according to the microdilution methods published by the National Committee for Clinical Laboratory Standards (NCCLS, M7-A6). Frozen reference plates were manufactured by TREK Diagnostic Systems; Lab Services Department.

Strains
Three clinical sites tested known ESBL + isolates characterized by phenotypic and molecular methods. Overall 513 strains were evaluated: 186 *E. coli*, 120 were confirmed ESBL+ and 337 *Kleb.spp*, 283 were confirmed ESBL+. A majority of the organisms tested were well-characterized strains including both ESBL producers and strains that produced other types of ß-lactamases. Many of the strains produced multiple ß-lactamases, an increasingly common characteristic of clinically occurring strains. This collection of strains was designed to provide a highly demanding assessment of the ability of the TREK plates to accurately confirm the presence of an ESBL.

Isolates Tested by Site

	Klebsiella spp. ESBL+/ESBL-	*Escherichia coli* ESBL+/ESBL-
Creighton	120/32	51/16
Wyeth-Ayerst	110/22	46/13
Summa Health System	53/0	23/27
Total Tested	**283/54**	**120/56**

RESULTS

SENSITITRE ESBL CONFIRMATORY TEST
3 SITES COMBINED
before/after reevaluation of 3 isolates

Table 1. *E. coli* Isolates

MANUAL (SENSITOUCH) READ

		Reference			Results
	No. Tested	Positive	Negative	Total	
Test	Postive	117	3/0	120/117	Sensitivity 97.5%
	Negative	3	53/56	56/59	Specificity 94.6/100%
	Total	**120**	**56**	**186**	

SENSITITRE (AUTOREADER) READ

		Reference			Results
	No. Tested	Positive	Negative	Total	
Test	Postive	119	3/0	122/119	Sensitivity 99.0%
	Negative	1	53/56	54/57	Specificity 94.6/100%
	Total	**120**	**56**	**186**	

Table 2. Kl. pneumoniae and Kl. oxytoca Isolates

MANUAL (SENSITOUCH) READ

		Reference			Results
	No. Tested	Positive	Negative	Total	
Test	Postive	275	2	277	Sensitivity 97.0%
	Negative	8	52	60	Specificity 96.3%
	Total	**283**	**54**	**337**	

SENSITITRE (AUTOREADER) READ

		Reference			Results
	No. Tested	Positive	Negative	Total	
Test	Postive	277	1	278	Sensitivity 97.8%
	Negative	6	53	59	Specificity 98.0%
	Total	**283**	**54**	**337**	

SENSITITRE ESBL CONFIRMATORY TEST
3 SITES COMBINED
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Table 3. All Clinical Isolates

MANUAL (SENSITOUCH) READ

		Reference			Results
	No. Tested	Positive	Negative	Total	
Test	Postive	392	5/2	397/394	Sensitivity 97.3%
	Negative	11	105/108	116/119	Specificity 95.4/98.0%
	Total	**403**	**110**	**513**	

SENSITITRE (AUTOREADER) READ

		Reference			Results
	No. Tested	Positive	Negative	Total	
Test	Postive	396	4/1	400/397	Sensitivity 98.3%
	Negative	7	106/109	113/116	Specificity 96.3/99.0%
	Total	**403**	**110**	**513**	

CONCLUSIONS

- Of the 403 confirmed ESBL positive strains tested, 392 (97.3% sensitivity – 97.3% agreement) were ESBL positive with a manual read result. With the autoreader read results 396 were ESBL positive (98.3% sensitivity – 98.3% agreement).
- Of the 110 isolates that tested negative for ESBL 105 (95.4% specificity – 95.4% agreement) tested negative with a manual read and 106 (96.3% specificity – 96.3% agreement) tested negative with the autoreader read results.

This multi laboratory evaluation of the Sensititre ESBL Confirmatory Test Plate establishes its performance to be equivalent to the NCCLS methodology. The collection of isolates tested in this study provided a suitable challenge for determining the capabilities of Sensititre's Test Plate to detect ESBL producing and non-producing strains.