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Evaluation of common ESBL screening methods in clinical settings

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Abstract

Purpose

An accurate detection of ESBL in clinical setting is needed for proper therapy, surveillance programs and infection control. The purpose of our study was the evaluation of common ESBL screening methods in clinical settings in Tunisia.

Methods

91 clinical cephalosporin-resistant-*Enterobacteriaceae* strains, were tested for ESBL production using Synergy test (ST), combined disks (CD), Vitek 2 (V2) and molecular method. Sensitivity and specificity were calculated for each evaluated method. The P value was determined using Epi- Info 7 statistical software.

Results

The V2 automated method showed the highest sensitivity and the lowest specificity. However, ST and CD tests showed 100% of specificity with less sensitivity. Low sensitivity was noted in all tested methods for the group 0 (*P. mirabilis*) and the lowest specificity was observed in the group 3 (*E. cloacae, P. stuartii, S. marcescens, E. aerogenes*) with the V2 method. Statistical analysis showed significant difference between ST results and molecular results ($P<0.05$) in contrast to the two remained methods (V2/CD) that showed no significant difference ($P> 0.05$). Our study showed that the most appropriate method for groups G0 (P. mirabilis), G1 (*E. coli*) and G2 (*K. pneumoniae C. koseri*) is the method of V2 in opposite to group 3 where the most reliable method is that of combined disks. Synergy test appear to be the imperfect method with high false negative results.

Conclusion

According to our results, we recommend to use the V2 and the CD in order to minimize the error rates of false positive and false negative results.

Keywords: ESBL*, Enterobacteriaceae,* Synergy Test, Combined Disks, Vitek 2

Résumé

Objectif Une détection précise des BLSE en milieu clinique est nécessaire pour la mise en route d'un traitement précoce et efficace des infections causées essentiellement par des entérobactéries productrices des BLSE. Le but de notre étude était l'évaluation des méthodes courantes de dépistage des BLSE dans les laboratoires cliniques en Tunisie.

Méthodes

Trois méthodes courantes de dépistage des BLSE (Test de synergie (ST), méthode des disques combinés (CD), et l'automate Vitek 2 (V2)) ont été évaluées en utilisant comme référence les résultats moléculaires de 91 souches cliniques des entérobactéries résistantes aux céphalosporines. La sensibilité et la spécificité ont été calculées pour chaque méthode évaluée. La valeur P a été déterminée à l'aide du logiciel statistique Epi-Info 7

Résultats

La méthode automatisée Vitek 2 a montré la plus grande sensibilité et la plus faible spécificité. Cependant, le test de synergie et la méthode des disques combinés ont montré une spécificité de 100% avec une moindre sensibilité.

Une faible sensibilité a été notée dans toutes les méthodes testées pour le groupe 0 (*P. mirabilis*) et la spécificité la plus faible a été observée pour la méthode V2 dans le groupe 3 (*E. cloacae, P. stuartii, S. marcescens, E. aerogenes*). L'analyse statistique a montré une différence significative entre les résultats du test de synergie (ST) et les résultats moléculaires ($P <0,05$) contrairement aux deux autres méthodes (V2 / CD) qui n'ont pas montré une différence significative ($P = 0,05$). Notre étude a montré que la méthode la plus appropriée pour les groupes G0 (*P. mirabilis*), G1 (*E. coli*) et G2 (*K. pneumoniae, C. koseri*) est la méthode V2, contrairement au groupe 3, où la méthode la plus fiable est celle des disques combinés. Le test de synergie semble être la méthode imparfaite avec des résultats faussement négatifs.

Conclusion

Selon nos résultats, nous recommandons d'utiliser la V2 et le CD afin de minimiser les taux d'erreur des résultats faux positifs et faux négatifs.

Mots-clés: BLSE*, Enterobacteriaceae,* Test de synergie, Disques combinés, Vitek 2

1. Introduction

Extended spectrum-β-lactamases was the common mechanism conferring the resistance to β-lactam antibiotics especially in *Enterobacteriaceae* and is rapidly increasing all over the world (1-6). Genes encoding ESBLs are carried on plasmids, which promote their quick spread among various *Enterobacteriaceae* species making therapeutic antibiotic choices more and more restricted. Several studies in Tunisia described the rapid spread of ESBL Enterobacteriaceae in community, patients and environments7-10. An accurate detection of ESBL in clinical setting is needed for proper therapy, surveillance programs and infection control11. Various phenotypic methods were developed until now for the detection of ESBL producing strains in clinical settings (12). However, several factors are behind the method used in each country or even in each hospital particularly the high cost of some methods especially automated ones. The main purpose of our study was to evaluate diverse phenotypic tests used in the Military hospital of Tunisia in order to select the adequate method for use in routine detection of ESBL in the laboratory and to minimize the error rate especially the false positive and the false negative rate. This study will provide more information about most adequate phenotypic methods for ESBL detection in clinical setting in our country and even other countries especially developing ones where molecular tests were rarely used because of their high costs.

2. Material and Methods

2.1. Enterobacteriaceae strains

Clinical cephalosporin-resistant-*Enterobacteriaceae* strains (n=91) isolated from various clinical samples taken from patients hospitalized in the Military university hospital of Tunisia (one strain per patient) were identified by automated system Vitek2 (BioMérieux®, France). All strains included in this study were susceptible to carbapenems.

2.2. Double disk diffusion test

Usually called synergy-test, it was performed as recommended by the European committee of antibiotic susceptibility testing (EUCAST). This test is based on the synergy between cefotaxime (30µg), ceftazidime (30µg), cefepime (30µg), aztreonam (30µg) and a disk containing β-lactamase inhibitor such as amoxicillin-clavulanic acid (AMC) (20 µg/10 µg).

Firstly, antibiotic disks were placed at 30 mm from AMC, then the interdisk distance were decreased to 20 mm in *Enterobacteriaceae* strains showing unclear results except for *Proteus mirabilis* and *Providencia stuartii* where the distance were increased to 40 mm.

2.3. Combined disks test

Disks containing 30µg of cefotaxime (CTX30) and 30µg of cefepime (FEP30) were placed separately with disks containing cefotaxime with clavulanate (CTX+C) and cefepime with clavulanate (FEP+C) on plates of Muller Hinton (MH) seeded with 0.5 McFarland of bacterial suspension. After incubation during 18 hours at 37°C, positive strains producing ESBL had inhibition zone around combined discs greater or equal to 5 mm compared to that around sample corresponding discs.

2.4. Automated system Vitek-2 Compact

All isolates were investigated for ESBL production using the Vitek 2 (BioMérieux®, France), according to the manufacturer's recommendations

2.5. Confirmation of ESBL production by molecular test

PCR of the common ESBL gene CTX-M-U was performed for all strains using primers (CTX-M-U-F: CGATGTGCAGTACCAGTAA; CTX-M-U-R: TTAGTGACCAGAATCAGCGG) as previously described (13). Other ESBL genes (SHV and TEM) were tested using primers (SHV-F: CACTCAAGGATGTATTGTG; SHV-R: TTAGCGTTGCCAGTGCTCG and TEM-F: ATTCTTGAAGACGAAAGGGC; TEM-R: ACGCTCAGTGGAACGAAAAC) as previously described (14, 15) and analyzed by sequencing in strains showing negative results with CTX-M-U PCR. This molecular test was considered as the reference method in statistical analysis and in the evaluation of various methods tested in this study.

2.6. Statistical analysis

Sensitivity and specificity were calculated for each evaluated method. P value was determined to compare proportions using Epi-Info 7 statistical software.

3. Results

A total of 91 *Enterobacteriaceae* strains showing resistance to third generation cephalosporins were isolated from diverse clinical samples from patients hospitalized in several services in the Military hospital of Tunisia and were identified as *Klebsiella pneumoniae* (n=38), *Escherichia coli* (n=31), *Enterobacter cloacae* (n=7), *Proteus mirabilis* (n=6), *Providencia stuartii* (n=4), *Serratia marcescens* (n=3), *Citrobacter Koseri* (n=1) and *Enterobacter aerogenes* (n=1). *Various* methods were used in the identification of ESBL producing strains as shown in table 1. The reference method used in our study showed that 75.82 % (69 of 91) of strains were ESBL producers, all of them harbored bla$_{CTX\text{-}M\text{-}15}$ gene.

79.71% (55 of 69) and 88.40% (61 of 69) of ESBL producing strains were detected by the double disk diffusion test and by the combined disks test respectively without false positive results for these two methods. However, 100% of ESBL producing strains with four false positive strains (three Providencia stuartii and one *Serratia marcescens*) were obtained by the Vitek 2 automated system.

Table 2 show sensitivity, specificity and P value of each

Table 1: Results of ESBL Phenotypic and molecular tests in 91 Enterobacteriaceae strains

	G0 n= 6		G1 n= 31		G2 n= 39		G3 n= 15		Total n= 91	
	+	-	+	-	+	-	+	-	+	-
ST	0	6	26	5	26	13	3	12	55	36
CD	0	6	26	5	31	8	4	11	61	30
V2	1	26	26	5	6	1	12	3	72	19
Molecular results	2	4	28	3	34	5	5	10	69	22

ST : Synergy Test ; CD : Combined Disks ; V2 : Vitek 2; G0 : *P. mirabilis* ; G1 : *E. coli* ; G2 : *K. pneumoniae* and *C. koseri* ; G3 : *E. Cloacae*, *P. stuartii, S. marcescens* and *E. aerogenes*

evaluated method for each group of *Enterobacteriaceae* tested strains. All results were obtained by comparing the evaluated methods to the reference method.

The Vitek 2 automated method showed the highest sensitivity and the lowest specificity. However, double disk diffusion test and the combined disks test showed 100 % of specificity with less sensitivity. Low sensitivity was also observed in all tested methods for the group 0 (*G 0: P. mirabilis*) of Enterobacteriaceae species and the lowest specificity (58.82%) was observed in the group 3 (G 3*: E. Cloacae, P. stuartii, S. marcescens* and *E. aerogenes*) with the Vitek 2 method.

The results of statistical analysis of each method compared to molecular one using Epi-Info 7 software showed that there was significant difference between Synergy test and molecular method ($P<0.05$), however, there was no significant difference comparing molecular results with combined disks and Vitek 2 automated method results ($P> 0.05$).

Table 2: Evaluation of ESBL phenotypic tests in each group of Enterobacteriaceae species

	G0 (N=6)			G1 (N=31)			G2 (N=39)			G3 (N=15)			Total (N=91)		
	S (%)	Sp (%)	P (%)	S (%)	Sp (%)	P (%)	S (%)	Sp (%)	P (%)	S (%)	Sp (%)	P (%)	S (%)	Sp (%)	P
ST	50	100	0.45	93.33	100	0.7	80.95	100	0.05	71.42	100	0.68	83.13	100	0.03
CD	50	100	0.45	93.33	100	0.7	91.89	100	0.54	83.33	100	1	89.61	100	0.25
V2	66.66	100	1	93.33	100	0.7	97.14	100	1	71.42	58.82	0.02	100	85.18	0.72

ST : Synergy Test ; CD : Combined Disks ; V2 : Vitek2 ; S : Sensitivity ; Sp : Specificity ; P : P value ; G0 : *P. mirabilis;* G1 *: E. coli* ;
G2 : *K. pneumoniae* and *C. koseri ;* G3 *: E. Cloacae, P. stuartii, S. marcescens* and *E. aerogenes*

4. Discussion

Three conventional methods usually used for ESBL detection in clinical setting in our region were evaluated using molecular as a reference method in our study. Several studies evaluating methods used for ESBL identification in clinical setting in diverse regions worldwide were reported (16-19). Nevertheless, in our knowledge none of these studies were performed in our country or neighbor countries. In this study, eight various species belonging to *Enterobacteriaceae* family usually isolated in clinical specimens were tested for ESBL production using the most three common phenotypic methods (two manual methods and one automated method) used in clinical settings. The reference method that has been adop-

ted in the evaluation of phenotypic tested methods was the molecular method. Our study showed that synergy test was the imperfect method showing significant difference with molecular results. These findings were completely in agreement with those of previous studies showing limitations of this method (17, 18, 20, 21). Although, the synergy test was the common method used for ESBL detection in several hospitals in our country, this method seem to be doubtful since it was depending to numerous factors such as disk spacing or coproducing of AmpC enzyme in some species (17,20). Our results showed that the combined disks method was more appropriate for ESBL production than synergy test method especially for the group 2 (G2) (100% specificity; 91.89% sensitivity) and group 3 (G3) (100% specificity; 83.33% sensitivity) of *Enterobacteriaceae* isolates. This method increased sensitivity and decreased the rate of false negative strains in G2 and G3 of the tested strains in our study. These findings were very similar to previous studies reported by several investigators showing that sensitivity and specificity of this test ranged between 86% and 100% (17, 19, 22, 23). In our research, the Vitek 2 automated method seem to be the most reliable method for detection of ESBL especially in the group 2 (100% specificity; 97.14% sensitivity). Our results showed that the detection of ESBL producing *Enterobacteriaceae* using Vitek 2 could be the most accurate method in the detection ESBL in various groups of *Enterobacteriaceae* except group 3 (G3) (58.82% specificity ; 71.42% sensitivity). In fact, the low specificity and sensitivity of Vitek 2 with species belonging to group 3 could be explained by the overproduction of chromosomal cephalosporinase which could affect the results. Several investigators suggest that the performance of Vitek 2 method in the detection of ESBL producing strains depended on both the AST card used and the type of ESBL produced (24-26). Regarding the results of statistical analysis for each group, it should be noted that the most appropriate method for groups G0 (*P. mirabilis*)), G1 (*E. coli*) and G2 (*K. pneumoniae* and *C. koseri*) is the method of Vitek 2 in contrast to group 3 where the most reliable method is that of combined disks. Synergy test appear to be the imperfect method with high false negative results. Because of some errors in these methods, some authors suggested to use more than one method (at least two methods) in routine screening of ESBL producing strains (27, 28). According to our results, we recommend to use the Vitek 2 and the combined disk methods in order to minimize the error rates of false positive and false negative results.

5. Conclusion

Suitable methods should be adopted for the detection of ESBL producing strains in our clinical setting for adequate antibiotic therapy in order to stop the quick spread of resistant bacteria.

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