Extended Spectrum Beta Lactamases (ESBLs) Detection in Enterobacteriaceae According to New CLSI Guidelines

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ABSTRACT

Introduction: *The production on ESBLs can lead to life-threatening infections with Increased morbidity, mortality and healthcare-associated costs.* ***Objectives*** *To investigate the value of different simple disc diffusion screening methods of ESBLs in comparison to semiautomated microbiology machines.* ***Material and methods:*** *Three commercially available microbiology identification and susceptibility testing systems were evaluated and compared with regard to their ability to presumptively or definitely detect ESBL production in Enterobacteriaceae. Themethods tested were disc diffusion screening tests using selected antimicrobial agents (Kirby-Bauer antibiotic testing) , combined disk test and MicroScan ESBL plus ESBL confirmation panels (Dade Behring Inc., West Sacramento, Calif.). Disk diffusion screening and combined discs were evaluated against the result of microScan ESBL plus ESBL confirmation panels.* ***Results:*** *Disk diffusion screening tests revealed 61 suspected ESBL. Reported ESBL by Combined ESBL Confirmatory test disks were 70. Confirmed ESBL by microscan were 79. All confirmed ESBL by microscan were resistant to Cefpodoxime and ceftazidime in disc diffusion method. Three test agreements reported in 58 isolates.* ***Conclusions:*** *Cefpodoxime (CPO) and ceftazidime(CAZ) show the highest agreement with microscan for ESBL detection followed by cefotaxime(CTX) & ceftriaxone (CRO)so we can rely upon them in detecting ESBL confirmed by microscan in our routine daily work.*
Key words: *Enterobacteriaceae, ESBL,CLSI guidelines.*

INTRODUCTION

The prevalence of extended-spectrum β-lactamase (ESBL) production in strains of the family Enterobacteriaceae such as Escherichia coli, Klebsiella spp. and Enterobacter spp., has been increasing continuously during the past decade in Europe and worldwide[(1)]. The production of ESBLs can lead to life-threatening infections with increased morbidity, mortality and healthcare-associated costs[(2)]. Extended-spectrum beta-lactamase (ESBL) production in members of the Enterobacteriaceae can confer resistance to extended-spectrum cephalosporins, aztreonam, and penicillin. As such, the accurate detection of ESBL producers is essential for the appropriate selection of antibiotic therapy[(3)].

The clinical microbiology laboratory has the task of screening and confirming isolates for ESBL production. This is a challenge for the laboratory to detect ESBL-containing gram-negative bacilli because they can appear susceptible in vitro to certain beta-lactam anti-microbial agents yet result in clinical treatment failure[(4)]. Currently the CLSI documents recommend screening of ESBL production in E. coli, K. pneumoniae, and Klebsiella oxytoca by using the antimicrobial agents cefpodoxime, CAZ, aztreonam, CTX, and ceftriaxone. The use of several antimicrobial agents increases the sensitivity of ESBL detection[(5)]. Confirmatory testing should be performed on organisms in which the ESBL screen may indicate ESBL production. Phenotypic confirmatory testing involves testing the E. coli, K. pneumoniae, or K. oxytoca isolates against CAZ and CTX alone and in-combination with clavulanic acid (CTX/CA and CAZ/CA, respectively).

The Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recently changed their recommendations concerning the interpretation and reporting of in vitro drug susceptibility testing (DST) results and brighten the importance in clinical microbiology lab.[(1)].

The aim of this study:

To investigate the value of different simple disc diffusion screening methods of ESBL (disc diffusion screening test& Combined ESBL Confirmatory test disks) in comparison to semiautomated microbiology machines (MicroScan ESBL plus ESBL confirmation) according to new CLSI guidelines.

MATERIAL & METHODS

***Study design*:**

Three commercially available microbiology identification and susceptibility testing systems were evaluated and compared with regard to their ability to presumptively or definitely detect ESBL production in Enterobacteriaceae.

One hundred and thirteen samples collected from National cancer institute-Cairo University from August 2010 to August 2011 from adult cancer patients. Different sample types were collected and included in this study; 26 pus, 29 urine, 18 sputum, 26 blood, 6 throat swab, 5 Drain,3 others (pleural effusion – mouth swab-catheter).

The methods tested were disc diffusion screening test using selected antimicrobial l agents (Kirby-Bauer antibiotic testing), combined disk test and MicroScan ESBL plus-ESBL confirmation panels (Dade Behring Inc., West Sacramento, Calif.),we consider the microscan detection of ESBL as the reference method.

1- For susceptibility testing using the disc diffusion screening method according to Kirby–Bauer was used. Antibiotic discs (Becton Dickinson, Franklin Lakes, NJ, USA) were selected, and results were interpreted according to the 2011 guidelines of EUCAST and CLSI[5,6].

Each Klebsiella pneumoniae, K. oxytoca, or Escherichia coli isolate should be considered a potential ESBL-producer if the test results are as follows:

Table 1: Interpretation of disc diffusion screening test according to CLSI guidelines[7].

Disk diffusion	MICs
cefpodoxime < 22 mm	cefpodoxime > 2 µg/ml
ceftazidime < 22 mm	ceftazidime > 2 µg/ml
aztreonam < 27 mm	aztreonam > 2 µg/ml
cefotaxime < 27 mm	cefotaxime > 2 µg/ml
ceftriaxone < 25 mm	ceftriaxone > 2 µg/ml

2- Combined ESBL Confirmatory test disks: The disk diffusion method with CTX and CAZ disks alone and in combination with CA was used to detect ESBL production. An ESBL producer had a ≥5-mm-zone size difference between the CTX/CA or CAZ/CA disks compared to disks without the CA.[8]

3- MicroScan ESBL plus ESBL confirmation: The MicroScan panel was performed in accordance with the guidelines of the manufacturer. Strain characterization and antimicrobial susceptibility testing with MicroScan WalkAway-96 system were performed with Neg/BP/Combo NM31 panels, according to manufacture's instructions. Panels were read following 16 to 20 h of incubation. Confirmatory testing uses both CTX and CAZ, alone and in combination with CA. ESBL production was determined by a ≥3 twofold-concentration decrease in MICs of either CAZ or CTX in the presence of a fixed concentration of CA versus the MIC when tested alone. Subsecquently strains were considered as ESBL-positive or –negative in accordance with CLSI recommendations[9].

RESULTS

Bacterial isolates in 113 sample are as follow: The clinical isolates include 52 E. coli isolates and 34 Klebseilla (32 K. pneumoniae isolates 1 klebseilla ozaena, 1 klebseilla oxytoca), 21 enterobacter, 4 proteus, 1 Kluvera ascorbata, 1 Morganella morganii.

<u>Results by each method were as follow:</u>

I Disk diffusion screening tests results using cefpodoxime, ceftazidime, aztreonam, cefotaxime, ceftriaxone (Kirby-Bauer antibiotic testing) revealed 61 isolates resistant to all these antimicrobial agents (suspected ESBL were 61). Resistance to CTX & CRO were 75 while resistance to Cefpodoxime and ceftazidime were 79.

II (II)Results of Combined ESBL Confirmatory test disks detected ESBL in 70 isolates.

III Results of Microscan Confirmed ESBL were 79 isolates.

Result of each method compared to microscan result:

I Confirmed Esbl by microscan and Disk diffusion screening tests were 58.

II Confirmed ESBL by microscan and combined ESBL confirmatory test disks positive were 60.

III Confirmed Esbl by microscan with resistance to CTX & CRO were 75.

IV Confirmed ESBL by microscan 79, Cefpodoxime and ceftazidime resistant isolates were 79. i.e all confirmed ESBL by microscan were resistant to Cefpodoxime and ceftazidime in disc diffusion method.

V Three test agreements reported in 58 isolates.

Table 2 shows Interpretation of result of different used methods

Microscan			Combined disks			Disk diffusion screening		
ESBL	**Not ESBL**	**Total**	**ESBL**	**Not ESBL**	**Total**	**ESBL**	**Not ESBL**	**Total**
79 (69.9%)	34 (30.1%)	113 (100%)	70 (61.9%)	43 (38.1%)	113 (100%)	61 (54%)	52 (46%)	113 (100%)

Table 3 shows Microscan versus other test methods to detect the most test which give the most close result to microscan and show the three test agreement.

Microscan ESBL vs +ve combined disc		Microscan ESBL vs dds**		Microscan ESBL vs R* CTX&CRO		Microscan ESBL vs R* CPO&CAZ		Microscan vs combined disc vs dds**	
79 (100%)	60 (76%)	79 100%	58 (73.4%)	79 (100%)	75 (95%)	79 (100%)	79 (100%)	79 (100%)	58 (73%)

R* Resistant -dds ** disk diffusion screening

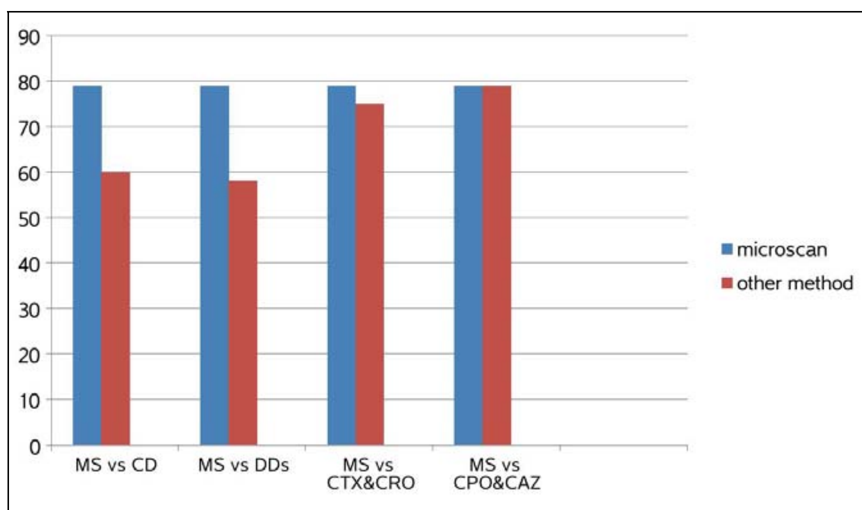

Figure 1: The results of different methods compared to microscan detection of ESBL in enterobacteriacae pathogens.
MS: Microscan- CD: Combined disc – DDS: Disk diffusion screening

DISCUSSION

In 2010 CLSI changed their guidelines concerning ESBL detection and interpretation. Reporting of penicillins and cephalosporins as resistant, independent of in vitro results, is no longer recommended[10,11] However, detection of ESBL is still considered useful or even mandatory for epidemiological purposes.[5,6]

Correct identification of ESBL-positive Enterobacteriaceae in due time is mandatory not only for optimal patient management but also for immediate institution of appropriate infection control measures to prevent the spread of these organisms[12].

The phenotypic confirmation of ESBL production is recommended by CLSI[5]. Isolates which are screen positive for ESBL production should be confirmed by testing with CTX and CAZ alone and in combination with CA. Both CTX and CAZ with and without CA are tested to ensure detection of ESBL production.

Although CAZ currently detects most ESBLs in the United States, the use of only one drug combination will not detect all ESBL producers. Furthermore; ESBLs of the CTX-M group are increasing and spreading througho-ut the world. These enzymes are more active against CTX than against CAZ(13,14,15,16).

In this study all confirmed ESBL by Microscan were resistant to cefpodoxime and ceftazi-dime in disc diffusion method.The sensitivity of screening for ESBLs in enteric organisms can vary depending on which antimicrobial agents are tested. The use of more than one of the five antimicrobial agents suggested for screening will improve the sensitivity of detection. Cefpodoxime and ceftazidime show the highest sensitivity for ESBL detection(7) and this is in agreement with our study. The use of semiautomated systems for identification and antimicrobial susceptibility testing of gram-negative rods is now common practice in many laboratories(8). The performance of these systems with respect to ESBL identification in comparison to conventional methods such as the disc approximation method (DAM), DDS, and Etest ESBL has been studied previously(17,18,19,20,21,22). Using the standard disk diffusion as screening test for identifying ESBL producer, cefopodoxime was found to be the most efficient antimicrobial agent in screening isolates as potential ESBL producer followed by cefatazidime in Klebseilla spp. Isolates(23) and this is in agreement with our finding. While the presence of the ESBL genes generally was associated with varying degrees of resistance to cephalosporins, the presence of a particular genotype could not predict the susceptibility pattern to a particular drug with the exception of blaSHV, which was associated with resistance to ceftazidime. Similarly, isolates that had blaCTX-M were more sensitive to ceftazidime than those without(24). The susceptibility of blaCTX-M containing isolates to ceftazidime has been documented by other authors who suggest that ceftazidime can be used in the treatment of community-acquired UTIs due to CTX-M ESBLs[25]. This presents a possible clinical application of genotyping ESBLs and for empiric therapy in UTIs suspected to be due to ESBL-producing E. coli. Half of the K. pneumoniae isolates carried blaSHV which predicted resistance to ceftazidime, making it unsuitable for use as treatment in this species(24). And this explains why there is difference in cephalosporin susceptibility testing in our finding.

Our investigation differs from those of other researchers in that : instead of evaluating a single method, we compared side-by-side the three methods (one semiautomated and two manual phenotypic methods that are currently commercially available, easy to perform,& cheap), we used isolates that were consecutively collected from routine clinical specimens instead of using a well-characterized strain collection of challenge strains with known β-lactamase types; and investigators were blinded to whether the isolate was an ESBL producer or not. Our study design is therefore suitable to optimally reflect daily clinical practice.

In conclusion: In the present study, we investigated the new CLSI guidelines decreasing the MIC values for cephalosporins in simple disc diffusion testing to screen for ESBL enterobactericae. Cefpodoxime and ceftazidime show the highest sensitivity for ESBL detection followed by CTX & CAZ so we can rely upon them in detecting ESBL confirmed by Microscan in our routine daily work. Especially for Egypt where many Labs have restricted resources with no available automated system to complement lab detection of ESBL we can rely upon this simple manual method in detecting ESBL.

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البكتيريا المعوية الموسعة الطيف بيتا لاكتاميز كشف في وفقا لمبادئ توجيهية حديثة من معهد المعايير الاكلينيكية والمخبرية

البكتيريا المعوية الموسعة الطيف بيتا لاكتاميز يمكن أن تؤدي العدوى بهذه البكتيريا الى الى إصابات تهدد الحياة مع زيادة معدلات الاعتلال والوفيات وتكاليف الرعاية الصحية المرتبطة بها.

الهدف من هذا البحث هو التحقق من مختلف الطرق البسيطة المتاحة للكشف عن هذه البكتيريا و مقارنتها بالطرق الحديثة المميكنة المستخدم بها جهاز ال microScan وتحديد الطرق الانسب والاقل تكلفة لتشخيص هذه البكتيريا.وجرى تقييم الثلاثة طرق المتاحة تجاريا لتحديد الأحياء الدقيقة ونظم اختبار الحساسية و تحديد قدرتها على الكشف عن و التأكيد على إنتاج الموسعة الطيف بيتا لاكتاميز في البكتيريا المعوي.

المواد والأساليب المستخدمة في هذا البحث هي وسائل بحث يدوية بسيطه مثل (disc diffusion screening tests) و هواختيار اقراص بها مضادات حيوية معينه فى هذه الطريقة وطريقة اخرى وهي (combined disk test) اختبار الاقراص المجمعة تأكيدا على ذلك جرى تقييم الطريقتين ضد نتيجة ا لmicroScan

و قد كانت نتائج هذا البحث كالتالي : عدد الحالات المستخدمة في هذا البحث ١١٣ حالة ، تم تاكيد وجود البكتيريا المعوية الموسعة الطيف بيتا لاكتاميز في مختلف الطرق كلاتي: ٦١ عزلة متوقعة عن طريق (disc diffusion screening tests) ، ٧٠ عزلة بالطريقة الثانية (combined disk test) اختبار الاقراص المجمعة، ٧٩ عزلة مؤكدة بال microScan وتم الكشف عن انه كل العزلات المؤكد انها الموسعة الطيف بيتا لاكتاميز بطريقة ال microScan فهى مقاومة ايضا للمضادات الحيوية Cefpodoxime (CPO) و ceftazidime (CAZ).و قد تم توافق الثلاثة طرق في ٥٨ من العزلات.

اهم الاستنتاجات من هذه الدراسة هى ان نتيجة الحساسية لاقراص السيفوبودوكسيم (CPO) ، والسيفتازيديم (CAZ) تظهر اعلى درجة من التوافق مع النتائج المؤكدة من ال microScan يليها الحساسيه لاقراص ال سيفوتاكسيم (CTX) والسيفترياكسون (CRO) و لذلك يمكن الاعتماد على هذه الطرق البسيطه اثناء عملنا اليومي في تشخيص هذا النوع من البكتيريا الخطيرة و خاصة في البلاد المحدودة الموارد .