A Simple disc diffusion method for detecting AmpC and extended-spectrum ß-lactamases in clinical isolates of Enterobacteriaceae.

Thesis

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Submitted by

Dr.Marwa Aref Mahmoud

Under supervision of:

Prof .Dr .Mona Abdel Aziz Wassef

Professor of Clinical Pathology, Cairo University.

Prof. Dr.Sahar Mohammed Khairat

Professor Of Clinical Pathology,Cairo University.

Dr.Rasha hamed Elsherif

Lecturer of Clinical Pathology,Cairo University.

Faculty of Medicine

Cairo University

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ABSTRACT

Background: We thought to determine whether extended spectrum β-lactamases (ESBLs) and AmpC β-lactamases (derepressed and inducible) ,alone or in combination,could be detected in clinical isolates of the Enterobacteriaceae using a simple, overnight disc diffusion test.

Methods: 178 of 400 Enterobacterial isolates were included according to selection criteria: resistance to third generation cephalosporins (CPD) & (CTX) and to cephamycins (FOX). A scheme for detecting these resistance mechanisms phenotypically using Approximation (DDST, induction test) and combined (clavulanate and boronic) confirmatory tests and E-test strip.

Results and Conclusion: By screening tests ESBLs were suspected in 178/400 (44.5%), AmpC were suspected in 98/400 (24.5%). By DDST ESBLs were confirmed in 173/276 (62.7%) while in combined clavulanate test ESBL alone were found in only 2 isolates while 253/276(91.7%) were found to be ESBL in presence of AmpC.

AmpCs were detected by the combined clavulanate method in 64/276 (23.2%) and by combined boronic method in 65/276 (23.5%). Comparing these results to the gold standard E-test combined clavulanate method found to be a reliable method (sensitivity 100% &PPV 96%) in relation to the approximation method DDST (sensitivity 64.7% & specificity 83.3%).

Key words:

(ESBL –AmpC β-lactamases – Enterobacteriaceae-disc tests)

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List of Abbreviations

3G	**third generation**
ACC	**Ambler class C**
AMC	**Amoxacillin clavulanic**
AmpC	**Class C Beta-lactamase enzymes**
ATM	**Aztreonam**
BEL	**(Belal) name of patient**
BES	**Brazil extended spectrum beta lactamase**
BR	**Boronic acid**
CAZ	**Ceftazidime**
CL	**clavulanic acid**
CLSI	**Clinical laboratory standards institute**
CMT	**Complex mutant TEM**
CMY	**Cephamycins**
CN	**E-test with Cefotetan**
CNI	**E-test with Cefotetan +Cloxacillin**
CPD	**Cefpodoxime**
CPM	**Cefepime**
CRO	**Ceftriaxone**
CT	**E-test with Cefotaxime**
CTL	**E-test with Cefotaxime+Clavulanate**
CTX	**Cefotaxime**
DDST	**Double disk synergy test**
DMSO	**dimethyl sulphoxide**
ESBLs	**Extended-spectrum ß-lactamases**

ESCS	**Extended Spectrum Cephalosporins**
FEP	**Cefepime**
Fig	**Figure**
FOX	**Cefoxitin**
GES	**Guiana-extended spectrum**
K. pneumoniae	**Klebsiella pneumoniae**
k.oxytoca	**Klebsiella oxytoca**
KPC	**Klebsiella pneumoniae carbapenemase**
MBL	**Metallo-ß-lactamases**
MDDT	**Modification of the double-disk test**
MHA	**Muller-Hinton agar**
MIC	**Minimal inhibitory concentration**
MIR	**Miriam Hospital**
NCCLS	**National committee for clinical laboratory standards**
OXA	**Oxacillinase**
P. aeruginosa	**Pseudomonase aeruginosa**
P. mirabilis	**Proteus mirabilis**
pAmpCs	**Plasmid mediated AmpC**
PCR	**Polymerase chain reaction**
PER	**Pseudomonas extended resistance**
PM	**E-test with Cefepime**
PML	**E-test with Cefepime+ Clavulanate**
SFO	**Serratia fonticola**
SHV	**Sulfhydryl variable**
TEM	**(Temoneira)name of patient**
TLA	**Tlahuicas (Indian tribe)**

TZ	**E-test with Ceftazidime**
TZL	**E-test with Ceftazidime+Clavulanate**
TZP	**piperacillin-tazobactam**
UK	**United kingdom**
VEB	**Vietnam extended-spectrum**
β-lactam	**Beta lactam**
β-lactamase	**Beta lactamase**

INTRODUCTION

ß-Lactamase production is the most common mechanism of ß-lactam drug resistance in gram-negative bacteria. Newer ß-lactamases that hydrolyze cephamycins, oxyimino and zwitterionic cephalosporins, monobactams, or carbapenems are of increasing concern because they restrict therapeutic options, cause treatment failures ***(Black et al, 2005)*.**

AmpC β-lactamases are clinically important cephalosporinases encoded on the chromosome of many Enterobacteriaceae where they mediate resistance to most penicillins, cephalothin, cefazolin, cefoxitin, third generation cephalosporins and ß-lactamase inhibitor/ß-lactam combinations. In many bacteria, AmpC enzymes are inducible and can be expressed at high levels by mutation ***(jacoby ,2009).***

Transmissible plasmids have acquired genes for AmpC enzymes, which consequently can now appear in bacteria lacking or poorly expressing a chromosomal blaAmpC gene,resistance due to plasmid- mediated AmpC enzymes is less common than ESBL production but may be both harder to detect and broader in spectrum ***(Jacoby , 2009).***

Current advise is to screen for ESBL production using both cefotaxime and ceftazidime,or to use cefpodoxime,and to carry out confirmatory tests on resistant isolates ***(Health protection Agency, 2008).***

Techniques for confirming ESBL production all depend on detecting synergy between clavulanic acid and the indicator cephalosporin that has been used as a primary screen,they are not ideal for detecting ESBLs in the presence of AmpC β-lactamases as these enzymes may be induced by Clavulanic acid and may attack the indicator cephalosporin,masking the inhibition of any co-present ESBL ***(Derbyshire et al,2009).***

Resistance to cefoxitin has been used as a marker for the Production of AmpC β-lactamases,but some AmpC types are susceptible to cefoxitin;further,cefoxitin resistance may also arise due to decreased permeability or to species-specific intrinsic resistance.Other methods for detecting of AmpC β-lactamases are time-consuming,do not detect all enzymes and cannot be incorporated into routine use ***(Brenwald et al,2005).***

Recent approaches to the detection of AmpC β-lactamases Involve the use of indicator cephalosporins and AmpC inhibitors Such as benzo(b)thiophene -2-boronic acid or cloxacillin. A further approach to detecting ESBLs in the presence of AmpC β – lactamases is to use AmpC-stable fourth- generation cephalosporins such as cefepime or cefpirome,but this generally necessitates prior identification of the organism ***(Derbyshire et al ,2009).***

AIM OF WORK

The aim of this study is to evaluate the reliability of a new single-plate, disc diffusion method for the detection of ESBLs and AmpC b-lactamases (derepressed and inducible), alone and in combination, in clinical isolates of Enterobacteriaceae in comparison to other convential phenotypic methods and comparing our results with a gold standard technique as the E-test for detection of both ESBLs & AmpCs aiming to determine a simple and appropriate method for their detection in clinical laboratory.