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THE PREVALENCE OF PLASMID MEDIATED AMPC B-LACTAMASE PRODUCTION IN ENTEROBACTEIACEAE LACKING CHROMOSOMAL AMPC B-LACTAMASES AT J L N MEDICAL COLLEGE AND ASSOCIATED GROUP OF HOSPITALS, AJMER,RAJASTHAN .

Microbiology

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ABSTRACT

Background: *Enterobacteriaceae produces AmpC β-lactamases that make them resistant to commonly used antibiotics. AmpC β-lactamases can be chromosomal-mediated or plasmid-mediated AmpC β-lactamases (PABLs). The present study was undertaken to determine the occurrence of PABLs production in clinical isolates in Klebsiella and proteus species.*

Objective: To determine the prevalence of plasmid mediated AmpC β-lactamase production in Enterobacteiaceae lacking chromosomal AmpC β-lactamases.

Methodology: In this prospective study all non-duplicate Enterobacteriacae 100 isolates recovered from various clinical samples submitted in microbiology laboratory of our hospital. Isolated over a period of 3 months were screened for AmpC β-lactamase production.

Results: Among the 100 clinical isolates tested only 21 isolates were found to harbour AmpC enzymes Confirmed by AmpC disk test and cloxacillin inhibitor based method.

Of these isolates , 15 isolates were identify as of Klebsiella spp. and 6 isolates were identify as a Proteus mirabilis.

Conclusion: Some of the AmpC isolates were sensitive to cefoxitin but were either resistant to cefotaxime or ceftazidime or were resistant to both antibiotics.These data indicate that although methods that use cefoxitin in standardised methods to detect AmpC-harbourng isolates,are useful ,they are not perfect. The results in the present study showed that screening should include all the clinical isolates showing resistance to any of the cephalosporins irrespective of their cefoxitin susceptibility status.

KEYWORDS

Enterobacteriaceae, Ampc B-lactamases

INTRODUCTION

AmpC enzyme is a β-lactamase that can hydrolyse cephamycins as well as other extended spectrum Cepholosporins and not inhibited by clavulanic acid.Although plasmid mediated AmpC β-lactamases.[1] Were first reported in the late 1980s,many infectious disease personnel remain unaware of their Clinical importance. This study detected plasmid mediated AmpC β-lactamases in organisms lacking chromosomal ly mediated AmpC β-lactamase that results in multiple antibiotic resistance leaving few therapeutic options and have been associated with false in vitro susceptibility to cephalosporins.[2]There is a paucity of data about the prevalence and clinical significance ofplasmid mediated AmpC β-lactamases because many laboratories do not test for this resistance mechanism and current tests are inconvenient,subjective,lack sensitivity and/or specificity,or require reagents that are not readily available.A simple disc based protocol using cefoxitin non-susceptibility as a screeningtool,followed by the AmpC disk test as well as cloxacillin inhibitor based method for confirmationwere used to detect AmpC β-lactamase production.[3,4]

MATERIALS AND METHODS

This study was conducted at Department of Microbiology of JLN Medical College,Ajmer,Rajasthan.

In this prospective study all non-duplicate Enterobacteriacae 100 isolates recovered from various clinical samples submitted in microbiology laboratory of our hospital.

Isolated over a period of 3 months were screened for AmpC β-lactamase production.

Screening for AmpC β-lactamase*:*

Screening done by using the disc diffusion method as per CLSI recommendations(CLSI,2015)and cefoxitin(30µg)non-susceptibleis isolates were considered to be screen positive isolates.

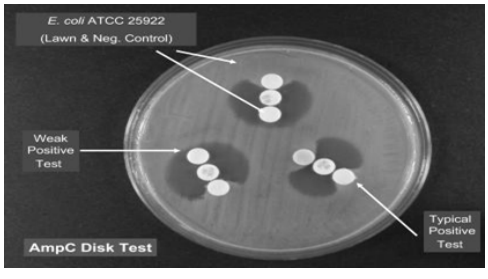
Further screening was carried out using the method ,in which strains sensitive to cefoxitin and resistant to amoxicillin-clavulalanic acid(30/10 µg),and either resistant or intermediate resistant to cefotaxime(30 µg) or ceftazidime(30 µg) and are negative for extended spectrum beta lactamase production,were also included.

Phenotypic confirmation tests for detection of AmpC β-lactamase:

1.The AmpC disk test,based on filter paper disks impregnated with EDTA, is a highly sensitive ,specific ,and convenient means of detection of plasmid mediated AmpC β-lactamases inorganismslacking a chromosomal ly mediated AmpC β-lactamase.

The test is based on use of Tris-EDTA to permeabilize a bacterial cell and release β-lactamases into the external environment. AmpC disks (i.e., filter paper disks containing Tris-EDTA,) were prepared in-house by applying 20 µl of a 1:1 mixture of saline and 100× Tris-EDTA to sterile filter paper disks, allowing the disks to dry, and storing them at 2 to 8°C. The surface of a Mueller-Hinton agar plate was inoculated with a lawn of cefoxitin-susceptible *E. coli* ATCC 25922 according to the standard disk diffusion method . Immediately prior to use, AmpC disks were rehydrated with 20 µl of saline and several colonies of each test organism were applied to a disk. A 30-µg cefoxitin disk was placed on the inoculated surface of the Mueller-Hinton agar. The inoculated AmpC disk was then placed almost touching the antibiotic disk with the inoculated disk face in contact with the agar surface. The plate was then inverted and incubated overnight at 35°C in ambient air. After incubation, plates were examined for either an indentation or a flattening of the zone of inhibition, indicating enzymatic inactivation of cefoxitin (positive result), or the absence of a distortion, indicating no significant inactivation of cefoxitin (negative result)

FIG. 1.



2.Inhibitor based methods for detection of AmpC β-lactamase:The standard disc diffusion method was applied using inoculated Mueller-Hinton agar plates and a 30 µg cefoxitin disc,alone or in combination with the test inhibitor,were placed on the surface of the medium and the plates were incubated overnight at 35C.An increase of 4mmin presence of cloxacillin(200 µg),in the inhibition zone diameter,was interpreted as a AmpC producer organism.

RESULT

Among the 100 clinical isolates tested only 21 isolates were found to harbour AmpC enzymes Confirmed by AmpC disk test and cloxacillin inhibitor based method.

Of these isolates ,15 isolates is of Klebsiella spp. and 6 isolates were identified as and Proteus mirabilis,respectively.

Table 1: Frequency of cefoxitin resistance among AmpC-positive and negative isolates

Sr No.	Tested organism(n)	AmpC positive isolates(n)		AmpC negative isolates(n)	
		Cefoxitin resistant	Cefoxitin susceptible	Cefoxitin resistant	Cefoxitin Susceptible
1.	K.pneumoniae(n=71)	7	3	5	56
2.	K.oxytoca(n=15)	4	1	4	6
3.	P.mirabilis(n=14)	3	3	0	8

Table2: Phenotypic methods for detection of plasmid-mediated AmpC β-lactamases

S.No.	Tested isolate	AmpC disk test		Inhibitor based method using cloxacillin	
		positive	Negative	positive	Negative
1.	K.pneumoniae	10	0	10	0
2.	K.oxytoca	5	0	5	0
3.	P.mirabilis	6	0	6	0

DISCUSSION

In this study the AmpC disk test provided a simple, convenient, and accurate means of detection of plasmid-mediated AmpC β-lactamases in organisms lacking chromosomal mediated AmpC β-lactamase, i.e.,K.pneumoniae,K. Oxytoca.,and P.mirabilis.These organisms are chosen for the study because they are convenient indicator organisms for this resistance mechanism in that a positive test can unequivocally indicate the presence of a foreign ,or plasmid mediated AmpC β-lactamase.E.coli isolates that produce r plasmid mediated AmpC β-lactamases were not Included in this study,because the test does not discriminate between positive results due to upregulated chromosomal ly mediated AmpC β-lactamases and those due to plasmid mediated AmpC β-lactamases.[5,6]

AmpC production in cefoxitin susceptible isolates may have a mechanism similar to that of ESBL producing organisms that appear susceptible to ceftazidime by dic diffusionmethod.Cefoxitin resistance in AmpC non-producers could be due to some other resistance mechanisms;lack of permeation of porins as one of the risistance mechanism has been reported. Thus,the test accurately distinguished between cefoxitin insusceptibility caused by AmpC production and non-β-lactamase mechanisms, such as reduced outer membrane permeability (porin mutations). Distinguishing between these types of mechanisms is a current diagnostic problem for laboratories wanting to detect AmpC β-lactamases. [7]

Coproduction of ESBLs did not interfere with the detection of the AmpC β-lactamases Care is required in interpreting the test with isolates exhibiting reduced carbapenem susceptibility, since this may be due to other, currently rare β-lactamases capable of hydrolyzing cefoxitin, e.g., carbapenemases.[8]

Paul R.Ingram etain(Feb2011) their study described a simple disc based protocol using cefoxitin non-Susceptibility as a screening tool,followed by the Tris-EDTA method for confirmation,detects BlaAmpC activity with95% sensitivity and 98% specificity[9]

In 2002 Philippon ,A.,G.Arlet,and G.A.Jacoby stated that plasmid mediated class C β-Lactamases have been discovered most frequently in isolates of K.pneumoniae and also In other naturally AmpC species such as K.oxytoca,Salmonella,and P.mirabilis.[10]

In Jan 2009,Tan etal found that for AmpC detection the best sensitivity(95%) and Specifici y(95%) were obtained using a combination of cefoxitin and cloxacillin and applying a cutoff of an increase in zone diameter of ≥4mm.[11]

Given the need for a test for AmpC β-lactamases and the fact that many clinical laboratories are often short staffed and overworked, the AmpC disk test could fill a current gap in diagnostic microbiology. Adoption of this test would make it possible to learn more about the clinical implications of plasmid-mediated AmpC β-lactamases and to contain the spread of Organisms possessing this resistance mechanism. The potential benefits would include better patient outcomes in terms of avoiding inappropriate therapy and a reduction in the escalation of antibiotic resistance through better infection control.

CONCLUSION

Some of the AmpC isolates were sensitive to cefoxitin but were either resistant to cefotaxime or ceftazidime or were resistant to both antibiotics.These data indicate that although methods that use cefoxitin in standardised methods to detect AmpC-harbourng isolates,are useful ,they are not perfect. The results in the present study showed that screening should include all the clinical isolates showing resistance to any of the cephalosporins irrespective of their cefoxitin susceptibility status.

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