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Detection of AmpC Beta-lactamases among Escherichia coli isolates at a tertiary care hospital in Karnataka

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Abstract: *Background & objective*: AmpC β-lactamases are clinically significant because they may confer resistance to a wide variety of β-lactam drugs, including α-methoxy-β-lactams, such as cefoxitin, narrow-, expanded- and broad-spectrum cephalosporins, β-lactam-β-lactamase inhibitor combinations and aztreonam. Although reported with increasing frequency the true occurrence in different organisms remains unknown. The present study was conducted to determine the occurrence of AmpC β-lactamases among the clinical isolates of *Escherichia coli*. *Methods*: A total of 100 non-repeat clinical isolates obtained from urine, pus, sputum, blood and body fluids were taken. All the isolates were screened for AmpC β-lactamases by standard disc diffusion breakpoint for cefoxitin (30μg). Isolates with zone diameter less than 18 mm were tested for AmpC activity by AmpC disc test. *Results*: Of the 100 isolates that were tested, 30 yielded cefoxitin zone diameters less than 18 mm (screen positive). Production of AmpC β-lactamase was detected in 24 isolates by AmpC disc test. *Conclusion*: AmpC disc test can be used as a simple, convenient and rapid screening test for detection of AmpC β lactamase in clinical laboratories.

Keywords: AmpC β-lactamase, AmpC disc test, Cefoxitin.

Introduction

The accumulation of bacterial antibiotic resistance is a dramatic demonstration of Darwin's dictum of the survival of the fittest with serious practical consequence for treatment failure. The predominant mechanism for resistance to β-lactam antibiotic in Gram negative bacteria is synthesis of β-lactamase, an enzyme which produces a biologically inactive product by hydrolyzing the β-lactam ring. To date, at least 400 different types of β-lactamases have been described [1].

Newer β-lactamases that hydrolyse, oxyminino and 7-α-methoxy-cephalosporin, monobactam or carbapenems are of increasing concern because they restrict therapeutic options, cause treatment failure, and are increasing in occurrence [2]. AmpC β-lactamases are clinically significant, since they confer resistance to cephalosporin in the oxyiminogroup (cefotaxime, ceftazidime, ceftriaxone), 7-α-methoxy-cephalosporin (cefoxitin or cefotetan) and monobactam. Most significantly they are not blocked by commercially available β-lactamase inhibitors (clavulanate, sulbactam) [3]. These enzymes are typically associated with multiple antibiotic resistance leaving few therapeutic options [2, 4]. Furthermore, in a strain with decreased outer membrane permeability such enzymes can provide resistance to carbepenem as well [1, 5].

Genes for AmpC β-lactamases are commonly found on the chromosomes of the several members of the family Enterobacteriaceae, including *Enterobacter, Shigella, Providencia, Citrobacter freundii, Morganella morganii, Serratia marsescens* and *Escherichia coli*. Plasmid mediated AmpC β-lactamases has arisen through the transfer of chromosomal genes for the inducible AmpC β-lactamases on to plasmids. The transfer has resulted in plasmid mediated AmpC β-lactamases in isolates of *Escherichia coli, Klebsiella pneumoniae, Salmonella species, Citrobacter freundii, Enterobacter aerogenes and Proteus mirabilis* [6].

Prevalence of this resistance mechanism appears to be increasing and has been responsible for nosocomial outbreaks, avoidable therapeutic failures (sometimes

fatal) and outbreaks of multidrug resistant Gram negative pathogens that require expensive control efforts [3]. In view of increasing reports of AmpC β-lactamase producing strains of *Klebsiella spp.* and *E. coli* from around the world, the present study was undertaken with an objective to examine the occurrence of Amp C β-lactamase producing strains of *E. coli* from various clinical specimens.

Material and Methods

A total of 100 non repetitive, non enteric clinical isolates of *E. coli* obtained from various clinical specimens were studied over a period of 6 months in Shri B. M. Patil Medical College, Bijapur. Isolates were obtained from various clinical specimens like urine, pus, sputum, blood and other body fluids. All the isolates were identified as *E. coli* by standard biochemical methods. The sensitivity of *E. coli* isolates were determined by Kirby Bauer disc diffusion method [7] (concentration/disc in µg) to ampicillin (10), amikacin (10), gentamicin (10), cefotaxime (30), ceftazidime (30), ceftriaxone (30), cefoxitin (30), amoxycillin-clavulanic acid (20/10), ciprofloxacin (5), piperacillin-tazobactum (100/10), imipenem (30) (Hi-Media, India). The results were interpreted as per CLSI recommendations [8]. *E. coli* isolates were screened for AmpC β- lactamases by standard disc diffusion breakpoint for cefoxitin. Isolates with zone diameter less than 18mm for cefoxitin were tested for AmpC activity by AmpC disc test [3, 9].

AmpC disc test: Here, a lawn culture of *E. coli* ATCC 25922 was prepared on MHA plate. Sterile discs (6mm) were moistened with sterile saline (20µl) and inoculated with several colonies of test organism. Inoculated disc was then placed beside a cefoxitin disc (almost touching) on the inoculated plate. The plates were incubated overnight at 35^0 C. A positive test appeared as a flattening or indentation of cefoxitin inhibition zone in vicinity of test disc. A negative test had an undistorted zone [10].

Results

Of the 100 clinical isolates of *Escherichia coli*, 53 isolates were from urine, 30 from pus, 7 from sputum, 5 from body fluids and 5 from blood. Of the100 isolates screened for AmpC β-lactamase production by standard disc diffusion breakpoint for cefoxitin, 30 showed zone diameter less than 18 mm (screen positive). These isolates were considered as presumptive AmpC producers and further confirmed by AmpC disc test. Of the 30 isolates, 24 showed positive result by AmpC disc test. Indentation indicating strong AmpC producer was observed in 19 isolates where as flattening indicating weak AmpC producer was observed in 5 isolates. Among the total 24 AmpC producing strains of *E. coli*, 12(50%) were from urine specimens, 7(29%) from pus, 3(13%) from sputum, 2(8%) from body fluids. AmpC producing strains showed high degree of resistance to gentamicin (95.8%), amoxycillin + clavulanate (95.8%), ciproflo-xacin (87.5%), piperacillin + tazobactum (83.4%). But all the AmpC producing strains were sensitive to imipenem.

Discussion

Organisms over expressing AmpC β-lactamases are a major clinical concern because these are usually resistant to all β lactam drugs except for cefepime, cefpirome and carbapenems [11]. Failure to detect AmpC β-lactamase producing strains has contributed to their uncontrolled spread and therapeutic failures. Hence their appearance in a hospital setting should be indentified quickly so that appropriate antibiotic usage and containment measures can be implemented [10]. Detection of AmpC β-lactamase is a challenge to clinical microbiologists. The current CLSI documents do not indicate the screening and confirmatory tests that should be used for detection of AmpC β-lactamases [1]. Phenotypic variations in the bacterial expression of plasmid encoded AmpC mediated resistance have to be addressed cautiously. The accurate detection of plasmid mediated AmpC is important to improve the clinical management of infection and to provide sound epidemiological data [12].

Prevalence of AmpC β-lactamases among *E. coli* in the present study was found to be 24%, while Ratna et al [3], Subha et al [13], Singhal et al [9] and Sinha et al [14], have reported prevalence ranging from 3.3% to 37.5%. Cefoxitin discs were used for screening

AmpC production. However, we observed that 6 cefoxitin resistant isolates did not produce AmpC, which may be attributed to other resistance mechanisms like porin channel alteration in these isolates.

Multidrug resistance (resistance to 3 or more drugs) was observed in most of the AmpC harboring isolates (92%). Similar findings are reported in studies conducted by Sinha et al [14], Taneja et al [15]. This emphasizes the need for detecting AmpC β-lactamase producing isolates so as to avoid therapeutic failures and nosocomial outbreaks. But all AmpC producing isolates in the present study were susceptible to imepenem. Carbapenems can be used to treat infection due to AmpC producing bacteria but carbapenem resistance can arise in some organisms by mutation that reduce influx or enhance efflux [16].

Though three dimensional test is gold standard for AmpC detection, it is labour intensive and cannot be performed routinely on all clinical isolates [17]. AmpC disc test can be used as a simple, convenient and rapid screening test for detection of AmpC β-lactamase in clinical laboratories. Potential benefits would include better patient outcome in terms of avoiding inappropriate therapy and a reduction in escalation of antibiotic resistance through better infection control.

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