Research Paper

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Excessive Use of Third Generation Cephalosporins May Increase in AmpC Producing Beta-Lactamase in Enterobacteriaceae

Medical Science

KEYWORDS :

Dr. Arijit Chatterjee C/o- Smt. Gouri Mitra, 6/1 A MATHUR BABU LANE, P.O. TANGRA, KOLKATA-700015 (WB)

ABSTRACT

Nowadays, Extended spectrum β-lactamase (ESBL) and AmpC β-lactamase -producing enterobacteriaceae has been observed largely.

In this study, the prevalence AmpC producing ESBL strains of enterobacteriaceae was done in urinary tract infections patients in SRL Reference Laboratory, Salt Lake, Kolkata-700092.

The prevalence of AmpC producing beta-lactamase is very important to know to prevent the misuse of antibiotics, especially third generation cephalosporins which may induce the production of this enzyme in enterobacteriaceae family.

INTRODUCTION

Urinary Tract Infection (UTI) causing microorganisms eg. Enterobacteriaceae family have the ability to produce Beta-Lactamase enzymes in large quantities. These enzymes are plasmid borne and confer multiple drug resistance, making urinary tract infection difficult to treat with common oral antibiotics.

ESBLs are enzymes capable of conferring bacterial resistance to the penicillins, first, second and third generation cephalosporins and monobactum groups of antibiotics such as aztreonam. They are inactive against the cephamycins groups (cefoxitin and cefotetan) but, are susceptible to β- lactamase inhibitors (clavulanic acid, sulbactum, tazobactum etc.).

The ESBL phenotypes have become more complex due to the production of multiple enzymes which include the inhibitor-resistant ESBL variants and plasmid-borne AmpC.

AmpC is normally produced in low levels by many organisms and is not associated with resistance, but it can be produced at high levels and cause resistance to all beta lactams, except carbapenems (Imipenem, meropenem) and 4th generation cephalosporins (Cefepime, cefpirome).

This study was done to explore the prevalence of AmpC producing ESBL strains of enterobacteriaceae in patients with urinary tract infections in SRL Reference Laboratory, Salt Lake, Kolkata-700092.

MATERIALS AND METHODS

Early morning and mid-stream urine is collected in a sterile container. The urine samples were routinely checked for the presence of pus cells per HPF under the compound light microscope to establish 'Significant Bacteriuria' and to avoid contaminated false growth.

The test is based on use with the application of Cefoxitin disc to show the release β- lactamases into the external environment. Cefoxitin (30µg) resistant enterobacteriaceae isolates tested by AmpC test as following: -

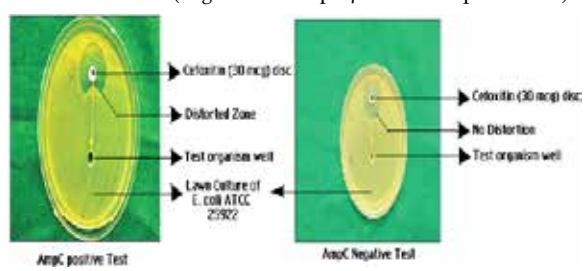
A lawn culture of a 0.5 McFarland's suspension of strain of *E. coli* (ATCC 25922, bioMérieux) was prepared on a Mueller-Hinton agar plate. A 30 µg cefoxitin disc was placed almost at the centre on the inoculated surface of the agar. The test organism was taken with a sterile nichrome loop and a heavy inoculum was prepared in nutrient broth.

The inoculum was centrifuged @ 3000 r.p.m. for about 10 minutes and the bacterial deposit was taken for freezing, discarding the supernatant. The deposit was thawed again and was freezed, 4-5 times the process of freezing and thawing was repeated to extract the crude enzyme, if present.

A small well was prepared at 12-15 mm distance from the cefoxitin disc and a narrow channel was prepared from the well towards the disc almost touching the cefoxitin disc with the help of a sterile nichrome straight wire or by a scalpel. The well was inoculated or poured with the test organism deposit.

After 24 hours of incubation at 37°C, the plates were examined. for either an indentation, flattening or distortion in the zone of inhibition of cefoxitin disc, which indicated the enzyme inactivation of cefoxitin (positive for AmpC β-lactamase production).

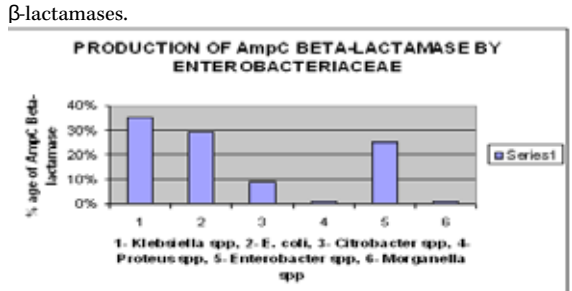
An absence of distortion, which indicated no significant inactivation of cefoxitin (negative for AmpC β-lactamase production).



RESULT AND DISCUSSION

In this study, we studied 100 urine sample from 100 UTI patients, samples yielded different isolates of enterobacteriaceae such as *Klebsiella spp*, *Escherichia coli*, *Citrobacter spp*, *Proteus spp*, *Enterobacter spp* and *Morganella spp*.

Out of 100 species *Klebsiella spp* 35 (35%), *Escherichia coli* 29 (29%), *Citrobacter spp* 9 (9%), *Proteus spp* 1 (1%), *Enterobacter spp* 25 (25%), *Morganella spp* 1(1%) produced AmpC β-lactamases.



AmpC β-lactamases producing Gram-negative bacteria are rapidly evolving group of plasmid-mediated enzymes emerging pathogens. Clinicians, microbiologists, infection control practitioners and hospital epidemiologists are concerned about AmpC-producing bacteria because of the increasing incidence of such infections.

CONCLUSION

The prevalence of AmpC producing beta-lactamase is very im-

portant to know to prevent the misuse of antibiotics, especially third generation cephalosporins which may induce this enzyme in enterobacteriaceae family which was our interest of study.

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