ORIGINAL RESEARCH PAPER

Microbiology

TO STUDY PREVALENCE OF EXTENDED SPECTRUM BETA LACTAMASE PRODUCTION IN ISOLATES OF E.COLI IN URINARY TRACT INFECTION.

KEY WORDS: Extended-spectrum beta-lactamase positive, *Escherichia coli.*

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ABSTRACT

AIM

1. To study prevalance of ESBL production in isolates of E. coli in urinary tract infection.

MATERIAL AND METHODS: This prospective study was carried out between January 2016 and September 2017. Follow-ups varied in patients according to their disease presentation and clinical outcomes. All strains from urine were cultured and identified by the Laboratory. In vitro presence of ESBL was confirmed by double disc method.

RESULTS: The most common isolated organism is E.coli.55 patients were studied out of which 26% are ESBL producer 76% are non ESBL.ESBLconfer multidrug resistance making UTI difficult to treat.

CONCLUSION ESBL prevalence is steadily increasing & confer multidrug resistant strains difficult to treat.

INTRODUCTION

The incidence of ESBL-producing strains among clinical isolates has been steadily increasing over the past years resulting in limitations of therapeutic option. Over the last fifteen years numerous outbreaks of infection with organisms producing extended spectrum -Lactamases (ESBL) have been observed world wide. These enzymes are plasmid-borne and confer multiple drug resistance, making UTI difficult to treat. There are not enough data on the prevalence of ESBL producers in UTI in India. Hence the present study was undertaken to find out prevalence of ESBL producers in urinary isolates of E.coli.

ESBL (Extended-Spectrum Beta-Lactamase)-producing E. coli are antibiotic-resistant strains of E. coli. E. coli are very common bacteria that normally live harmlessly in the gut. ESBL-producing strains produce an enzyme called extended-spectrum beta lactamase, which makes them more resistant to cephalosporin antibiotics Infections caused by ESBL-producing E. coli are a growing worldwide phenomenon.

ESBL E.coli are antibiotic resistant strains of e. coli UTIs have been reported to affect up to 150 million individuals annually worldwide[1]. A complicated UTI is an infection associated with a condition, such as a structural or functional abnormality of the genitourinary tract, or the presence of an underlying disease that interferes with host defense mechanisms, which increases the risk of acquiring infection or of therapeutic failure[2]

The World Health Organization and the EuropeanCommission have recognized the importance of studying the emergence and determinants of acquired anti-microbial resistance and the need to devise appropriate strategies for their control[3,4,5]

Resistant bacteria are emerging world wide as a threat to the favourable outcome of common infections in community and hospital settings. - Lactamase production by several gram negative and gram positive organisms is perhaps the most important single mechanism of resistance to penicillins and cephalosporins. In the past it was believed that cephalosporins were relatively immune to attack by - lactamases. It was surprising to find cephalosporin resistant *Klebsiella* spp. among the clinical isolates. The mechanism of this resistance was production of extended spectrum -lactamases (ESBLs)[6]. The ESBL enzymes are plasmid - mediated enzymes capable of hydrolyzing and inactivating a wide variety of lactams, including third generation cephalosporins, penicillins and aztreonam. These enzymes are the result of mutations of TEM-1 and TEM-2 and SHV-I. All of these - lactamase enzymes are commonly found in the *Enterobacteriaceae* family. Normally, TEM-1, TEM-2 and SHV-1 enzymes confer high level resistance to early pencillins and low level resistance to first generation cephalosporins. Widespread use of third generation cephalosporins and aztreonam is believed to be the major cause of the mutations in these enzymes that has led to the emergence of the ESBLs. These enzymes mediate resistance to cefotaxime, ceftazidime and other broad spectrum cephalosporins and to monobactams such as aztreonam, but have no detectable activity against Cephamycins and imipenem. Because, of their greatly extended substrate range these enzymes were called extended spectrum -lactamases.3 The first ESBL isolates were discovered in Western Europe in mid 1980s and subsequently in the US in the late 1980s[7]. The resistant organisms are now a worldwide problem. They can be found in a variety of *Entrerobacteriaceae* species, however, the majority of ESBL producing strains are *K. pneumoniae*, *K. oxytoca* and *E.coli.* Other organisms reported to harbour ESBLs include *Enterobacter* spp., *Salmonella* spp., *Morganella morganii, Proteus mirabilis, Serratia marcescens* and *Pseudomonas aeruginosa*. However, the frequency of ESBL production in these organisms is low. Major risk factors for colonization or infection with ESBL producing organisms are long term antibiotic exposure, prolonged ICU stay, nursing home residency, severe illness, residence in an institution with high rates of ceftazidime and other third generation cephalosporin use and instrumentation or catheterisation.

MATERIALS AND METHODS

DETECTION METHOD FOR ESBL (ACCORDING TO CLSI GUIDELINE) ESBL can be done according to criteria recommended by CLSI. Phenotypic confirmatory test for ESBL was preformed using modified double disc method. Enterobacteriaceae isolates resistant to any indicator cephalosporin in the screening tests outlined above should be subjected to confirmatory tests. Confirmation of ESBL production depends on demonstrating synergy between clavulanate and those indicator cephalosporin(s) to which the isolate was initially found resistant. Three methods can be used:

(I) Double disc tests. A plate is inoculated as for a routine susceptibility test. Discs containing cefotaxime and ceftazidime 30 µg (or cefpodoxime 10 µg) are applied either side of one with co-amoxiclav 20+10 µg; and c. 25-30 mm away from it. ESBL production is inferred when the zone of either cephalosporin is expanded by the clavulanate. The method is cheap, but the optimal disc separation varies with the strain and some producers may be missed. We therefore do not recommend this method.

(ii) Combination disc methods. (Oxoid or Becton Dickinson 'Combination Discs' and Mast 'MAST D52C ESBL'). These compare the zones of cephalosporin discs to those of the same cephalosporin plus clavulanate. According to the supplier, either the difference in zone diameters, (Oxoid or MAST) or the ratio of diameters (BD), is compared with zone diameter increases of >5 mm or >50% in the presence of the clavulanate implying ESBL production. These tests are cheap

and do not require critical disc spacing. (*LABORATORY DETECTION AND REPORTING OF BACTERIA WITH EXTENDED SPECTRUM -LACTAMASES Issue no: 2.2 Issue date: 19.05.08)*

(iii) Etest ESBL strips (AB Biodisk, Solna, Sweden; Bio-Stat, Stockport,). These have a cephalosporin gradient at one end and a cephalosporin + clavulanate gradient at the other. Users should follow the manufacturer's instructions, including for a heavier inoculum than in BSAC disc tests. ESBL production is inferred if the MIC ratio for cephalosporin alone: cephalosporin + clavulanate MIC is >8. These are accurate and precise, but more expensive than combination discs.

Recent Criteria recommended by CLSI. (2010) Single disc method

Cefpodoxime <22 mm
Ceftazidime <22 mm
Azteonam <27mm
Cefotaxime <27mm
Ceftriaxone <25 mm

DETECTION OF ESBL BY DOUBLE DISC METHOD

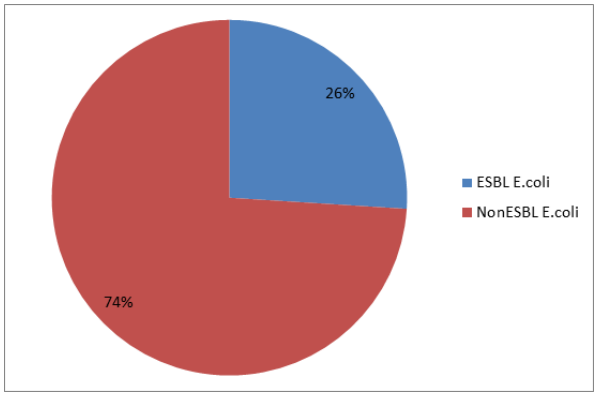
All isolates showing zone diameter of < 27mm for cefotaxime and < 25mm for ceftriaxone were selected for ESBL production. ESBL production was tested by DDST using a disc of ceftazidime-clavulanate along with four cephalosporins; 3GC-cefotaxime, ceftriaxone, cefoperazone and 4GC-cefepime. A lawn culture of the organisms was made on Mueller-Hinton agar plate as recommended by CLSI. A disc containing ceftazidime-clavulanate was placed in the centre of the plate. The discs of 3GC and 4GC were placed 15 and 20 mm apart respectively, centre to centre to that of ceftazidime-clavulanate disc . Any distortion or increase in zone towards the disc of ceftazidime-clavulanate was considered positive for ESBL production. K. pneumoniae ATCC 700603 was used as a control strain for positive ESBL production and E. coli 25922 was used as a negative control for ESBL production. (Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; 15th informational supplement. CLSI/NCCLS M100-S15. Wayne (PA): Clinical and Laboratory Standards Institute; 2009.)

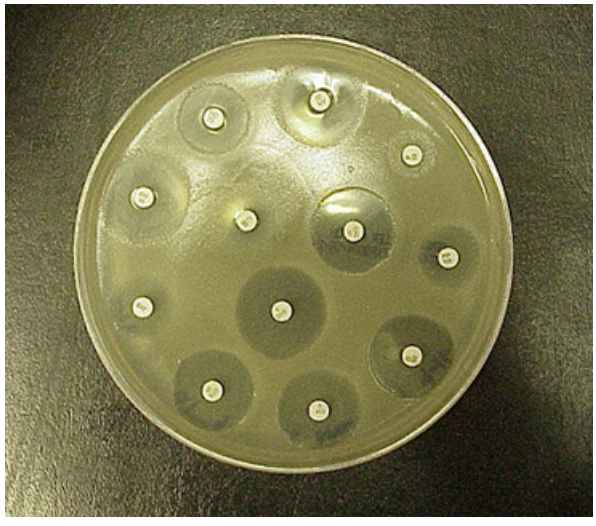
OBSERVATION AND RESULTS

PERCENTAGE OF ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *E.COLI*

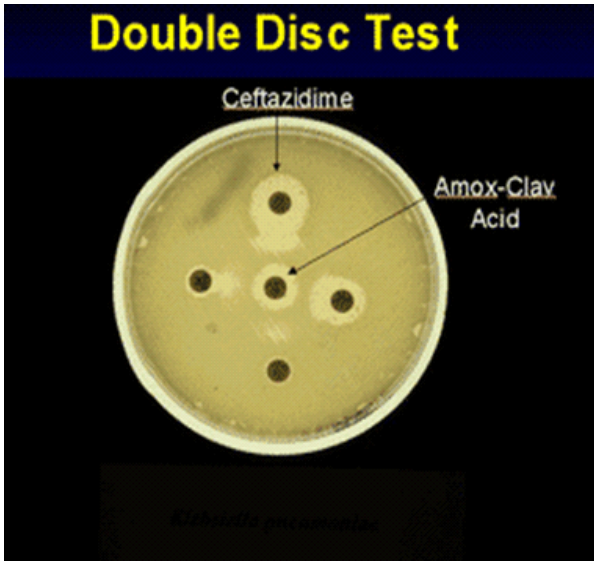
ANTIBIOTICS	ABBREVIATION	SENSITIVE	MODERATE SENSITIVE	RESISTANCE
Penicillin G	PnG	17	16	67
Methicillin	MET	28	9	63
Naficillin/Oxacillin	Naf	50	-	50
Nitrofurantoin	Nf	20	12	68
Ampicillin	Amp	12	25	63
Levofloxacin	Le	25	12	63
Ciprofloxacin	Cf	46	-	54
Gatifloxacin	Gf	50	19	31
Cephalosporin III generation (Ceftazidime)	Ca	12	19	69
Vancomycin	Vn	31	06	63
Amikacin	AK	69	06	25
Cefoperazone/ Salbactum	(7530)	60	27	13
Erythromycin	Er	50	19	31
Meropenem	Mem	27	13	60
Aztreonam	Azt	54	27	19
Imepenem	I	50	-	50
Tetracycline	Te	50	20	30
Fluoroquinolones	Flr	36	24	40
Fluconazole	Flu	10	-	90

CHART (1)-Proportion of ESBL and non ESBL producing Esch. coli





PICTURE 16 : KIRBY BAUER S AST USING MULLER HILTON AGAR



PICTURE 17: ESBL DETECTION USING- DOUBLE DISC METHOD

DISCUSSION

A total number of 55 urine samples were processed. Out of these samples 26% E.coli strains are ESBL producers. Similar study conducted at the Department of Microbiology, Kasturba Medical College, Manipal (2009). Showing 32% isolates of E. coli were ESBL producer which is similar to our study. In our study leucocytes

esterase test show pus cells - 27%, leucocytes esterase-15 %, nitrite -13%, as compared to the report of sunetha et al which is much more similar to our study. amikacin and nafcillin are sensitive antimicrobials. As compared to the study conducted by J.D.D. Pitout, (2007) 3rd generation cephalosporin's are sensitive .

CONCLUSION

Gram negative pathogens harbouring Extended Spectrum Beta Lactamases (ESBLs) have caused numerous outbreaks of infection and are becoming an increasing therapeutic problem in many countries. The incidence of ESBL producing E.coli strains among the clinical isolates has been steadily increasing over the past years resulting in limitations of therapeutic option.

REFERENCES

1. Stamm WE. The epidemiology of urinary tract infections: Risk factors reconsidered. Inter Sci Conf Antimicrob Agents Chemother. 1999;39:769.
2. Nicolle LE AMMI Canada Guidelines Committee. Complicated urinary tract infection in adults. Can J Infect Dis Med Microbiol. 2005;16:349–60. [PMC free article] [PubMed]
3. World Health Organization. Report on infectious diseases 2000: overcoming antimicrobial resistance. [Last accessed on 2000 Sep 23]. Available from: http://www.who.int/infectious.disease.report/index.html .
4. Centers for Disease Control and Prevention. Preventing emerging infectious diseases. [Last accessed on 2000 Sep 20]. Available from: http://www.cdc.gov/ncidod/emergplan/plan98.pdf .
5. European Community. A strategy against the microbial threat. Official Journal of the European Community. Council Resolution of 8 June 1999 on antibiotic resistance. Official Journal C 195, 13/07/1999. [Last accessed on 2000 Sep 29]. pp. 1–3. Available from: http://europa.eu.int/eurex/en/lif/dat/1999/en_399Y0713_01.html.
6. Aarestrup FM, Hasman H, Agersř Y, Jensen LB, Harksen S, Svensmark B. First description of blaCTX-M-1-carrying Escherichia coli isolates in Danish primary food production. J Antimicrob Chemother. 2006; 57(6):1258-9.
7. Arpin C, Coulange L, Dubois V, André C, Fischer I, Fourmaux S, et al. Extended-spectrum- beta- lactamase- producing Enterobacteriaceae strains in various types of private health care centers. Antimicrob Agents Chemother. 2007;51(9):3440-