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Research Article

EXTENDED SPECTRUM β-LACTAMASES AND AmpC β-LACTAMASES PRODUCING STRAINS OF *ESCHERICHIA COLI*: A CHALLENGE TO THERAPEUTICS IN CURRENT ERA OF ANTIBIOTICS

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A B S T R A C T

Objective: To determine the prevalence and presumptive antibiogram of ESBL and AmpC beta lactamases producing strains of *E. coli* isolated from different clinical specimens from a Tertiary Care Hospital of Punjab.

Material and Methods: Study was done from January 2014 to June 2014. A total 2069 various clinical samples received in Microbiology Department of GGSMCH, Faridkot were processed. *Escherichia coli* was the predominant bacteria and was identified as per CLSI. The antibiotic sensitivity pattern of *E.coli* was tested by Kirby Bauer's disc diffusion method. The isolates found resistant to one or more 3rd generation cephalosporins as per CLSI guidelines were further processed for confirmation by combination disc technique. All *Escherichia coli* isolates were tested for cefoxitin susceptibility (30μg). Isolates with zone of inhibition ≤ 18 mm were taken to be putative AmpC producers and further processed for confirmation by Boronic acid disc potentiation method.

Results: Of the 298 GNB isolates, predominant was E.coli (51%) followed by Pseudomonas (26.8%), Citrobacter (12%), Klebsiella (4.7%), Acinetobacter (3.7%) and Proteus (1%).

E.coli showed dreadful resistance to third generation cephalosporins (91%) as compared to other GNB isolates (57%). ESBL positivity was found to be 43% and AmpC β-lactamases (9.1%) among E.coli isolates.

Conclusion: The study concludes that there is a high prevalence of ESBL producing E.coli in our hospital. **So,** along with detection of ESBL, detection of AmpC β-lactamases is also important. Strategies to keep a check on the emergence of such drug resistant microorganisms by hospital environmental surveillance and laboratory monitoring should form an important aspect of Hospital Infection control policy guidelines.

INTRODUCTION

The Extended-Spectrum beta-lactamase (ESBL) and AmpC beta-lactamase producing *Escherichia coli* are emerging worldwide.[1] The havoc is created by ESBL-producing strains, as they are resistant to all penicillins, cepahlosporins (third and fourth generation agents) and to aztreonam; furthermore by AmpC β-lactamases which confer resistance to alpha methoxy β-lactams such as cefoxitin and β- lactamase inhibitors such as clavulanic acid. [2,3,4]

ESBL and AmpC producing *Escherichia coli* is unquestionably the frequently recovered bacterial pathogen from both hospital and community patients.[2,4]

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Beta lactamases producing *Escherichia coli* are distributed worldwide and their prevalence is increasing. Their incidence and antibiograms differ from country to country and within the same country between different geographical areas. [5,6]

The World Health Organization formulates appropriate strategies for determinants of acquired anti-microbial resistance and their control. Continuous monitoring of the changing pattern of resistance is required to choose appropriate empiric therapy. Beta lactamases producing strains are probably more prevalent than currently recognized and constitute a serious threat to currently available antibiotics and lapses in effective control measures. So vigilance and timely recognition of infection with resistant bacteria and their antibiogram for that area is useful for clinicians to make appropriate choice of empirical therapy.[6,7] Hence the study was designed with the aim to determine the prevalence and presumptive antibiogram of ESBL and AmpC beta lactamases producing strains of *E. coli* isolated from different clinical

specimens from a Tertiary Care Hospital of Punjab (North India).

MATERIAL & METHODS

From January 2014 to June 2014, a total 2069 clinical samples (urine, sputum, pus, blood and body fluids) received in Microbiology Department of GGSMCH, Faridkot were processed, out of which 532 were found to be culture positive among which 298 were gram negative bacilli. *Escherichia coli* were identified as per standard operating procedures including microscopy, culture and biochemical tests, which were further subjected for antibiotic sensitivity testing.[8] The antibiotic sensitivity pattern of *E.coli* was tested by Kirby Bauer's disc diffusion method following CLSI guidelines.[9]

ESBL detection

Screening test:- All *Escherichia coli* isolates were tested for susceptibility to 3rd generation cephalosporins by Kirby-Bauer method. The isolates found resistant to one or more 3rd generation cephalosporins as per CLSI guidelines were considered as suspected ESBL producers and further processed for confirmation by combination disc technique.[9]

Combination disc technique for detection of ESBL:- Ceftazidime (30µg) alone as well in combination with clavulanic acid (30/10µg) and Cefotaxime (30µg) alone as well in combination with clavulanic acid (30/10µg) were used. A ≥ 5 mm increase in the zone diameter for ceftazidime or cefotaxime in combination with clavulanic acid (30/10µg) versus that for ceftazidime or cefotaxime alone was taken as positive for ESBL production.

AmpC beta lactamase detection

Screening test:- All *Escherichia coli* isolates were tested for cefoxitin susceptibility (30µg) by Kirby-Bauer method as per Clinical Laboratory Standard Institute (CLSI) guidelines. Isolates with zone of inhibition ≤ 18 mm were taken to be putative AmpC producers and further processed for confirmation by Boronic acid disc potentiation method.

Boronic acid disc potentiation method:- Discs of cefoxitin alone and in combination with boronic acid were used. A ≥ 5 mm increase in the zone diameter around the disc containing boronic acid+ cefoxitin than around cefoxitin alone was considered positive for AmpC production.

Preparation of disc containing boronic acid+ cefoxitin:- The stock solution was prepared by dissolving 120 mg of phenylboronic acid in 3 ml of dimethylsulphoxide and then by adding 3 ml of sterile distilled water to this solution. 20µl of this stock solution was dispensed onto cefoxitin disc (30µg). Discs were allowed to dry for 30-60 min and used immediately.

RESULTS

Of the 298 GNB isolates, predominant was *Escherichia coli* (154; 51%) followed by *Pseudomonas spp.* (80; 26.8%), *Citrobacter spp.* (36; 12%), *Klebsiella spp.* (14; 4.7%), *Acinetobacter spp.* (11; 3.7%) and *Proteus spp.* (3; 1%).F

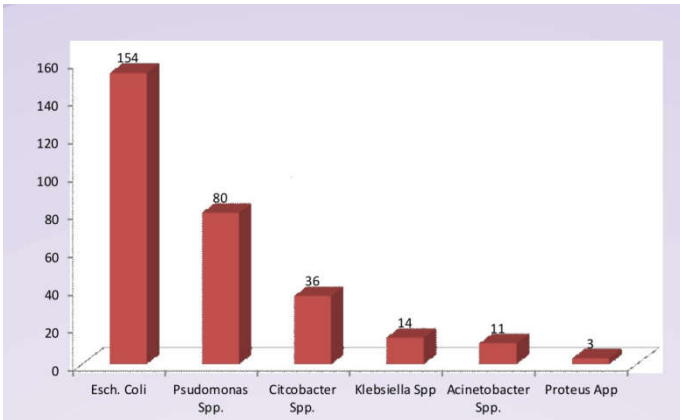

Figure 1 Graph showing number of various gram negative bacilli isolated from clinical specimens From total of 154 *Escherichia coli* isolates, the majority were from urine 88 (57%), followed by pus 43 (28%), body fluids 7 (4.5%) and blood 1 (0.6%). (Table 1)

Table 1 Specimen wise distribution of ESBL and AmpC producing E. coli isolates

Clinical specimen	Number of *Escherichia coli isolates*	ESBL producers	AmpC screening test positive
Urine	88	39	16
Pus	43	17	4
Catheter tip	11	5	1
Body fluids	7	3	0
Respiratory	2	1	0
Vaginal swab	2	1	1
Blood	1	0	0
Total	154	66	22

The clinical isolates of E.coli showed resistance to multiple antimicrobial agents. Maximum resistance was observed against ampicillin (89.6%), followed by cefotaxime (84.4%), ceftazidime (83.2%), ceftriaxone (82.5%). This was found to be co-existing with resistance to two or more antibiotics that is ciprofloxacin (57%), co-trimoxazole (59%), norfloxacin (56%), amoxyclav (55.4%), piperacillin+ tazobactam (53%), gentamicin (53%), amikacin (50.6%), chloramphenicol (31%). Least resistance was observed with imipenem (1.3%) and nitrofurantoin (4%) in cases of urinary isolates. (Fig. 2) ESBL production was found to be in 66 (43%) among *E.coli* isolates. Twenty two (14.3%) strains were positive for AmpC screening test. Fourteen (9.1%) were confirmed AmpC producers by Boronic acid disc potentiation method. AmpC genes were found in ten phenotypically positive strains.

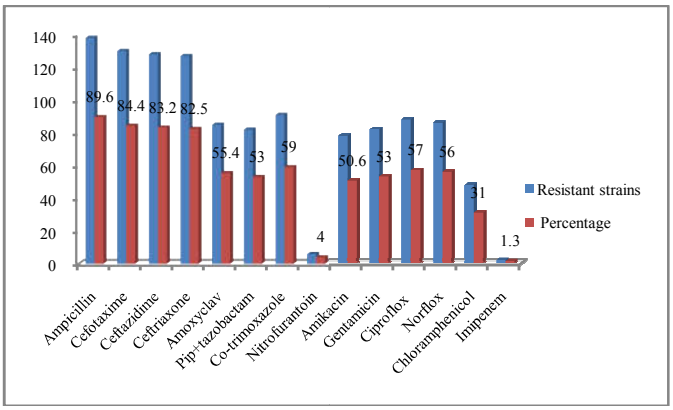

Figure 2 Antibiogram of E.coli isolates

DISCUSSION

The prevalence of ESBL, AmpC β-lactamases among common isolates like *E. coli* is increasing worldwide. In our study ESBL production was found to be in 66 (43%) *E.coli*. In India, the prevalence rate varies in different institutions from 28 to 84 %. [12,13]

In present study 14 (9.1%) E.coli isolates were AmpC producers. In last one decade, varied prevalences (3.4% - 47.8%) of AmpC enzyme has been reported from various parts of the country.[14-17] Moreover lack of standard guidelines for AmpC detection hinders investigation of their epidemiology and the clinical significance.[18] Multidrug resistance among beta lactamases enzyme producing strains has also been documented, which demonstrates the plasmid mediated spread of drug resistance genes.[3] This has led to treatment failures to commonly used antibiotics. This also is adding to increased use of high end antibiotics like carbapenems and with the loss of outer membrane porins, these strains become resistant even to carbapenams.[19]

Hence continuous surveillance for the mechanisms of resistance in the common pathogens is the needed for prevention of spread of the resistant strains. This will aid the physicians to prescribe the most appropriate antibiotics, which will curtail the misuse of high end antibiotics.

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