Original Article

Frequency of AmpC β-Lactamases in Multi-Drug Resistant Isolates of Escherichia Coli at Tertiary Care Hospital

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ABSTRACT

Objective: To determine the frequency of plasmid mediated AmpC beta-lactamases (PABLs) in multi-drug resistant isolates of E.coli.

Study Design: Cross sectional study

Place and Duration of Study: This study was conducted in a 450- bed Tertiary Care Hospital of Lahore, (Ittefaq Hospital, Lahore), Pakistan from January 2014 to December 2014.

Materials and Methods: Total 2610 clinical specimens were obtained from different wards and outdoor patient department (OPD) of hospital. Out of 2610 cultured samples only 1200 specimen showed significant growth of pathogens. These isolates were further proceeded for identification through colonial characters, gram staining and biochemical reactions. Standards protocols were used for inoculation, identification and isolation of E.coli. Cefoxitin resistance was used as a primary screening protocol for AmpC enzyme production. AmpC disk test (EDTA disk) was used as a confirmatory test for AmpC enzyme production.

Results: Out of 2610 culture samples only 1200 specimen showed significant growth of pathogens. Out of 1200 positive cultures, 421 (35.08%) isolates were identified as E.coli. Male patients 230 (54.63%) and 191 (45.37 %) female patients having mean ages 44.23 + 10.89 years. AmpC producing strains were more prevalent in surgical specimens than others. Out of 421 E.coli isolates, only 19.95% (n=84) E.coli were selected as plasmid mediated AmpC β-lactamase (PABLs) producers. After the confirmatory AmpC disk test only 8.07% (n=34) E.coli were isolated as PABLs producers and 47.05% (n=16) as ESBL producers. Total 20% (n=84) cefoxitin resistant was detected and 40.48% (n=34) of E.coli were isolated as PABLs producers from cefoxitin resistant. Out of (n=34) the percentage of PABLS and ESBLs co-producers was 26.47 % (n=9).

Conclusion: The isolates that involved in coproduction of PABLs and ESBL showed increased MICs against the applied cephalosporin drugs. This study noted high frequency of PABLs producing bacteria from hospital that may contribute to serious therapeutic problems.

Key Words: Hospital acquired infection (HAI), E.coli, Antimicrobial sensitivity testing, PABLs, ESBLs

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INTRODUCTION

Family of Enterobacteriaceae is the major cause of hospital acquired infections (HAI) and the microorganisms present in this family are acquiring multidrug resistance against the routinely used drugs by the production of AmpC β-lactamases[1,2]. These are worldwide distributed.

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The AmpC β-lactamases could be either plasmid mediated or chromosomal mediated. No doubt the development of drug resistance in bacteria is a major threat to antimicrobial chemotherapeutic interventions[3,4]. Generally, three categories of antibiotic resistance are presented by bacteria; Intrinsic, acquired and adaptive. The important mechanism of drug resistance to drugs that contain β-lactam ring structure, is the synthesis of β-lactamases. The β-lactamases are AmpC β-lactamases and ESBLs[5]. The genes involved in the synthesis of AmpC β-lactamases are mainly chromosomal mediated. However, in addition to these chromosomal mediated genes, plasmid mediated AmpC β-lactamases (PABLs) have been arisen by chromosomal gene transfer to plasmids and can lead to spreading of antibiotic resistance in different bacterial populations such as E. coli, Klebsiella spp, Proteus mirabilis and Salmonella spp[6,7]. The diagnostic and therapeutic challenges are being offered by PABLs and

ESBLs to the health care experts. The detection of PABLs and ESBLs can play an important role in several surveillance and epidemiological studies[8,9]. It is also important for infection control because may be all these types of resistant genes transfer from one organism to another organism in hospital environment[10].

The present study was carried out to disclose the prevalence of PABLS that have been produced by E.coli in a tertiary care hospital of Lahore. The AmpC disk test is a specified test which performed for detection of PABLs production. PABLs producing bacteria were further tested for antibiotic susceptibility and ESBLs production. This study will help out for the adaptation of better and effective hospital antibiotic policy against the pathogens that are involved in production of PABLs. The results of this study will help to create awareness among healthcare professionals for effective management of such pathogens in their clinical practice which will reduce burden of antimicrobial resistance in our population owing to rational drug therapy.

MATERIALS AND METHODS

The study was executed from January 2014 to December 2014 in a 450- bed tertiary care hospital of Lahore, (Ittefaq Hospital, Lahore), Pakistan. Total 2610 clinical specimens such as catheter tips, sputum, pus, blood, tracheal tips, tracheal secretions, sputum, swabs from wounds, urine and body fluids were obtained from different wards and OPDs of hospital. All pathological specimens were inoculated on Blood agar and MacConkey except urine samples which were inoculated on CLED medium and plates were incubated aerobically at 35 + 2 ºC for 18 hours. Out of these samples only 1200 specimen showed significant growth of pathogens. These isolates were further proceeded for identification through, colonial characters, gram staining and biochemical reactions using API 20E (BioMerieux France). Confirmed isolates were further tested for production of PABLs and ESBLs. Mueller-Hinton agar was used to test the bacterial susceptibility against cefoxitin disk (30μg) by following the standard disc diffusion protocol[11].The isolates which showed resistance or <18mm zone diameter were further analyzed for PABLs production[12-13].The E.coli strain ATCC 25922 was applied as negative control which was phenotypically β-lactamase negative.

PABLs producing isolates were further investigated for ESBLs production by following the double-disk diffusion synergy technique[11-14]. The isolates were swabbed on Mueller-Hinton MHA ager after making 0.5 McFarland dilution of isolates in nutrient broth. A Amoxicillin/clavulanic (AMC 20/10 μg) disk was positioned in the center of MHA plate while the disk of ceftriaxone CRO (30μg), Aztreonam ATM (30μg), ceftazidime CAZ (30μg), cefepime FEP (30μg) and cefpodoxime CPD (10μg) were placed in a close proximity of 20-30 mm distance. The results were interpreted as ESBL production by the clear extension of zone of inhibition of cephalosporin towards Amoxicillin/clavulanic (AMC 20/10 μg) disk.

Kirby-Bauer method was used to determine antimicrobial susceptibility pattern of E.coli. Mueller-Hinton ager plates were used with 0.5 McFarland dilution of already refreshed cultures[11]. Different antibiotics of known concentration were applied on Mueller-Hinton (MHA) for checking the susceptibility. These plates were further incubated at 35 + 2ºC for overnight under aerobic conditions[15]. Following drugs were employed to observe their susceptibility; "Ampicillin 10ug, Amoxicillin/Clavulanic acid 20/10μg, Amikacin 30μg, Aztreonam 30μg, Ceftazidime 30μg, Cefpodoxime 10μg, Chloramphenicol 30μg, Ceftriaxone 30μg, Cefepime 30μg, Cefoxitin 30μg, Ciprofloxacin 5μg, Cefoperazone/Sulbactam 95/10μg, Imipenem 10μg, Norfloxacin 10ug, Nitrofurantoin 300μg, Piperacillin 100μg, Trimethoprim-sulfamethoxazole 1.25/23.75μg, Piperacillin/Tazobactam 100/10μg , Tetracycline 30μg, and Tigecycline 15μg discs (Oxoid)"[16]. Antibiotics zone of inhibition were measured and interpreted by following the guidelines of Clinical Standard Laboratory Institute (CLSI)[17]. The isolate were considered as susceptible to an antimicrobial agent concentration when the zone of growth inhibition was in the range displayed by wild-type strain. Similarly, the isolates were considered as resistant to an antimicrobial agent when zone of growth inhibition was higher than that of wild-type strain. Two-fold agar dilution plate method was used to determine the minimum inhibitory concentrations of different drugs such as Imipenem, Cefepime, Cefoxitin and ceftazidime[18].

RESULTS

Out of 2610 culture samples only 1200 specimen showed significant growth of pathogens. These isolates were further proceeded for identification through Gram staining and biochemical reactions. Out of 1200 positive cultures, 421 (35.08%) isolates were identified as E.coli from 230 (54.63%) male patients and 191 (45.37 %) female patients having mean ages 44.23 + 10.89 years. From surgical specimens 20.1% (n=85), from non-surgical samples 60.80% (n=256) and from OPD 19% (n=80) E.coli were isolated from total (n=421) isolates. Out of total (n=34) AmpC producing strains were isolated from surgical specimens at the frequency of 44.12% (n=15), from OPDs 20.58% (n=7) and from non- surgical specimens 35.29% (n=12). AmpC producing strains were more prevalent in surgical specimens than others. Frequency of 421 E. coli was observed from different types of samples such

as sputum 4.75% (n=20), fluid 2.37 % (n=10), pus 30.87% (n=130), tissue 0.5% (n=2), urine 48.93% (n=206), blood 3.33% (n=14), tracheal secretions (n=7) 1.66%.

Out of 421 E.coli isolates, only 19.95% (n=84) E.coli were selected as plasmid mediated AmpC β-lactamase (PABLs) producers. After the confirmatory AmpC disk test only 8.07% (n=34) E.coli were isolated PABLs producers. Moreover 26.47 (n=9) of E.coli were ESBL producers. Almost 26.47 % (n=9) of E.coli were producing both ESBLs and PABLs. Out of n=34 AmpC producing strains of E.coli recovered from pathological specimens at the frequency of blood 8.82% (n=3), urine 17.64% (n=6), pus 52.94% (n=18) tips 8.82 (n=3), fluids 8.82 (n=3) and tracheal secretions 2.94% (n=1). AmpC producing strains of E.coli had been isolated from 21 (61.76%) male out of 34. AmpC producing strains were more prevalent in males than females.

PABLs producing strains of E.coli were resistant to Ampicillin, Piperacillin, Tetracycline, Amoxicillin/ Clavulanic acid, Aztreonam, Ceftriaxone, Ceftazidime and Cefpodoxime. PABL and ESBL co-producing E.coli were resistant to Cefepime. E. coli showed the susceptibility to Ciprofloxacin 17.64%, Amikacin 67.64%, Chloramphenicol 26.47%, Cotrimoxazole 20.58%, Imipenem 100%, Tigecycline 100%, Cefepime 67.6%, Cefoperazone/sulbactam 29.41%, Piperacillin /Tazobactam 41.17% (Fig.1). Norfloxacin and Nitrofurantoin were applied on isolates from urine samples. Nitrofurantoin was 71.42%and 100% susceptible and Norfloxacin was 100% resistant against isolates from urine. Cefepime was less susceptible; Imipenem and Tigecycline were susceptible against all isolates.

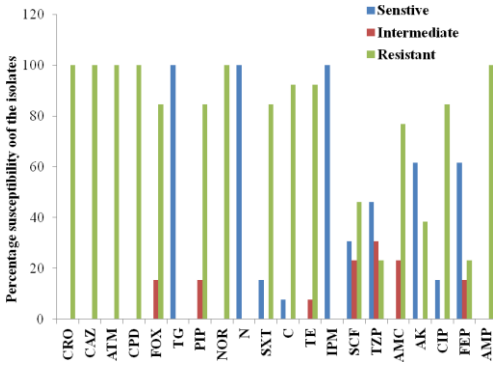

Figure No.1: Percentage Susceptibility pattern of PABLs producing E. coli.

Cefoxitin minimum inhibitory concentration for E.coli were 256 to ≥512 µg/ml. PABLs and ESBLS Co-producer showed increased MICs for Cefoxitin. High Ceftazidime MICs, 512 to ≥512 µg/ml were observed against AmpC producing strains. Cefepime MICs ranges from 4 to256 µg/ml against the isolates and is considered effective. High MICs Values were observed against ESBLs and PABLs co-producers and few of co-Producers showed intermediate to resistant MIC results (16 µg/ml to32 µg /ml) for cefepime. Imipenem MICs ranges from 0.5 to 2µg/ml were observed against all the isolates.

DISCUSSION

The current study detect the frequency of plasmid-mediated AmpC β-lactamases among the E.coli isolates from the hospital sites. Enterobacteriaceae play a major role in causing the hospital acquired infection and emergence of resistance in these organisms is a great challenge for health-care professionals[9]. The plasmid mediated AmpC beta-lactamases producing organism are very problematic in clinical point of view as they inhibit the action of most of the β-lactam drugs and make them ineffective. The mechanism of emergence of resistance in these organism is the production of ESBLs and PABLs[19].

In current study 8.07% (n=34) E.coli were isolated as AmpC producers. A similar study was carried out in a hospital of Pakistan, where 40.74% (n = 33) E. coli were PABL producers[9]. A study reported 39.3% AmpC β-lactamase producers in E.coli[20]. Another study reported 28.5 % AmpC producers in the E.coli isolates[21]. PABL producing strains were isolated from surgical specimens at the frequency of; 44.12% (n=15) E.coli. The frequency of PABL producers was high from surgical sites[22]. From OPDs 20.58% (n=7) E.coli had been isolated. From non- surgical specimens 35.29% n=12 E.coli were isolated. This may represent referral cases from other hospitals that indicate the frequency of PABL producers in community[23].

Almost 26.47 % (n=9) of E.coli were producing both ESBLs and PABLs. These findings were consistent with the previous study which reported PABLs and ESBLs in E. coli (24.24%)[17]. In my study 19.95% E. coli were observed as showing resistance to cefoxitin[24]. Another study demonstrated that 1.6 % of E.coli had been resistant to Cefoxitin. In my study 67.75% E.coli were sensitive to cefepime. 22.4% susceptibility was reported to Cefepime by the ESBL and AmpC producing strains[25]. All ESBL and AmpC β-lactamase co-producing isolates had been resistant to cefepime. Intermediate susceptibility to cefepime was exhibited by 8.24% of E.coli. Only two of the AmpC β-lactamase producing isolates of E.coli showed resistant to cefepime.

In our study, Ampicillin, piperacillin, tetracycline, Amoxicillin/Clavulanic acid, Aztreonam, ceftriaxone, Ceftazidime and cefpodoxime had no susceptibility against AmpC producing isolates. 94.3%, 80.2%, 77.6%, 78.6%, 8.6%, 10.2% resistant was reported to amoxicillin/clavulanic acid, Aztreonam, Cefepime, ampicillin/sulbactam, imipenem, piperacillin/ Tazobactam respectively by the ESBL and AmpC producing isolates[25]. 96%, 92%, 83%, 77%, 71% and 69% resistance to cefoxitin, ampicillin, ceftriaxone, ceftazidime, Cotrimoxazole and ciprofloxacin was

reported in isolates of E.coli respectively[21]. A study reported 59.7%, 92.7%, 90.72%, 86.6%, 39% and 79.3% resistant to Cefoxitin, Ceftriaxone, Ceftazidime, Aztreonam, Imipenem and Cefepime respectively among the members of the Enterobacteriaceae family. In my study, Ampicillin, piperacillin, tetracycline, Amoxicillin/Clavulanic acid, Aztreonam, ceftriaxone, Ceftazidime and cefpodoxime had no susceptibility against AmpC producing isolates. 94.3%, 80.2%, 77.6%, 78.6%, 8.6%, 10.2% resistant was reported to amoxicillin/clavulanic acid, Aztreonam, Cefepime, Ampicillin/Sulbactam, Imipenem, Piperacillin/ Tazobactam respectively by the ESBL and AmpC producing isolates[26]. 96%, 92%, 83%, 77%, 71% and 69% resistance to Cefoxitin, Ampicillin, Ceftriaxone, Ceftazidime, Cotrimoxazole and Ciprofloxacin was reported in isolates of E.coli respectively[20]

In our study isolates showed 100% susceptibility to Imipenem, and Tigecycline Worldwide studies also authenticate the imipenem efficiency against these most challenging organisms and even in serious infections, it was recommended as a drug of choice[25]. No resistance was reported to Imipenem by AmpC producers[20].

CONCLUSION

This study shows the high frequency of plasmid mediated AmpC beta-lactamases (PABLs) producing isolates. The important aims of study were to detect the possible source of emergence of these PABLs producing strains for the prevention and treatment of infections. It is required to establish the standard procedures in order to overcome the controversies in susceptibility reporting of PABLs producers. Indiscriminate use of antibiotics should be discouraged to control the development of multi-drug resistant microorganisms.

Author's Contribution:

Concept & Design of Study:	Whied ul Hussan Shahzad
Drafting:	Ali Abuzar Raza, Mehvish Javeed
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Revisiting Critically:	Whied ul Hussan Shahzad, Ali Abuzar Raza
Final Approval of version:	Whied ul Hussan Shahzad

Conflict of Interest: The study has no conflict of interest to declare by any author.

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