Original Article

Epidemiology of Extended Spectrum Beta Lactamase Producing Gram Negative Rods

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ABSTRACT

OBJECTIVE: To estimate the distribution of ESBL producing capacity among different Gram negative isolates along with specimen and department wise prevalence in a tertiary care hospital.

METHODOLGY: This cross-sectional study was conducted at the Microbiology section of Pathology Department, Allama Iqbal Medical College, Lahore, Pakistan, from August 2016 to January 2017. Total of 437 clinical samples were collected from different wards of Jinnah Hospital, Lahore. Sample was cultured on Blood agar, MacConkey's agar, chocolate agar, CLED agar. After the identification every Gram negative isolate was further processed for the antibiotic susceptibility testing following Modified Kirby Bauer disc diffusion method. ESBL detection was performed by the combination drugs disc method using ceftriaxone + clavulanic acid (30/10 µg) in case of Enterobacteriacae, ceftazidime + clavulanic acid (30/10µg) in case of Pseudomonas species.

RESULTS: Among total 437 patients male and female were 61% and 39% respectively, overall 21.5% (94/437) were ESBl producers. Distribution of ESBL producers was as followed Acinetobacter spp 22.3% (31/139), Escherichia coli 37.5% (27/72), Klebsiella spp 31.9% (15/47), Pseudomonas aeruginosa 8.4% (13/154) and Proteus spp 32.0% (8/25). Department wise ESBL positivity was as followed Surgical units 25.6% (32/125) Medical unit 31.9% (30/94), Burn centre 10% (8/80), ICU 20% (8/40) Surgical Allied 11.7% (4/34), Medical Allied 6.2% (2/32), Neurology 36.3% (8/22), Gynaecology 28.5% (2/7) and Dermatology 33.3% (1/3).

CONCLUSION: High percentage of ESBL positivity seen in Escherichia coli and Proteus spp, among departments neurology and medical unit is holding maximum positivity. High frequency of ESBL producing Gram negative isolates strongly recommend its detection and management accordingly. It can lead to treatment failures in Gram negative rods associated infections.

KEYWORDS: ESBL, Gram Negative Rods, Escherichia coli.

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INTRODUCTION

Inspite of several solid steps taken to reduce the emergence of drug resistance, still it is prime threat, our hospitals are full of infected people with no appropriate treatment due to drug resistant isolates, endangering the efficacy of antibiotics[1,2]. Clinicians have nothing for them but wait, pray and watch them die. Extensive and inappropriate use of β-lactam drugs in our health care settings has twisted foremost resistance problem leading to increase in health care cost, morbidity and mortality in all over the world. Production of β-lactamases is the most common cause of drug resistance against β-lactam group of antibiotics. There are more than 1300 beta lactamases in the arsenal of these enzymes and extended spectrum β-lactamases (ESBL) is one of them[3].

ESBL has generally been defined as transmissible beta-lactamases resistant to 3rd generation cephalosporin, monobactam that can be inhibited by clavulanic acid, tazobactam or sulbactam which are encoded by gene that can be exchanged between bacteria[4]. ESBL has generally been a group of plasmid mediated, diverse, complex and rapidly evolving emergency that is posing a major therapeutic challenges in the hospital acquired and tertiary care setups. ESBL majorly comes in the class B of AMBLER'S molecular classification[5]. ESBL lies in 2be, 2ber, 2de, 2e, according to Bush-Jacoby functional classification[6].

Detection of ESBL is tough job in developing countries[7]. According to few studies almost 37% of ESBL producers were misreported[8]. Moreover according to Tenover FC 1999[9] out of 38 laboratories only 07 were correctly identified and reported ESBL. These scenarios suggest that perfection in the skill of clinical laboratories to detect ESBL is required.

Therefore detection of ESBL is routinely involved in routine practice, which may lead to result in misreporting and henceforward treatment failures. Furthermore in low ESBL endemic areas it may not be cost effective to test for ESBL on a routine basis.

ESBL detection usually done via phenotypic and genotypic methods. Phenotypic methods include MIC by E-Strip, Agar dilution and by broth dilution[10]. The most common and sensitive method of genotypic detection of ESBL is PCR (Polymerase Chain Reaction). Many types of PCR are involved in the detection of ESBL i.e Duplex, Multiplex, Real-time, Pyro-sequencing, Reverse-line hybridization [11].

Detection of ESBL is a paramount as it provides clinicians with supportive information. Although ESBL producers may showed susceptibility to extended spectrum drugs i.e Cephalosporin or Aztreonam but they are intrinsingly resistant. Moreover the patients inhabited or infested with ESBL producer should be isolated under contact precautions to avoid spread of nosocomial infections. These benefits summon the detection of ESBL-producing organisms in clinical laboratories. Clinically the treatment option for ESBL producing isolates is limited to Carbapenems among beta lactam drugs. Spectrum of ESBL producer differs in accordance with geographic location[7]. Therefore present study was conducted to determine the spectrum of ESBL producers Gram negative rods among clinical specimens received from different departments of a tertiary care hospital.

METHODOLOGY

This cross-sectional study was conducted at Microbiology section of pathology department at Allama Iqbal Medical College, Lahore, Pakistan, from August 2016 to January 2017.Non probability consecutive sampling technique was used and a total of 439 clinical samples were collected from different wards of Jinnah hospital, Lahore.

All clinical samples both from male and female patients from indoor/admitted patients, Gram negative rods resistant to 3rd generation cephalosporin were included while duplicate samples from same patient during same episode of illness, all Gram negative rods susceptible to 3rd generation cephalosporin were excluded. Every sample was processed according to standard protocol and cultured on Blood agar, MacConkey's agar, Chocolate agar, CLED agar. Organisms were identified on the basis of colonial morphology (Lactose fermenter, non-lactose fermenter, late lactose fermenter, size, color of colony) Gram staining reaction and biochemical tests i.e. oxidase test, catalase test, Triple sugar iron, Citrate utilization test, Urease test, Indole test, semisolid motility agar.

ESBL: Detection by combination drugs disc method

After the confirmation, every Gram negative isolate was further processed for the antibiotic susceptibility testing following Modified Kirby Bauer disc diffusion method, ESBL detection was performed by the combination drugs disc method using ceftriaxone + clavulanic acid (30/10 µg) in case of Enterobacteriaseae and ceftazidime + clavulanic acid (30/10µg) in case of Pseudomonas species. Results were interpreted as followed, zone size of ceftriaxone + clavulanic acid disc is 5mm bigger than the zone size of ceftriaxone (30 µg) disc was tagged as ESBL producer while the zone size of ceftriaxone + clavulanic acid disc is not 5mm bigger than the zone size of ceftriaxone (30 µg) disc were considered as non ESBL producer. Same interpretation protocol was adopted for ceftazidime + calvulanic discs.

Data was entered in SPSS version 21.0 and descriptive analysis was done.

RESULTS

Among total 439 patients male and female were 61% and 39% respectively. Distribution of these 3rd Generation cephalosporin resistant isolates was as followed *Acinetobacter* spp 31.6%, *Escherichia coli* 16.4%, *Klebsiella s*pp 10.7%, *Pseudomonas aeruginosa* 35.0% and *Proteus* spp 5.6%. While distribution of ESBL producers was as followed *Acinetobacter* spp 22.3% (31/139), *Escherichia coli* 37.5% (27/72), *Klebsiella s*pp 31.9% (15/47), *Pseudomonas aeruginosa* 8.4% (13/154) and *Proteus* spp 32.0% (8/25) **Figure I.**

Figure II depicted department wise distribution of total samples and ESBL producers, maximum number of samples were received from surgical units 28.6% (12/437), followed Medical unit 21.5% (94/437), Burn centre 18.3% (80/437), ICU 9.1% (40/437) Surgical Allied 7.7% (34/437), Medical Allied 7.3% (32/437), Neurology 5.0% (22/437), Gynecology 1.6% (7/437) and Dermatology 0.6% (3/437). ESBL positivity was followed surgical units 25.6 (32/125), followed Medical unit 31.9% (30/94), Burn centre 10% (8/80), ICU 20% (8/40) Surgical Allied 11.7% (4/34), Medical Allied 6.2% (2/32), Neurology 36.3% (8/22), Gynecology 28.5% (2/7) and Dermatology 33.3% (1/3) **Figure II.**

FIGURE I: ORGANISMS BASED FREQUENCY DISTRIBUTION OF ESBL PRODUCERS (n=437)

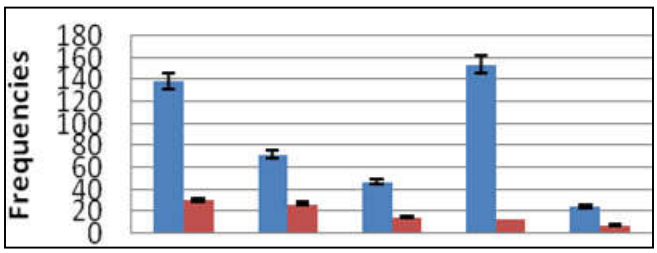
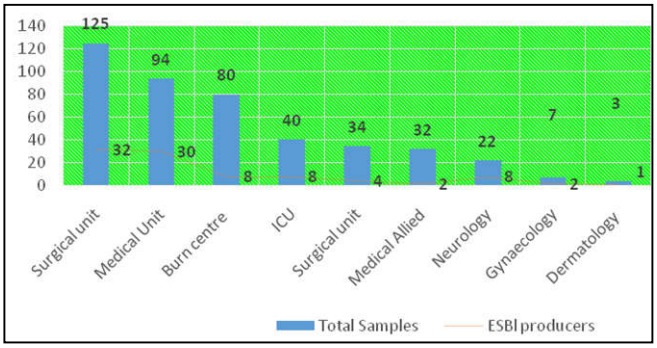


FIGURE II: DEPARTMENT WISE FREQUENCY DISTRIBUTIONS OF ESBL PRODUCERS (n=94)



DISCUSSION

Since 1970's when the first description was done, drug resistance surveillance has a key role among all policies to control the problem of drug resistance[12]. Globally ESBL have been isolated and form a major contributor of antimicrobial resistance in Gram negative isolates[13]. Now a days ESBL producers are prime threat as nosocomial infections for hospitalized patients throughout around the globe[14]. Frequency and distribution of ESBL producers among different clinical samples vary greatly around the globe. Present study reported 21.4% (n= 94) ESBL producers, *Acinetobacter* spp 22.3%, *Escherichia coli* 37.5%, *Klebsiella* spp 31.9%, *Pseudomonas aeruginosa* 8.4% and *Proteus* spp 32.0% **Figure I.** Similarly Ali AM 2004[15] from Army Medical College, Rawalpindi reported very high rate of ESBL producers i.e; 45% (n=366) *Escherichia coli* with 45% was the most frequent organism isolated followed by *Klebsiella pneumoniae* with 21%, *Pseudomonas aeruginosa* with 19.2%, *Enterobacter cloacae* 4.6% and *Acinetobacter baumannii* 4.4% Multiple studies reported similar results i.e Ahmad N 2016[16] reported out of 209 Enterobacteriaceae isolates, 32% were ESBL producers, Enterobacter species 47.8% Klebsiella species 40% and *E. coli 29.5%.* Kausar A et al[17] reported out of 199 Gram negative isolates 61% were ESBL producers, *E. coli* 56% Pseudomonas aeruginosa 18%, Klebsiella species 17% and Proteus species 8%. Ghazal L 2015[18] reported frequency of ESBL producing organisms 66%, among these GNRs, *Klebsiella pneumoniae* 52%, *Escherichia coli* 38%, *Enterobacter cloacae* 4.6%, *Citrobacter freundii* 3.6% and *Proteus mirabilis* 0.9%. Hafeez R 2009[19] reported 35% ESBL producers, with the highest frequency of *Escherecia coli* 44%, followed by *Klebsiella pneumoniae* 38%, *Proteus mirabilis* 31% and *Acinetobacter baumannii* 7%. Jabeen K 2005[7] reported 40% ESBL producers , 71% *E. coli, 15% K. pneumoniae and 9% Enterobacter species.* Sasirekha B 2010[20] reported 65.8% ESBL producers with maximal incidence in *E. coli* 73.8% followed by *Klebsiella pneumoniae* 51.1%. Rahman MM 2004[21] used double disk test to detect ESBL production, and reported *Escherichia coli* and *Klebsiella pneumoniae* 43.2% and 39.5% respectively. Jain et al from India reported distribution of ESBL producers as followed *Klebsiella* spp 87.2%, *Enterobacter* spp 72.5%, *E. coli 65.3%* and *Acinetobacter* spp 33.3%. and in none of the isolates of *Citrobacter* or *Pseudomonas* spp[22].

Excessive and imprudent use of antibiotics, lack of the knowledge for the development of new formula drugs by the pharmaceutical industries contributes in the emergence of bacterial resistance[23]. Although drug resistance is naturally observed in bacterial isolates of remote places irrespective of excessive human intervention of antibiotics. However antibiotic biosynthetic genes and resistance-conferring genes are also involved. Other mechanisms of drug resistance include, enzyme production causes inactivation of drug, modification of target sites, mutation in porin proteins and efflux pump[24].

Bacteria produces enzymes ß-lactamases inactivates beta lactam drugs by cleaving the ß-lactam ring of the drug and inactivate antimicrobial drug. Modified target sites are developed against which the drug has no effect. A mutant protein in the 30S ribosomal subunit can result in resistance to certain, and a methylated 23S rRNA can result in resistance to many antibiotics[25]. Bacteria decreases their cell membrane permeability so that effective intracellular concentration of the drug should not achieved such changes in porin proteins of cell membrane can reduce the amount of certain antibiotics to enter bacterium causes antibiotic resistance. Bacteria actively export drugs using a multidrug resistance pump or efflux pump that import protons and in an exchange-type reaction, export a variety of foreign molecules including certain antibiotics results in no antibiotic effect on the active site.

Genetic mechanisms of drug resistance include chromosome-mediated resistance, plasmid-mediated resistance, and transposon-mediated resistance[4]. CLSI recommended methods of screening of ESBL production are not much sensitive and can detect false positive ESBL producers. Double disc synergy method which CLSI recommends for epidemiological studies should be used in routine for detection of ESBL. Because treatment options are limited for ESBL producing Gram negative rods and are difficult to treat.

CONCLUSION

High percentage of ESBL positivity seen *Escherichia coli* and *Proteus* spp, among departments of

neurology and medical unit is holding maximum positivity. High frequency of ESBL producing Gram negative isolates strongly recommend its detection and management accordingly. It can lead to treatment failures in Gram negative rods associated infections.

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