Prevalence of Extended Spectrum Beta Lactamase amongst clinical specimens at a tertiary care hospital, Valsad

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Abstract

Background: Extended Spectrum Beta Lactamase (ESBL) are a variant of β-lactamase enzymes that open the β- lactam ring thus inactivating the antibiotic and conferring resistance.

Objective: To determine the prevalence of ESBL producing Gram Negative Bacilli isolated from various clinical specimens and to study their antibiotic susceptibility patterns.

Methodology: A cross sectional study was carried out at the Department of Microbiology, GMERS Medical College, Valsad for a period of 2 months. The antibiotic susceptibility patterns were tested using the Kirby- Bauer disc diffusion method following CLSI guidelines.

Results: 221 specimens were examined. Out of which 39 (18%) were Gram positive isolates, 63(28%) were Gram negative isolates and the rest 119 (54%) were no growth. Out of the 63 Gram Negative, 30(48%) were ESBL positive while the rest 33 (52%) were ESBL negative

Conclusion: Prevalence of ESBL in Gram negative bacilli was 55% and thereby it sends a signal among the medical fraternity for the rational use of antibiotics.

Keywords: Antibiotic susceptibility testing, Bacterial resistance, Extended Spectrum Beta Lactamase, Gram Negative Bacilli

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Introduction

As aptly quoted by Paul Ehrlich (1854-1915) "Drug resistance follows the drug like a faithful shadow".

ESBL are a variant of beta lactamase enzyme. They are enzymes that mutate the beta-lactam ring, inactivating the antibiotic[1] and conferring broad resistance to antibiotics like penicillins, cephalosporins and monobactams. They are not resistant to carbapenems and are commonly inhibited by beta lactamase inhibitors like clavulinic acid, sulbactum and tazobactum.[2,3] The first strain of ESBL was discovered in 1983 in Germany from Escherchia coli. Later they were isolated from England, USA and France from strains of Klebsiella pneumoniae. In India it was first discovered in Delhi in 2001 containing CTX-1 gene in an isolate of Klebsiella pneumoniae.[4]

The major cause of outburst of ESBL producing organisms is the inappropriate and irrational use of antibiotics in a healthcare setup. To reduce the rate of widespread resistance amongst antibiotics an appropriate antibiotic needs to be ensured for treatment. This can be done using the knowledge of different bacteria that cause infections and their antibiotic susceptibility patterns. The Gram negative bacteria causing ESBL resistance commonly encountered in the specimens are Klebsiella pneumoniae, Escherichia coli, Proteus mirabilis, Acinetobacter spp, Pseudomonas spp, Proteus vulgaris, etc. Bacterial resistance is the capacity of bacteria to withstand the effects of antibiotics that are intended to kill or control them. The mechanisms by which organisms acquire resistance are namely:[5] Alteration in target proteins reducing the affinity for antibiotic, impermeablity to antibiotic or its efflux so that it does not reach the site of action and elaboration of beta-lactamase which destroy the specific drugs. ESBL positive and ESBL negative organisms are calculated from time to time all over the globe to keep a check on the spread of resistant organisms.

Objectives

- To determine the prevalence of Extended Spectrum Beta-Lactamase producing Gram Negative Bacilli isolated from various clinical specimen
- To study the antibiotic susceptibility patterns in Gram negative bacilli isolated from various clinical specimen

Materials and Methods

Study type: Cross Sectional Study
Duration: 2 months (June- July 2015)
Study site: Department of Microbiology, GMERS Medical College and Hospital, Valsad, Gujarat

Inclusion criteria:

1. Clinical Specimens: Blood, urine, sputum, pus, stool, wound, swab, CSF, pleural fluid, ascetic fluid, Broncho alveolar lavage.
2. Culture showing presumptive growth of Gram Negative Bacilli.

Exclusion criteria:

1. Clinical specimen giving no growth result
2. Clinical Specimens with Extended Spectrum Beta Lactamase negative result
3. Unsterile clinical specimen
4. Improperly labelled clinical Specimen

Ethical Consideration: Permission from the Institutional Ethics Committee (IEC) was obtained.

Clinical specimens labelled and collected aseptically were received at the Microbiology laboratory. Gram's staining was performed using the standard method to differentiate between Gram positive and Gram negative bacteria. The specimens were cultured on appropriate bacteriological media and incubated at 37°C for 18-24 hrs.

On (Day 2) Gram's staining was performed on a single colony to confirm Gram Negative Bacilli.

(For Antibiotic susceptibility testing) A pure isolated colony was picked up and added to peptone water or nutrient broth and kept for 2 hours at 37°C. It was compared with 0.5 Mc Farland's standard and adjusted accordingly. It was inoculated in Muller-Hinton agar using sterile cotton swab. Appropriate antibiotic susceptibility discs (taking into consideration the clinical specimen) were placed at the distance of 15 mm from the edge of petri dish and leaving 30mm distance between each disc. It was incubated at 37°C for 18-24 hours.

(Day 3) The Antibiotic Susceptibility was measured using the zone measurement scale. For knowing the quality of antibiotic susceptibility discs we performed the similar tests using the ATCC strains.

Antibiotic susceptibility was performed using two methods:

Double disc approximation

- The susceptibility to Ampicillin sulbactam; piperacillin/tazobactam; Cefotaxime; Cefepime; Cefaclor; Cefixime; Imipenem; Gentamycin; Amikacin; Minocycline; Levofloxacin; Ofloxacin ; Nalidixic acid; Cotrimoxazole; Chloramphenicol; nitrofurantoin; polymyxin B; Cefotaxime were determined by Kirby-Bauer disc diffusion method according to CLSI Guidelines.[6]
- In a single petri dish a disc of amoxyclav(20 microgram amoxycillin and 10 microgram clavulinic acid)and 30 microgram of ceftazidime was placed at a distance of 15 mm.
- Bacterial susceptibility was checked after overnight incubation. If the organism was sensitive to amoxyclav but not to ceftazidime then it was considered to be ESBL positive.

Combination disc method

- The combination disc test would be done using both cefotaxime and ceftazidime (both 30 microgram) alone and in combination with clavulinic acid would be performed for ESBL detection according to CLSI guidelines.
- Cefotaxime (30 microgram) and cefotaxime-clavulinic acid (30/10 micro gram)discs were placed at a distance of 15 mm on an agar plate
- Incubate it overnight at 37°C
- >= 5 mm increase in the zone diameter of either antimicrobial agent which will be tested in combination with clavulinic acid versus its zone when tested alone would be checked for Extended Spectrum Beta-Lactamase production

After the growth takes place in the Muller Hinton agar the zone of inhibition is measured using the zone measurement scale. The results would be determined from the interpretation table given below.

Interpretation for ESBL

Drugs	Sensitive	Intermediate	Resistant
Cefotaxime	>=26	23-25	<=22
Cefepime	>=18	15-17	<=14
Ampicillin/ Sulbactam	>=15	12-14	<=11

If there is zone of inhibition seen in cefotaxime, cefepime, ampicillin/sulbactam is in the sensitive zone then the organism is sensitive to broad spectrum beta lactam antibiotics and if the zone of inhibition is resistant in any one of the above given drugs the sample is organism is Extended spectrum beta lactamase resistant.

Results

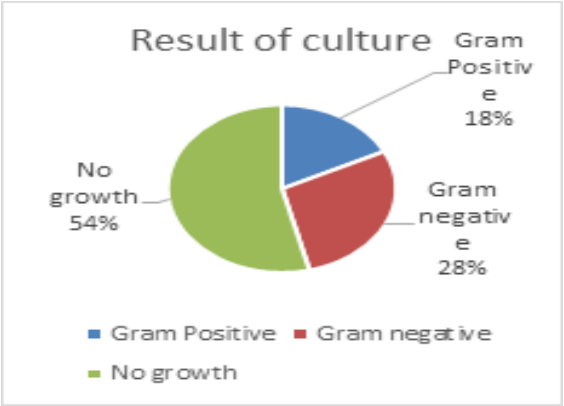

Fig. 1

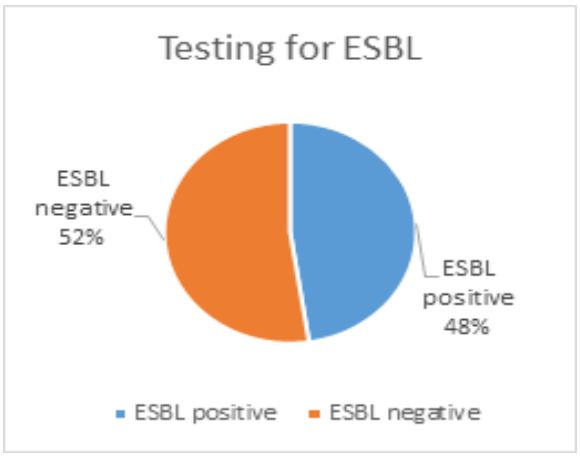

Fig. 2

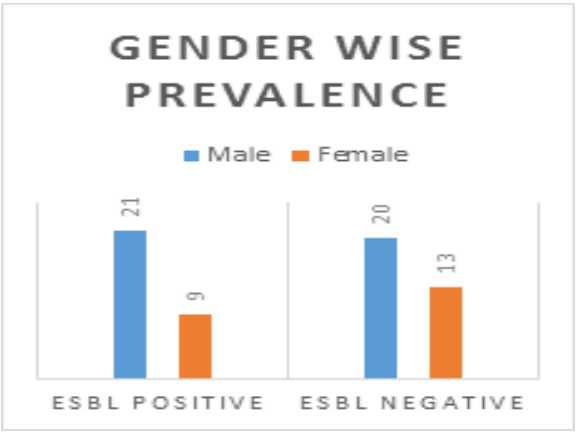

Fig. 5

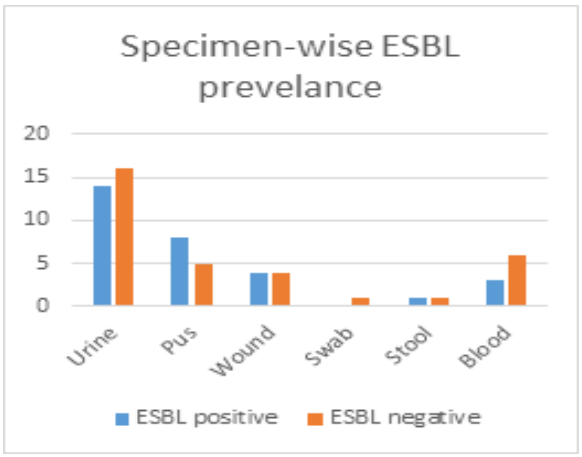

Fig. 3

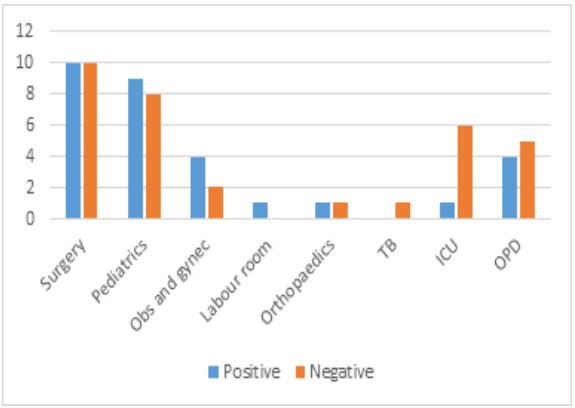

Fig. 6

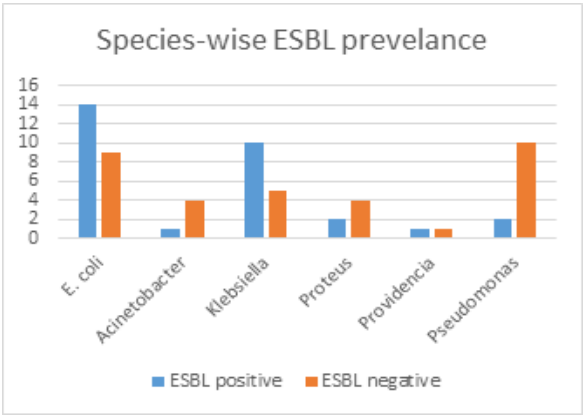

Fig. 4

Table 1

Organism/ ESBL	Urine		Pus		Blood		Wound		Stool		Swab	
	p	n	p	n	p	n	p	n	p	n	P	n
E.coli	7	4	3	1	2	2	2	0	1	1	0	0
Klebsiella	6	2	2	2	1	2	1	0	0	0	0	0
Proteus	2	1	0	0	0	1	0	1	0	0	0	1
Acinetobacter	0	2	1	0	0	0	0	1	0	0	0	0
Providencia	1	0	0	0	0	1	0	0	0	0	0	0
Pseudomonas	0	7	1	2	0	0	1	1	0	0	0	0

Here, p stands for positive and n stands for negative

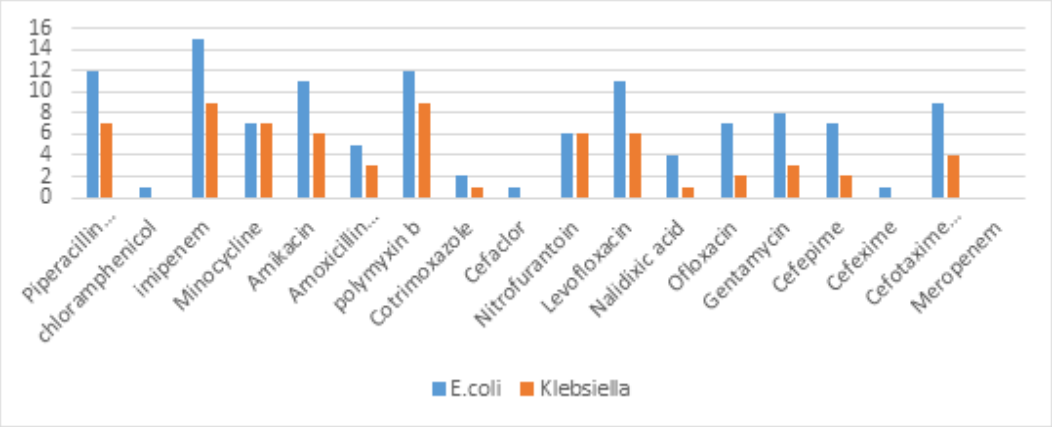

Fig. 7

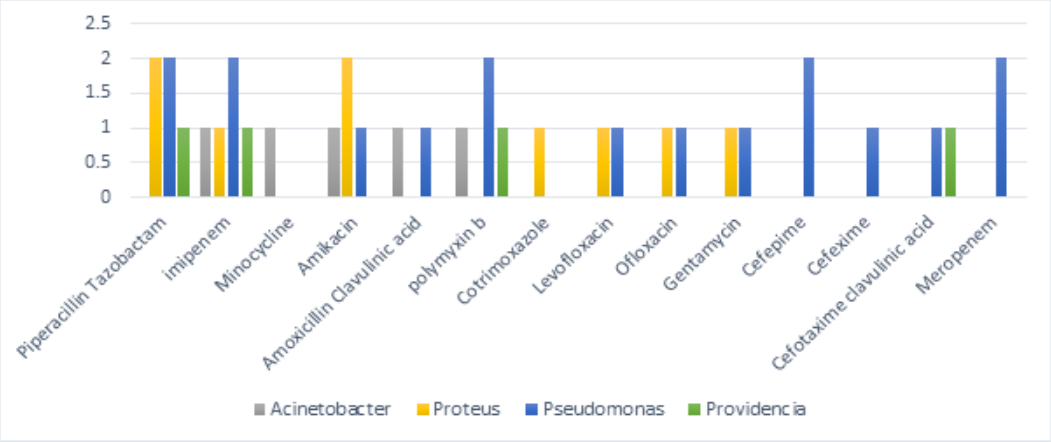

Fig. 8

Discussion

The results show that organisms harbouring ESBL enzymes are resistant to cephalosporins thus, creating challenges in the treatment of patients with special reference to complicated cases and ICU patients.[7] The results also state that the drugs which could easily treat Gram Negative infections are now difficult to treat.[8] The outbreak and spread of antimicrobial resistance has been well talked about problem world wide[3].

Fig. 1: out of the total 221 clinical specimens, 63(28%) were Gram negative while 39(18%) were Gram positive.

Fig. 2: ESBL positive and ESBL negative organisms isolated from various clinical specimens were 30(48%) and 33(52%) respectively. A study carried out at Puducherry in 2009 showed that there were 69% ESBL positive strains.[9] While a similar study carried out in Aurangabad in 2008-09 showed that 61% strains isolated were ESBL positive in a similar tertiary care hospital.[10] From this we can easily say that there is increase in the resistant strains which sends an emergency signal across all the medical fraternity to take steps towards antibiotic stewardship and make stringent control over the use of antibiotics.

Fig. 3: the most common site where ESBL resistance is seen is in the pus specimen where the extent is up to 61.5% which is followed by wound swab 50%, stool 50%, urine 46% and blood 33.3%. When we compare this data with that from the study carried out by Iroha, Amadi et al urine, blood, stool isolates had 34.9%, 54.2%, 26.3% prevalence.[10] While the study carried out in Ludhiana showed that the prevalence of ESBL in different specimen like urine, blood, pus and stool was 37%, 11%, 70% and 22% respectively.[11] Here in pus sample the most common organisms isolated were Escherchia coli and Klebsiella spp where the resistance was 75% and 50% respectively.

Fig. 4: shows the species wise prevalence of ESBL positive organisms. The most notorious species for acquiring the resistance were Klebsiella spp and Escherichia coli which have 66% and 61% prevalence respectively. While the resistance seen in Proteus spp, Providencia spp, Acinetobacter spp and Pseudomonas spp was 50%, 33%, 20% and 17% respectively. On comparison with study carried out at a tertiary care hospital in Lucknow showed that prevalence of Escherichia coli and Klebsiella pneumoniae was 37.5% and 63.1%.[12] A study carried out in Puducherry showed that there was 14% prevalence of ESBL in Pseudomonas spp. Pseudomonas spp shows less resistance to the 3rd

generation Cephalosporins because their resistance is mediated by different mechanisms.[9]

Fig. 5: shows that there is no correlation between the establishment of resistance by the organism and the sex of the patient as the prevalence among males is 51% and females is 41%.

Fig. 6: The resistant strains have been isolated from the Obstetrics and Gynaecology ward followed by paediatrics, Surgery and lastly the ICU, where the prevalence is 66%, 50%, 53% and 14% respectively. Indoor admitted patients have the maximum chances of getting the infection from resistant strains it may be due to cross infection from the patients or the hospital staff (nosocomial infections) or due to chronicity of infections. The main cause of microorganisms commonly infecting the wards may be due to frequent intervention by the means of indwelling urinary catheters, patients on I.V cannula, highly infectious wounds and open surfaces for infections due to trauma or burns.

The OPD isolates have shown 44% resistance which is significantly high confirming high resistance in the community. This may be due to lack of hygiene, may be lot of cross infection among the large populations, across the counter availability of antibiotics, lack of awareness and drug administration from quacks who frequently abuse antibiotics.

Fig. 7: almost all the ESBL positive Escherichia coli & Klebsiella spp are susceptible to imipenem. Among Escherichia coli & Klebsiella spp. 80% and 70% sensitivity is seen for piperacillin tazobactam. Minocycline was more sensitive for Klebsiella spp 70% but Escherichia coli was less susceptible to it 47%. Further the susceptibility for amikacin for these organisms was 73% and 60% respectively. The combination of Amoxicillin and clavulinic acid had 33% sensitivity to Escherichia coli and 30% to Klebsiella spp. While, the combination of cefotaxime and clavulinic acid had 60% sensitivity to Escherichia coli and 40% to Klebsiella spp. Similar sensitivity for Polymyxin B and Levofloxacin was 80% and 73% respectively for Escherichia coli while that for Klebsiella spp was 90% and 60% respectively. A study for ESBL producing organisms was carried out by K aruna et al at Mumbai showed similar sensitivity patterns for antibiotics like amikacin, cotrimoxazole, polymyxin B and amoxicillin / clavulinic acid.[13] Comparing it with similar study carried out at Punjab and Haryana by Singh et al also showed similar antibiotic susceptibility patterns.[11]

Fig. 8: Acinetobacter spp is sensitive to Imipenem, minocycline, amikacin, amoxicillin clavulinic acid and polymyxin B. Proteus spp are sensitive to Piperacillin Tazobactam, Imipenem, amikacin, cotrimoxazole, levofloxacin, ofloxacin and gentamycin. Pseudomonas spp are sensitive to Piperacillin Tazobactam, Imipenem, amikacin, amoxicillin clavulinic acid, polymyxin B, levofloxacin, ofloxacin, cefepime, cefexime, cefotaxime, meropenem and gentamycin. Providencia spp is sensitive to Piperacillin Tazobactam, Imipenem, polymyxin B and cefotaxime clavulinic acid combination.

Conclusion

The prevalence of ESBL in Gram negative bacilli in our tertiary care hospital is found to be 55%. It is important to detect them at a very early stage which would help in keeping a check on hospital acquired infections caused by them. The first step towards the goal of reducing the incidence of ESBL resistance is to reduce the inappropriate use of antibiotics and stopping their availability over the counter. In a hospital setting a strict antibiotic policy is the need of the hour. As far as the laboratories are concerned the ESBL reporting should be done diligently so that it helps the clinicians to prescribe the appropriate antibiotic. Another step to be taken is to deescalate the broad spectrum antibiotic as soon as culture and antibiotic sensitivity is available and shift the patient to the narrow spectrum drug.

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