ORIGINAL ARTICLE

Increasing Prevalence of ESBL Producing Bacteria from Ganga Raam Hospital, Lahore: Threatening remark

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Abstract

Background
Antimicrobial resistance occurs when microbes develop physical, physiological and behavioral responses against all chemical substances that normally kill them or check their growth. Extended Specturm Beta Lactamse-producing organisms exhibit co-resistance to wide range of antibiotics, moreover, inoculum effect and substrate specificity, their detection has become a major challenge The data obtained from this study is important for identifying emerging new pathogens in patients of Lahore and enables the development of targeted approaches to help control this threatening medical concern

Methods
Clinical samples (n=60) of patients were collected from Sir Ganga Ram Hospital Lahore and most of the samples were of the urine (n=25), followed by blood (n=14), pus (n=14), and sputum (n=7). Multidrug resistant bacteria were isolated and biochemical tests were performed for the genus and species identification. Further, combination disc test and *Epsilometer test* (*E- test*) were performed for detection of Extended spectrum beta lactamase production (ESBLs).

Results
The frequency of bacterial isolates *E. coli*, *Enterobacter* and *Klebsiella pneumonia* were 59%, 23%, 18% respectively. After ESBL detection test 60% (n=36) were found ESBLs positive and about (n=24) were ESBLs negative respectively. ESBL producing strains of *Enterobacteriaceae* have now become as a significant issue in hospitalized and community patients. These ESBL producing bacteria are major cause of urinary tract infection, septicemia, hospital-acquired pneumonia, intra-abdominal abscess, brain abscess and other diseases.

Conclusion
The frequency of ESBL producing bacteria is highest because of recommendation of broad spectrum antibiotics by doctors and misuse of prescribed medicines by patients. The results from this study helped the health care practitioner to use beta lactamse inhibitor alongwith prescribed antibiotics for treatment of these patients. E-test should be used as it yield qualitative results like classification of organisms as being resistant, susteptible or intermediate and easiness of setting up.

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Key Words
Antibiotic resistance, E- Test, Beta- Lactamases, *E. coli, Enterobacter, Klebsiella, pneumonia.*

Background
The E test (PDM epsilometer; AB Biodisk, Solna, Sweden) is an invitro method of determining the MICs of various antimicrobial agents. It is based on diffusion of antibiotic coated plastic strip into agar medium inoculated with test organisms. Previous studies with E-test concluded that it was the accurate method of determining Minimum inhibitory Concentration (MIC) of various antibiotics for *Staphylococci*,[1,2] gram negative bacilli[1] *Helicobacter pylori*,[4,8] and several genera of anaerobes.[3] Murray and Niles,[11] however concluded that minor error observed during application of E test made it an unfavorable method for testing against *Bacteroides fragilis*. Similar findings were reported with *Streptococcus pneumoniae*[7] demonstrated problems with certain organisms drug combination such as *H.influenzae* and ampicillin. Recent studies have observed good correlation between E-test and agar dilution MIC for penicillin and other drugs.[20]

Materials and Methods
Total 60 isolates were collected from Sir Ganga Ram Hospital Lahore from the clinical samples of urine (n=25), followed by blood (n=14), pus (n=14), and sputum (n=7). Bacteriological media including MacConkey's Agar, blood Agar, Chocolate Agar and CLED were used to culture the samples. All samples were cultured according to standard protocol. Suspension was lawned on to agar plates. When the surface of each plate had dried, E test strips of Ampicillin, ceftazidime, cefipeme and ceftriaxone were placed on the agar plate. After application of the E test strips, plates were incubated at 35°C in ambient air for 18 to 20 h (for Enterobacter) or for a full 24 h (for *Pseudomonas* species). MICs were read directly from the test

strip at the point where the elliptical zone of inhibition intersected the MIC scale on the strip (Figure 7). Combination disc test (Figure 8) was also applied for confirmation in some strains. Isolation, characterization and confirmation of beta lactamase production by infection producing isolates were done according to Standard Operating procedure recommended by health protection agency (HPA, 2006). Each strain was identified on the basis of gram staining and biochemical tests. The bacterial strain resistant to two or more antibiotics (carbapenems, fluoroquinolones, penicillins/cephalosporins, and aminoglycosides) is considered to be multi-drug resistant (MDR) bacteria.

Results

A total of 60 multi drug resistant bacteria (figure 4) were identified using Oxidase, indole, citrate utilization, sugar fermentation (Kligler iron agar medium) and urease tests (Figure 5,6). In all of above mentioned samples frequency of occurrence of *E. coli*, *Enterobacter spp* and *Klebsiella spp* were 59%, 23%, 18% respectively (figure. 1). Most of them (n=36) were ESBLs positive and about (n=24) were ESBLs negative and their percentage were 60% and 40% respectively (figure.2). In total 60 collected samples (n=25) were of urine, of which 64% were ESBLs producers and 36% were non-producers. Total (n=14) samples of blood were collected of which 57% are ESBLs producers and about 43% were non-producers. Total (n=14) samples of pus were collected of which about 64% were ESBLs positive while 36% were negative. There were (n=7) out of (n=60) samples were of sputum and their percentage is about 43% and 57% are ESBLs producers and non-producers respectively (figure 3). It was found that *E. coli* was 64%, 57%, 57% and 42% in samples of urine, blood, pus and sputum respectively. The results were found that 20%, 43%, 7% and 29% *Enterobacter spp.* were in samples of urine, blood, pus and sputum respectively (figure.4). It was estimated that about 16%, 0%, 36% and 29% of *Klebsiela spp.* were found in *samples* of urine, blood, pus and sputum respectively (Table 1).

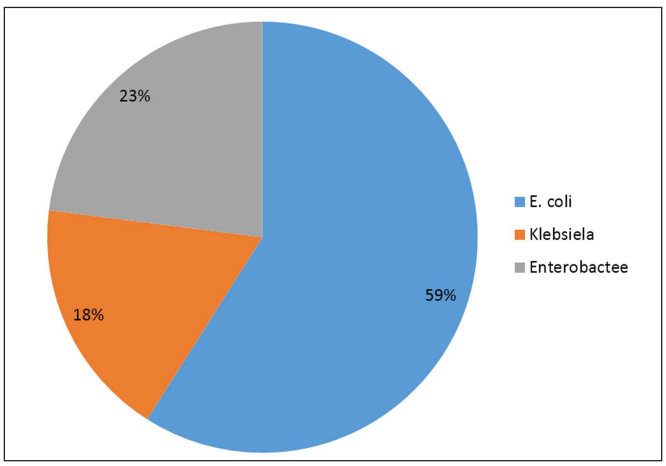


Fig1. Frequency of occurrence of different bacterial strains

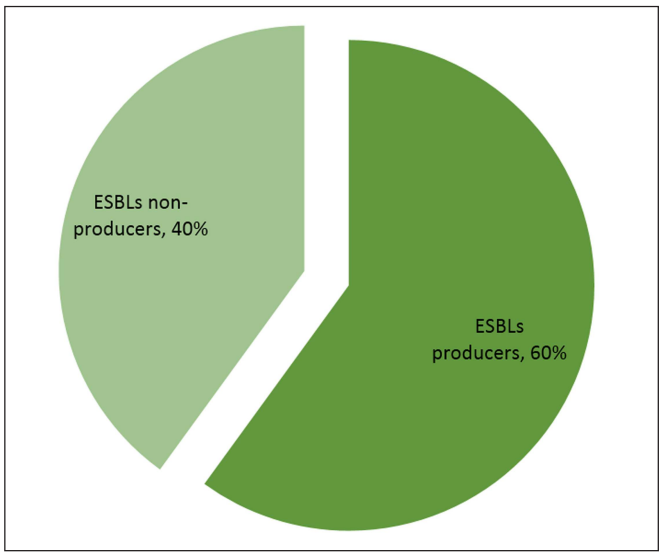


Fig 2. Prevalence of ESBLs producers and ESBLs non-producers

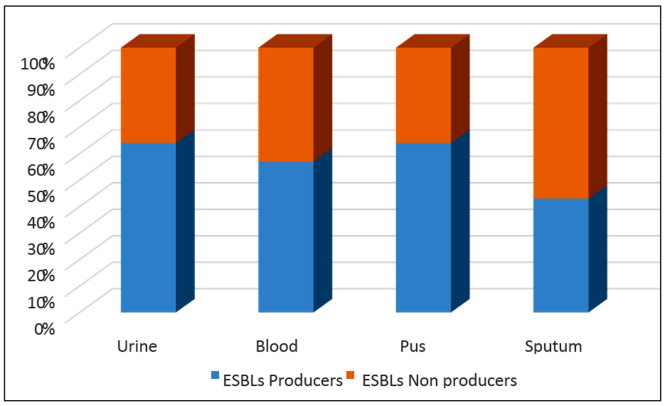


Fig 3. Prevalence of ESBLs producers and ESBLs non-producer in different clinical samples

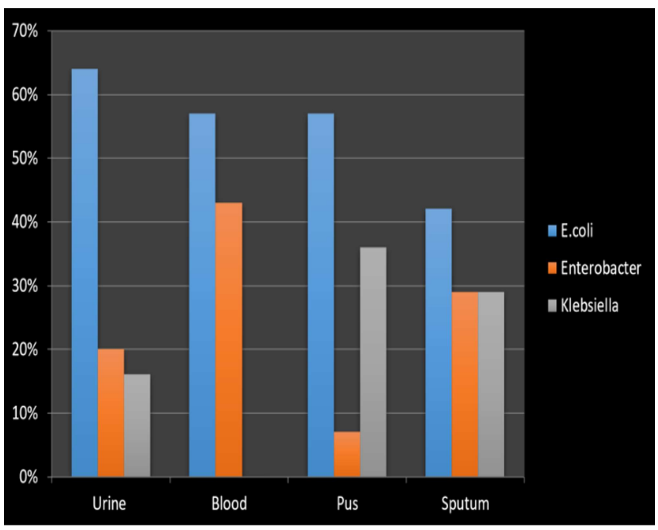


Fig 4. prevalence of different bacterial strains in different clinical samples

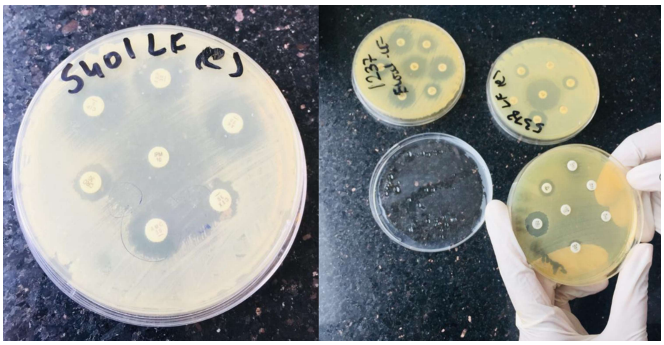
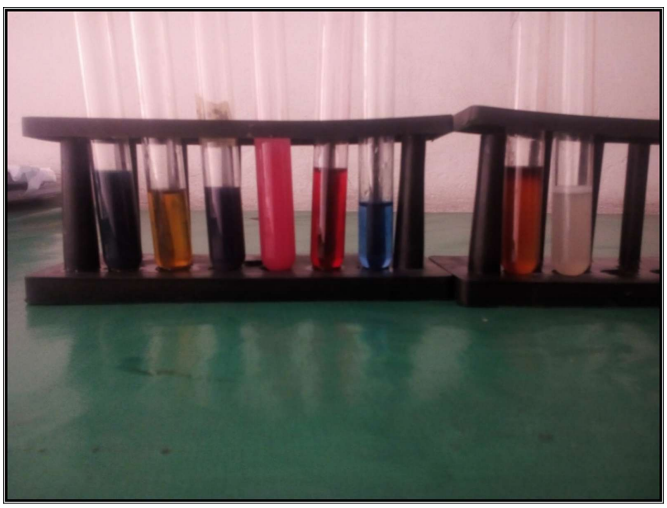


Fig 4. Multi drug resistant bacteria

CIT	TSI	LIA	URE	RM	VP	MAL	IND	MOT	GAS+
-	A/A	K/K	-	+	-	-	+	+	H2S-

Fig 5. Biochemical test for *E.coli*



CIT	TSI	LIA	URE	RM	VP	MAL	IND	MOT	GAS+
+	A/A	K/K	+	-	+	+	-	-	H2S-

Fig 6. Biochemical tests for *Klebsiella pneumoniae*

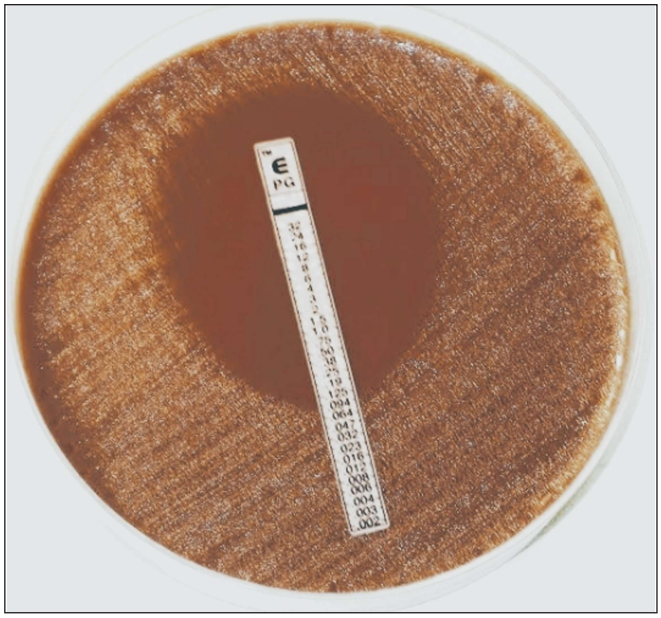


Fig 7. E test

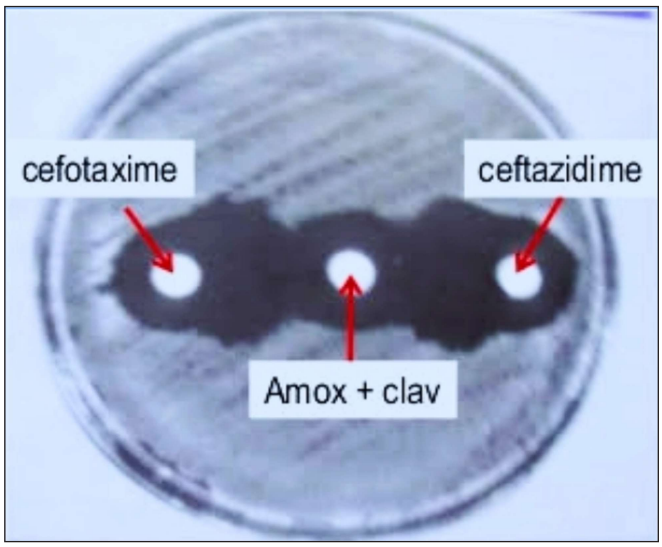


Fig. 8. Combination dic test

Table 1. Prevalence of ESBLs + and ESBLs – Bacteria in different clinical samples

Samples	No. of samples	ESBLs +	ESBL-
Urine	25	16	9
Pus	14	9	5
Blood	14	8	6
Sputum	7	3	4
Total	**60**	**36**	**24**

Discussion

Resistance in bacterial strains is of deep scientific concern. Microorganisms are getting resistant to antibiotics day by day. Over exposure to antibiotics chooses the antibiotic resistant trait which in return have challenge the accurate detection of ESBL.[15] Therefore, accurate detection of ESBL production will quicken the process of treatment therapy.

Urine samples n=25(30%) were collected from patients suffering from infections of urinary tract. It includes the infections of kidneys, ureters, Urinary bladder, and urethra.[25] The prominent gram–negative bacteria isolated from clinical samples were members of family Enterobacteriacae and P*seudomonas aeruginosa*.[24] Among gram negative bacteria, the emergence of resistance to expanded spectrum cephalosporins has been a major concern. Many of ESBL producing bacteria showed multidrug resistance. Resistance against antibiotics is appeared in bacterial species that do not naturally produce AmpC enzymes (*K. pneumoniae*) due to the production of TEM- or SHV-type ESBLs.[15]

E. coli was most prevalent isolate (64%) in clinical samples. The resistance recorded in these isolates was much greater than the previous studies conducted by other researchers. The study has confirmed that antibiotic resistance is continuously increasing in isolates collected from patients suffering from Urinary tract infections.[26] *Klebsiella pneumoniae* was second most abundant isolate (22%) in clinical samples. *Klebsiella pnemoniae* is widely distributed in all natural environments. This pathogen is responsible for urinary tract infections, blood born infections and severe form of pneumonia. This microbe can exist in two forms in environments conditions it loses its mobility, comes to surface of water bodies and becomes non-motile.[27]

Two methods for ESBL detection were compared. Major difference was observed between two methods. However, combination disc method needs to be revised. Combined disc method is more reliable method with ability to detect presence of ESBL. A study documented by Zali *et al*.,[23] recommended use of both ceftazidime and cefotaxime combinations to increase the sensitivity up to 93%.

A study conducted by World Health Organization using disc diffusion technique found that 5.4% of laboratories found an ESBL producing strain to be susceptible to all cephalosporins. Bacteria keep on assuming a significant part as a reason for medical care related diseases. Bacterial antibiotic resistance has become a significant clinical worry all through the Globe.[18] Recently, the utilization of second and third generations cephalosporins has prompted the determination of gram negative bacteria resistant to extended spectrum cephalosporins. This opposition is because of the production of extended spectrum β-lactamases.[11] The present study showed alarming rate of ESBL producers which is 60%. The failure of clinical treatment happens over and again, especially when wrong antimicrobial treatment is utilized to treat infections brought about by ESBL producing microbes.[12]

The treatment of infections caused by ESBL strains is a therapeutic challenge because of the limitations posed by high level resistance to various groups of antibiotics. Resistance pattern against a broad group of antibiotics may indicate formation of many such enzymes by ESBL positive group of bacteria.[21] Longer hospital stays, prolong use of antibiotics, unawareness of most clinicians, poor infection control practices, and over counter availability of antibiotics are major factor for spreading ESBLs. However, in all cases where infection with β-lactamase-producing bacteria is suspected, the choice of a suitable β-lactamase antibiotic should be carefully considered prior to treatment. In particular, choosing appropriate β-lactam antibiotic therapy is of upmost importance against organisms with inducible β-lactam-lactamase expression.

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