Original Article

Bacteriological Profile of ESBL Producing Bacteria With Their Antibiotic Resistance Pattern

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Abstract

Background: *Extended Spectrum Beta-Lactamase (ESBL) can cause infections, such as kidney infections, wound infections or in severe cases, blood infection. If a patient is prone to infection and the infection is caused by ESBLs, it can be more difficult to treat, because many of the commonly used antibiotics will not work against ESBLs.* ***Materials and Method:*** *This descriptive cross-sectional study was carried out to evaluate samples submitted for culture and sensitivity at Clinical Laboratory of North Bengal Medical College Hospital, Sirajganj the general objective identify the causative organisms and their antimicrobial resistance pattern.* ***Results:*** *Majority of subjects (37.5%) were from 46-60 years age group. ESBL resistance was significantly related with age groups and educational status. Resistance was not related with gender distribution, occupation, family size and yearly family income. Detection rate of ESBL production among Pseudomonas spp were (91.67 %), followed by Klebsiella spp. (83.33 %), Proteus spp. (69.49%), Esch. Coli (64.42 %) and others (82.35 %). Aztreonam, Ampicillin, Amoxyclave and Piperacillin were more resistant antibiotics against ESBL producing organisms.* ***Conclusion:*** *ESBL antibiotic resistance pattern should be determined in chronic Gram negative infection for effective treatment.*

Key words: *ESBL producing organism, Antibiotic resistance pattern.*

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Introduction

Extended Spectrum Beta-Lactamase (ESBL) are gram negetive bacteria that produce an enzyme; beta- lactamase that has the ability to breakdown commonly used antibiotics, such as penicillin, cephalosporins resulting ineffective treatment. Antibiotic resistance is global concern for two to three decades. It is resulted with increased length of hospital stay, a significant amount of healthcare related costs and most significantly, a high rate of morbidity and mortality. Global dose of antibiotic consumption had been estimated at more than 70 billion doses per annum. The link between inappropriate use of antimicrobials and development of antimicrobial resistance was acknowledged in different scientific studies and global proceedings. In Bangladesh, prescribers generally diagnose microbial infection on clinical judgment and select antimicrobial on empirical basis, which unfavorably affected the sensitivity pattern of microbes.[1-3] Moreover, reluctance of the lawmakers and regulators to enact law to overcome inadequacy in rules and regulation to control antimicrobial prescribing and dispensing led to worsening of the situation. Early treatment is usually based on the patient's clinical symptoms rather than diagnostic results in Bangladesh. Therefore, patient's early prognosis to final outcome might be much improved by available epidemiologic data for the most frequently isolated pathogenic organisms.[3-6] Studies have shown that, in Bangladesh, antibiotics are prescribed the most in cases of acute respiratory tract infections, acute watery diarrhea, acute trauma and gastrointestinal symptoms. The most prescribed antibiotics are Ceftriaxone 30.1%. Cefixime 18.87% and Amoxycillin 16.98%.[7] However more than 50% resistance was found against pseudomonas aeruginosa infections with commonly

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used antibiotics, including ciprofloxacin, gentamicin, ceftriaxone and cefixime. Azithromycin could not show any effectiveness in wound and urine infections, while ceftriaxone, cefixime was cent percent ineffective in tracheal infectionsthere. E. coli was observed to be resistant in 40% of cases to the commonly used antibiotics- ceftriaxone, levofloxacin, ciprofloxacin, amoxicillin, ampicillin and 95% resistant to azithromycin. The same pattern was observed in Klebsiella Pneumoniae.[8-10] Over the year, shigellosis has developed great propensity to resistance. Cholera, one of the most prevalent and threatening water borne diseases of the country, has eventually acquired resistance against tetracycline. A research by Johns Hopkins University discovered that 67% of hospitalized patients in Bangladesh received antibiotics, even though in at least 50 % cases they were not required.[11] A study suggested that Pseudomonas aeruginosa responsible for wound, urine, ear, throat and other infections were less than 50% sensitive to commonly-used antibiotics in Bangladesh, including ciprofloxacin, gentamicin, ceftriaxone, cefixime and azithromycin.[4] Azithromycin was 100% ineffective in wound and urine infections, while ceftriaxone and cefixime was 100% ineffective in tracheal infections.[5] Another study also reports that Escherichia coli was resistant in 40% of cases to commonly used antibiotics ceftriaxone, levofloxacin, ciprofloxacin, amoxicillin and ampicillin and 95% resistant to azithromycin. Klebsiella pneumoniae also showed similar patterns.[6] It was observed that 43.2% and 39.5% of isolated E. coli and K. pneumoniae respectively had ESBL phenotypes.[7]

This rate is higher than in countries of the Western Pacific Region, North America or Europe and some South American nations. Cholera germs have acquired resistance to a number of antimicrobials including tetracycline.[10] Over the year, shigellosis have shown great propensity to develop resistance to antibiotics. In 1996, reports from Matlab and Dhaka showed that more than 95% Shigella dysenteriae isolated were resistant to ampicillin, cotrimoxazole and nalidixic acid and 14%-40% were resistant to methicillin.[11] During the last 70 years, the development of effective antimicrobial drugs has reduced the incidence of life-threatening infections. However, that achievement has steadily been eroded by the emergence of antibiotic resistance especially against, ESBL producing bacteria. Antimicrobial resistance is a natural consequence of exposure to antimicrobial drugs and is not a new phenomenon, but the rate at which resistance emerges has been massively increased by the inappropriate and irrational overuse of antimicrobials in health and veterinary sectors.[3-5] Therefore, this present study was carried out to see bacteriological profile of ESBL producing organisms with antibiotic resistance pattern among submitted materials in clinical laboratory of North Bengal Medical College Hospital, Sirajganj, Bangladesh so that the study result could reflect the current ESBL resistance status, help the clinician to choose appropriate antibiotic and aware Government to make necessary policies regarding rational use of antibiotics.

Materials and Methods

This descriptive cross-sectional study was carried out to evaluate samples submitted for culture and sensitivity at Clinical Laboratory of North Bengal Medical College Hospital, Sirajganj the general objective identify the causative organisms and their antimicrobial sensitivity pattern. The objective of the study was discussed in details with the patients or their attendants before their decision to enroll themselves into the study. Demographic information was prospectively recorded and substantiated by means of inspection of medical record. Information included was the subject's age, gender, medical and clinical history, followed by conduction of the study. Samples included pus, wound swab, urine and CSF. During the eight months January 2017 to August 2017 period, a total of 304 Gram negative isolates from various clinical specimens were included in the study. At first, total 829 samples were selected including both Gram positive, negative bacteria and from 829 samples on basis of Gram stain, 304 Gram negative bacteria were isolated.

They were further characterized for their antibiogram, MIC, production of ESBLs. Urine was collected from random urine specimen, more commonly first-void morning specimen, about 1 mL by Midstream clean catch urine. Cerebrospinal Fluid was collected by lumbar Puncture. Wound swab was collected by rubbing of sterile swab over the wound and collection of swab stick within sterile test tube, proper sealing. Antimicrobial resistance was reported as the minimal inhibitory concentration (MIC), which was the lowest concentration of drug that inhibits the growth of the organism. The standard interpretation categorized each result as susceptible (S), intermediate (I), resistant (R), sensitive-dose dependent (SD), or no interpretation (NI). The procedural steps of each method were followed strictly in order to obtain reproducible results. The standardized components of were include bacterial inoculum size, incubation conditions (atmosphere, temperature, duration) and Quality control testing with reference quality control strains Routine QC testing with a range of QC strains was the backbone of the internal QC testing. All the relevant collected data were compiled on a master chart first. Then organized by using scientifically calculated and standard statistical formulas, percentage was calculated to find out the proportion of the findings. Data entry and analysis were done using SPSS for windows version 20 (IBM). Output of data and graphical representation was done using Microsoft Office chart and Microsoft-Word.

Results

Majority of subjects 114 (37.5%) were from 46-60 years age group. About 15.47% study subjects were 29 years age group. 85 (27.96%) subjects had age above 61 years (Table I).

Table I: Age distribution of study subjects (n=304)

Age groups in years	n	%
29 years and below	47	15.47
30-45	58	19.07
46- 60	114	37.50
61 years and above	85	27.96

Majority of the subjects were females 163 (53.62%). In this study (Table II) most of the subjects were illiterate 53.3%. Rests were graduates 3.3%, few crossed SSC 6.3% and HSC 5.6% levels. Among female's majority 51.6% housewives, rests of the subjects were day laborers 11.5%, farmers 13.5% and businessman 9.5%. Mean yearly family income were 10000/= (BDT). ESBL resistance was significantly related with age groups and educational status. Resistance was not related with gender distribution, occupation, family size and yearly family income (Table III).

Table II: Socio demographic characteristics of the study subjects (n=304)

Variables		Frequenc	Percentage/ Median(range)
Gender	Male	141	46.38
	Female	163	53.62
Education	Illiterate	162	53.3
	SSC	19	6.3
	HSC	17	5.6
	Graduate	10	3.3
	Others	96	31.6
Occupation	Day laborers	35	11.5
	Farmer	41	13.5
	Business man	29	9.5
	House wife	157	51.6
	Official worker	9	3.0
	Fisher man	1	.3
	Others	32	10.5
Family income (yearly)			100000.0 (1500 -350000)

Table III: Relationship between ESBL resistance pattern and socio demographic characteristics of the study subjects

Variables	ESBL resistance, p-value
Age (in years)	0.018^{S}
Gender	0.646^{NS}
Educational status	0.031^{S}
Occupation	0.498^{NS}
Family income (yearly)	0.106^{NS}
Family size	0.121^{NS}

** p value was achieved by ANOVA test between groups; S=Significant, NS=Not significant.

Table IV: Distribution of sample from various specimens (n=304)

Type of specimen	(n / %)
Urine	214 (70.39)
Wound swab	42 (13.81)
Pus	40 (13.15)
CSF	08 (02.65)
Total	304 (100)

The specimens were urine 214 (70.39%), wound swab 42 (13.81 %), pus 40 (13.15%) and CSF 08 (02.65%) (Table IV). Out of 304 Gram negative isolates in this study majority were Esch. coli 151 (49.67%), followed by Proteus spp. 59 (19.40%), *Klebsiella spp.* 48 (15.78%), Pseudomonas spp. 12 (03.94%) and others (Enterobacter spp., *Citrobacter spp.*) 34 (11.18%) (Table V).

Table V: Detection rate of different isolates in the study population.

Name of the organisms	n	%
Esch. coli	151	49.67
Proteus spp.	59	19.40
Klebsiella spp.	48	15.78
Pseudomonas spp.	12	03.94
**Others*	34	11.18
Total	304	100.0

* Others - Enterobacter spp., *Citrobacter spp*

Table VI showed prevalence of ESBL producing isolates from different clinical specimen by double disc diffusion test (DDDT). Among them Pseudomonas spp were the leading bacteria 11/12 (91.67 %), followed by Klebsiella spp. 40/48 (83.33 %), Proteus spp. 41/59 (69.49%), Esch. coli 97/151 (64.42 %) and others 28/34 (82.35 %).

Table VI: Rate of detection of ESBL production by DDDT from different organisms.

Name of organism	Total	ESBL positive (%)
Pseudomonas spp	12	11 (91.67)
Klebsiella spp	48	40 (83.33)
Proteus spp	59	41 (69.49)
E.coli	151	97 (64.42)
Others	34	28 (82.35)
Total	304	217 (71.38)

Note: Figures in parentheses represent percentage; *Others - Enterobacter spp., Citrobacter spp

Table VII showed detection rate of ESBL production among E.coli and Pseudomonas spp. were highest in urine sample were 82.47%, 74.74% respectively. But in case of Proteus spp. ESBL production was higher in pus 21.45%.

Table VII: Detection rate of isolation of ESBL positive Esch.coli, Klebsiella spp. Proteus spp. Pseudomonas spp. from different clinical specimen

Type of specimen	Rate of ESBL positive *Esch. coli*(n=97)	Rate of ESBL positive *Proteus spp* (n=41)	Rate of ESBL positive *Klebsiella spp* (n=40)	Rate of ESBL positive *Pseudomonas spp.*(n=11)
Urine, n=174	80 (82.47)	24 (58.53)	25 (62.50)	08 (74.74)
Pus, n=33	14 (14.43)	09 (21.45)	08 (20.22)	02 (18.19)
Wound swab, n=18	03 (03.90)	08 (19.51)	06 (15.00)	01 (09.90)

Note: Figures in parentheses represent percentage; Others-Enterobacter spp., Citrobacter spp.

Antimicrobial resistance pattern in ESBL positive strain by disc diffusion method revealed that Aztreonam, Ampicillin, Amoxyclave and Piperacillin were more resistant. Among them in Klebsiella spp. was more resistant than Esch. coli. The entire isolated organisms in this study were 100% sensitive to Imepenem and 98% sensitive to Nitrofurantoin (Table VIII).

Table VIII: Antimicrobial resistant pattern in ESBL positive strain by disc diffusion method

Antimicrobials	ESBL positive strain			
	Esch. coli	*Proteus spp.*	*Klebsiella spp*	*Pseudomonas spp.*
Aztreonam	67 (100)	89 (100)	17 (100)	28 (100)
Piperacillin	62 (92.5)	85 (95.5)	15 (88.3)	27 (96.4)
Amoxiclave	63 (94.0)	83 (93.2)	16 (94.1)	26 (92.8)
Ampicillin	64 (95.5)	80 (89.8)	16 (94.1)	25 (89.2)
Ceftriaxone	57 (85.0)	70 (78.6)	15 (88.2)	23 (82.1)
Ciprofloxacin	56 (83.5)	64 (71.9)	15 (88.2)	22 (78.5)
Cefotaxime	53 (79.1)	63 (70.7)	14 (82.3)	22 (78.5)
Ceftazidime	41 (61.1)	63 (70.7)	14 (82.3)	22 (78.5)
Azethromicine	54 (80.5)	52 (58.4)	14 (82.3)	16 (57.1)
Gentamicin	39 (58.2)	42 (47.1)	11 (64.7)	17 (60.7)
Amikacin	12 (17.9)	11 (16.4)	3 (17.6)	6 (21.4)
Nitrofurantoin	2 (2.9)	3 (3.3)	1 (5.8)	1 (3.6)
Imipenem	0 (0)	0 (0)	0 (0)	0 (0)

Discussion

Extended spectrum beta-lactamases (ESBL) are a type of enzyme by some bacteria. ESBL enzymes cause some antibiotics not to work for treating bacterial infections. Common antibiotics, such as cephalosporin and penicillin, are often used to treat bacterial infections. With ESBL infections, these antibiotics can become useless. Bacteria use ESBLs to become resistant to antibiotics. The most common types of bacteria that produce ESBLs include Escherichia coli, Klebsiella.[12-14] E. coli and Klebsiella infections can usually be treated with normal antibiotics like penicillin and cephalosporin. But when these bacteria produce ESBLs, they can cause infections that can no longer be treated by these antibiotics. So this present study was conducted to identify ESBL producing bacteria and to see antibiotic resistance pattern among submitted materials in Microbiology laboratory of North Bengal Medical College Hospital. The findings of this study are discussed according to objectives of present study based on related previous study. Majority of subjects [114 (37.5%)] were from 46-60 years age group in current study. About 15.47% study subjects were 29 years age group. 85 (27.96%) subjects had age above 61 years. Majority of the subjects were females [163 (53.62%)]. In this study (Table VII) most of the subjects were illiterate 53.3%. Rests were graduates 3.3%, few crossed SSC 6.3% and HSC 5.6% levels. Among female's majority 51.6% housewives, rests of the subjects were day laborers 11.5%, farmers 13.5% and businessman 9.5%. Mean yearly family income were 10000/= (BDT). ESBL resistance was significantly related with age groups and educational status. Resistance was not related with gender distribution, occupation, family size and yearly family income. Previous studies revealed that ESBLs were most commonly isolated from female patients 64.3% suffering from urinary tract infections 41.5%, as compared to male patients 35.7% in which the organisms were most commonly isolated from pus samples 54.2 %. ESBLs-producing Enteric Gram-Negative rods were most frequent at later part of life where they were most common 27.9% at 61-70 years, followed by 41-50 years of age group 20.0%. Another peak 13.3% was also seen at younger age group 11-20 years. The least prevalence 5.5% was seen in two age groups (0-10 and 31-40 yrs). In case of female patients, ESBLs-producing bacteria were most frequently 29.2% isolated from middle age group 41-50 years followed by later age groups 51-60 and 61-70 years, 15.1% and 25.5%.

The specimens were urine 214 (70.39%), wound swab 42 (13.81 %), pus 40 (13.15%) and CSF 08 (02.65%). From this study finding, it was revealed that Gram negative organisms caused mostly UTI, wound infection and meningitis. Previous studies[5,8,10,12] revealed the same finding that, major causes of UTI, RTI, wound infection, nosocomial infection were Gram negative bacteria. Malignant necrotizing otitis due to Pseudomonas aeruginosa had been encountered with increasing frequency as the number of older diabetic patients has increased. Nosocomial sinusitis and bacteremia due to Escherichia coli, Klebsiella pneumoniae, Enterobacter species, or P. aeruginosa developed in hospitalized patients. Bacteremia due to E. coli, K. pneumoniae, or P. aeruginosa often followed instrumentation of the urinary, respiratory, or gastrointestinal tracts in the hospitalized patient. Infections of skin structure, particularly decubitus ulcers in debilitated, bedridden patients, were due to a mixed gram-negative and anaerobic flora; frequently, P. aeruginosa and Enterobacteriaceae resistant to many older agents were the major pathogens. Similarly, osteomyelitis in patients who have undergone previous surgical procedures is caused by various multiply resistant Enterobacteriaceae and P. aeruginosa.[13] In current study it was seen that, out of 304 Gram negative isolates in this study majority were Esch. coli 151 (49.67%), followed by Proteus spp. 59 (19.40%), Klebsiella spp. 48 (15.78%), Pseudomona

s spp. 12 (03.94%) and others (Enterobacter spp., Citrobacter spp.) 34 (11.18%). Double disc diffusion test (DDDT) showed that Pseudomonas spp were the leading bacteria 11/12 (91.67 %), followed by Klebsiella spp. 40/48 (83.33 %), Proteus spp. 41/59 (69.49%), Esch. coli 97/151 (64.42 %) and others 28/34 (82.35 %) and detection rate of ESBL production among E.coli and Pseudomonas spp. were highest in urine sample were 82.47%, 74.74% respectively. But in case of Proteus spp. ESBL production was higher in pus 21.45%. In a study conducted by Yesmin[15] revealed that, collected specimens were urine 216 (72%), wound swab, 45 (15 %) pus 39 (13%). Out of 300 Gram negative isolates in this study majority were Escherichia coli 52% followed by Proteus species 18.3%, Klebsiella species 15%, Pseudomonas species 3% and others 11.7%. This study finding was similar to our current study. Another study[14] revealed that among wound swabs most common ESBL producing bacteria were Esch. coli 61.5%, Proteus species 78.3% and Klebsiella species 88.9%.

Antimicrobial resistance pattern in ESBL positive strain was done by disc diffusion method. Aztreonam, Ampicillin, Amoxyclave and Piperacillin were more resistant. Among them in Klebsiella spp. was more resistant than Esch. coli. The entire isolated organisms in this study were 100% sensitive to Imepenem and 98% sensitive to Nitrofurantoin. These findings were similar to previous studies[14,15] where it was seen that all the isolates were sensitive to imipenem and nitrofurantoin followed by amikacin 92.9%. Jose et al.[16] in a prospective study which was conducted in Department of Microbiology, DM WIMS, Wayanad obtained from 160 isolates from various exudates. The samples were processed based on standard laboratory techniques. Antibiotic susceptibility of the isolates was determined against various antibacterial agents by Kirby Bauer Disk Diffusion method. Among the 160 isolates, 68 (42.5%) were E.coli, and 35 (21.8%) were K. pneumoniae, 28 (17.5%) were MRSA, 14 (8.7%) were Pseudomonas, 8 (5%) were Proteus spp., 4 (2.5%) were Acinetobacter spp. and 3 (1.8%) were Citrobacter spp. Of these 160 strains tested, 45 (28%) were found to be ESBL producers, of which 22 (48.8%) were E.coli, 18 (40%) were K. pneumoniae, 3 (6.6%) were Acinetobacter spp. and 2 (4.4%) were Proteus spp. Saha et al.[17] isolated 186 Gram-negative organisms from various samples. Among the 186 Gram negative bacteria, 120 (64.5%) were Esch. coli while 33 (17.7%), 20 (10.8%) and 11 (5.9%) were Pseudomonas sp, Klebsiella sp and Proteus sp respectively. Out of total 186 isolates, 77 (41.4%) and 73 (39.2%) isolates were found ESBL producers by DDST and E-test method (p=0.674) respectively. Compared to Escherichia coli, Pseudomonas and Proteus, significantly high ($p<0.01$) proportion of Klebsiella were ESBL positive by both DDST and E-test methods. The detection rate of ESBL producing organisms was not significantly different by DDST and E-test 41.4% vs 39.2%. Non-determinable result was obtained for 4 (2.2%) isolates by E-test method. In another study[18] out of 232 E. coli isolates, 70 (30.2%) were found to be positive for ESBL by the applied phenotypic methods. ESBL-producing isolates yielded high resistance rates for trimethoprim-sulfamethoxazole 98.6%, tetracycline 88.6%, nalidixic acid 81.4% and ciprofloxacin 81.4%. The highest antimicrobial activities of ESBL-producing isolates were observed for amikacin 95.7%, followed by tobramicin 74.3% and nitrofurantoin 68.6%. Resistance to quinolones, aminoglycosides, trimethoprim-sulfamethoxazole,tetracycline, nitrofurantoin and chloramphenicol was higher in ESBL than non-ESBL isolates ($p<0.05$). The frequency of ESBL-producing isolates varied among hospitals 18.2% to 45.1%, although a high prevalence was recorded as 45.1% at Khartoum Teaching Hospital. Wound specimens were the most common source of ESBL-producing isolates. The proportion of ESBL-producing E. coli did not differ significantly between adults and children 31% vs. 27%.

Conclusion

From the present study it would be concluded that in chronic gram negative infection ESBL resistance pattern should be treatment for effusive.

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