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Research Article

Frequency and Susceptibility Pattern of Extended Spectrum Beta Lactamase Producing Aerobic Gram Negative Bacteria in Post-Operative Infections

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Abstract

Extended spectrum β-lactamases occur commonly in the aerobic Gram negative bacteria (AGNB) such as *E. coli*, *Klebsiella* spp., *Proteus* spp., *Pseudomonas aeruginosa*, *Acinetobacter* spp., etc. and have the ability to make these organisms resistant to cephalosporins (e.g. ceftazidime, ceftriaxone, cefotaxime etc.), penicillins, and monobactams, i.e. aztreonam. However theses antibiotics become sensitive in the presence of clavulanic acid, an extended spectrum beta-lactamase (ESBL) inhibitor. ESBL enzymes do not influence cephamycins or carbapenems, i.e. meropenem, imipenem, etc. Major problems in surgery is wound infections after operations. High risks of wound infections are due to being immune-comprised on antibiotics, prolonged hospitalization and other factors in many cases. The current research determined various aerobic gram negative bacteria in post-operative wound infections at the College of Medical Laboratory Technology, the National Institute of Health, Islamabad, Pakistan. It also determined the frequency of ESBL in the organisms and established their susceptibility profile during the time period of the research. Infections caused by ESBL producers are a major problem in our post-operative patients. The commonest isolate was *E. coli* and the commonest ESBL producer was *Klebsiella* spp. Double disk synergy test is an effective method for screening of such isolates, and the practice of incorporating this test along with the routine sensitivity is recommended.

Keywords: Extended spectrum beta lactamase (ESBL); Antimicrobial sensitivity pattern (ASP); Double disk synergy test (DDST)

Introduction

Extended-spectrum β-lactamase (ESBL) produced by gram negative bacteria *Escherichia coli* (*E. coli*) and *Klebsiella* species (*Klebsiella* spp.) [1]. The incidence of extended spectrum beta lactamase-*Escherichia coli* (ESBL-EC) infection has increased in community hospitals throughout the Southeastern United States [2]. ESBLs are mostly plasmid-mediated beta lactamases (β-lactamases) that efficiently hydrolyze oxyimino-cephalosporins and monobactams, yet are inhibited by β-lactamase inhibitors [6]. The introduction of third generation of cephalosporin's in 1980's was a breakthrough in the fight against β-lactamases producing bacterial resistance towards antibiotics. The β-lactamase enzymes produced by

the organisms act by hydrolyzing the beta lactam ring of b-lactam antimicrobials [8]. Theses enzymes are first discovered in Germany in 1983 from *Klebsiellae pneumonia* and they are group of enzymes that can even hydrolyze the third generation oxyimino-cephalosporins such as (cefotaxime, ceftazidime, ceftriaxone), the monobactams (aztreonam) but not the cephamycins (cefoxitin, cefotetan) or carbapenems (imipenem, meropenem) etc. [8, 9, 10, 22]. Antibiotic resistance among bacteria is a most prevalent issue worldwide both in hospital settings and in the community. β-lactamases production by various gram negative isolates is perhaps resistance mechanism towards b-lactam agents. Major risk factors are prolong exposure to antibiotics, long-term hospital stay, severe illness, resistance with third generation of cephalosporins, intubation and catheterization [10]. There are many risks for wound infections. Most hospital acquired infections (HAIs) arise from surgical wounds, improper hand washing, incomplete sterilization and air vents. In the field of surgery, post-operative wound infections is a major problem. Small plasmids are present in some resistant strains of *E. coli* that are responsible for the production of enzymes such as TEM-1 which acts as beta lactamases, that make it resistant to amoxicillin/clavulanic acid. Past studies shows that resistance against organisms in post operative wound infections are increasing [15]. ESBLs producing organisms are clinically relevant and are resistant with cephalosporins [22]. According to Centers for Disease Control and Prevention (CDC), by careful techniques 33% of wound infections can be prevented [26]. The main reason of infections in post-operative infections is bad healthcare practices in hospitals, no proper care of wounds, unsterilized instruments are used in surgical procedures and sometimes extensive use of antibiotics in treatment that predispose in resistance of microbes. Members of Enterobacteriaceae produce ESBLs, and are responsible for nosocomial infections [29]. ESBLs producing organisms are *Klebsiella pneumoniae* and *E. coli.* Less common members of Enterobacteriaceae and *Pseudomonas* spp. are also well known for ESBL production. ESBLs Gram negative bacteria have become a challenge in hospitals as well as community acquired infections caused by these organisms [36]. In the USA, 14 to 16% infections are hospital acquired in post operative wound infections, and 77% deaths occur due to surgical wound infection. Production of β-lactamase, can be detected by various molecular testing methods are gold standard but technically difficult to handle and lack facilities. For confirmation of ESBLs, CLSI recommends DDST using disk containing third generation cephalosprins with and without clavulanic acid [41].

In this study we determined various aerobic Gram-negative bacteria in post-operative wound infections at Islamabad Pakistan. We also determine the frequency of ESBL producing aerobic gram-negative bacteria isolated during this time and establish the susceptibility profile of these isolates.

Experimental

This study was descriptive and undertaken from January 2017 to February 2018. The research study was carried out in the Microbiology Department of College of Medical Laboratory Technology, National Institute of Health Islamabad. A total of 200 samples were processed. Non-probability random sampling technique was followed. All samples from post-operative patients were included irrespective of age and sex. All samples other than post-operative patients were excluded. All Gram positive isolates after identification were excluded. In all procedures, surgical masks, surgical gloves, and lab coat were used to prevent any sort of serious infections.

Sample processing

Samples were collected from patients and cultured on blood agar and MacConkey's agar plates and then incubated at 37 °C for 24 h. Then, Gram staining and biochemical tests were done for identification. Antimicrobial sensitivity test was carried out by Kirby-Bauer disc diffusion method.

Identification of isolates

Identification of aerobic Gram negative bacteria *Acinetobacter* spp., *E. coli*, *Klebsiella* spp., *Pseudomonas aeruginosa*, and *Proteus* spp. was performed by using different tests like Gram staining and biochemical tests. *E. coli* was considered as pathogenic micro-organism, and bio-safety level 2 was used during handling the samples, in which safety cabinet was used.

Preservation of isolates

Isolates were preserved on the nutrient agar slants. Pure growth of pathogen was inoculated on blood agar. After overnight incubation, well isolated colonies were taken and were inoculated on the slants. These slants

were incubated overnight at 36 ± 1 °C. Then these slants were placed at 4 °C.

Gram staining

Clean grease free slides were purchased from market and slides susceptible as greasy were cleaned by washing with hot water and soap, after which the slides were rinsed with distilled water and the excess water was blotted out with blotting paper or with lint-free cloth. With the help of an inoculating needle, a bacterial colony was placed on the drop of distilled water that was placed on the slide, making a smear and fixing it on Bunsen burner flame. Crystal violet was applied for 1 min and was washed with distilled water. Lugol's iodine solution (mordant) was applied for about 1 min, and then slides were washed with distilled water. Decolorizer was applied for 30 sec and then washed off the slide. Finally, safranin solution (counter stain) was applied on the slide and left for about 1 min, washed off with distilled water, and the smear was dried in air and examined under light microscope [17].

Quality control

On the same slide, known Gram positive and Gram negative smears were also stained with test organisms.

Biochemical tests

Following biochemical test, isolation and confirmation of aerobic gram negative bacterial colonies were performed.

Indole production test

The indole test was performed by growing the isolates in 10 mL sterile Tryptone water for 24 h at 37 °C; then, Kovacs' reagent (0.5 mL) was added to the culture. After 1 minute, the test tube was examine, and appearance of a red layer in the medium indicated positive results [17].

The methyl red-Voges Proskauer (MR-VP) test

The methyl red (MR) test was done by inoculating the isolate into a labeled methyl red-Voges Proskauer (MR-VP) broth by means of a sterile loop. The test tubes were then incubated at 37 °C for 72 h. After incubation, the content of each tube was divided into two equal portions; one of which was used for the methyl red (MR) test and the other for the Voges-Proskauer (VP) test. Two drops of methyl red indicator were added to the portion meant for MR test. Appearance of red color in the medium was recorded as a positive reaction. Barrett's method was employed in the VP test. 0.6 mL of α-naphthol and 0.2 mL of 40% potassium hydroxide solutions were added to the second portion designated for VP test. The appearance of red color denoted a positive test [17].

Citrate utilization test

Using a straight platinum wire, the isolate was inoculated into Koser's citrate medium and incubated at 37 °C for 48 h. Citrate utilization was denoted by turbidity and color change in the medium from light green to blue. Citrate negative cultures showed neither growth nor color change in the medium [7]. Positive control was *Klebsiellae pneumoniae* and negative control was *E. coli* ATCC 25922 [12].

Oxidase test

The test organism was taken from the nutrient agar plate with sterile glass rod and smeared across the surface of filter paper on reagent, Appearance of dark purple color within 10 sec was taken as positive test [12].

Biochemical identification Gram negative rods using API 20E (Biomeriuex, France)

API 20E is a standardized identification system for *Enterobacteriaceae* and other Gram negative rods. It has 20 miniaturized biochemical tests. A strip contains 20 micro tubes containing dehydrated substrates (Fig. 1). These tests were inoculated with bacterial suspension. After incubation for18 to 24 h at 35 ± 2 °C, reagents were added in respective wells, positive results were noted, and seven-digit numerical profile was determined which was looked up in Analytical Profile Index for a code equivalent to organism identification.

Other tests include b-galactosidase test; arginine dihydrolase (ADH) test; lysine decarboxylase (LDC) test; ornithine decarboxylase (ODC) test; citrate utilization test; H_2S production test; urea test; indole production test; voges Proskauer (VP) test; and gelatin hydrolysis test.

Antibiotic sensitivity test

Kirby-Bauer disc diffusion method was used according to CLSI 2011 guidelines, for identification of isolates for amikacin (AK), ciprofloxacin (CIP), cefepime (FEP), amoxicillin-clavulanic acid (AMC), imipenem (IPM), levofloxacin (LEV), piperacillin-tazobactam (TZP) and ceftazidime (CAZ), ceftriaxone (CRO).

Tops of 2 to 3 similar colonies were touched and emulsified in 4 mL of sterile peptone water to achieve a 0.5 Mac F standard. Using sterile swab, suspension

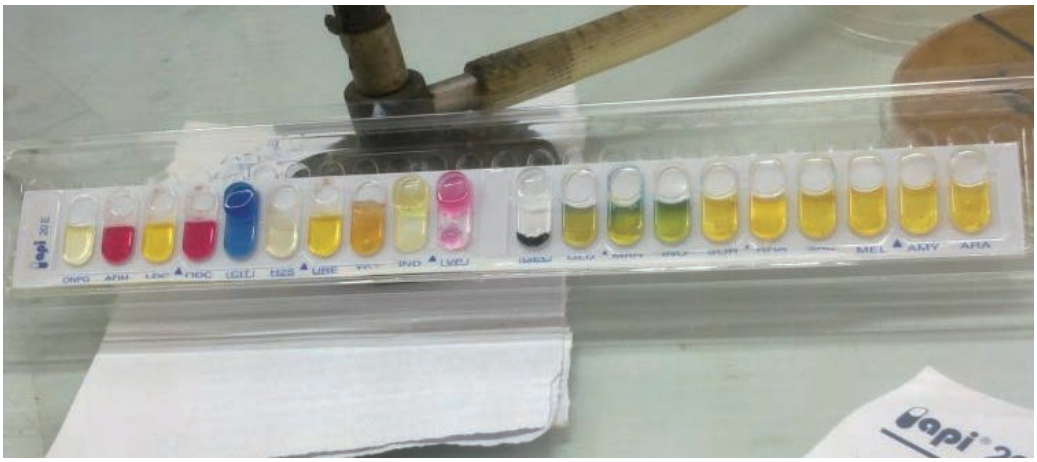

Fig. 1 API 20E strip used for identification of different micro-organisms belonging to the *Enterobacteriaceae* group. It was based on biochemical tests that shows changes in color on fermentation of different sugars and enzymes which indicate production of urease, indole, citrate, maltose, lactose, and fructose produced by different micro-organisms.

Table 1 Interpretation of biochemical tests on API 20E strip

Test	Active ingredients	Reactions / Enzymes	Result	
			Negative	Positive
ONPG	2-nitrophenyl-β D-galactopyranoside	beta-galactosidase	Colorless	Yellow
ADH	L-arginine	Arginine dihydrolase	Yellow	Red / Orange
LDC	L-lysine	Lysine decarboxylase	Yellow	Red /Orange
ODC	L-ornithine	Ornithine decarboxylase	Yellow	Red / Orange
CIT	Trisodium citrate	Citrate utilization	Pale green/yellow	Blue-green / Blue
H2S	Sodium thiosulfate	H_2S production	Colorless	Black deposit / Thin line
URE	Urea	Urease	Yellow	Red / Orange
TDA	L-tryptophane	Tryptophane deaminase	Yellow	TDA / Immediate (1) Reddish brown
IND	L-tryptophane	Indole production	Colorless	Jammes / Immediate (2) Pink
VP	Sodium pyruvate	Acetoin production	Colorless	Vp1+Vp / 10 min (3) Pink / Red
GEL	Gelatin	Gelatinase	No diffusion	Diffusion of black pigment
GLU	D-glucose	F/O (4), glucose	Blue-green/blue	Yellow
MAN	D-mannitol	F/O, mannitol	Blue-green/blue	Yellow
INO	Inositol	F/O, inositol	Blue-green/blue	Yellow
SOR	D-sorbitol	F/O, sorbitol	Blue-green/blue	Yellow
RHA	L-rhamnose	F/O, rhamnose	Blue-green/blue	Yellow
SAC	D-sucrose	F/O, saccharose	Blue-green/blue	Yellow
MEL	D-melibiose	F/O, melibiose	Blue-green/blue	Yellow
AMY	Amygdalin	F/O, amygdalin	Blue-green/blue	Yellow
ARA	L-arabinose	F/O, arabinose	Blue-green/blue	Yellow

Note: (1), (2), and (3) refer to reagents added after incubation and time to note the results. (4) refers to fermentation / oxidation reaction.

was inoculated on the surface of Mueller Hinton agar (Oxoid, Basingstoke, UK) with the petri dish lid in place; about 10 minutes were allowed for the surface of the agar to dry. Antibiotic discs were placed on the surface of agar medium. After incubation at 37 °C for 18 h, the diameter of the zones of inhibition were

measured in millimeter.

Extended spectrum β-lactamase (ESBLs) production

Screening of ESBLs was done by using disks of amoxicillin-clavulanic acid. Ceftazidime 30 µg, ceftriaxone and discs were placed around a disc containing clavulanic acid (in this case amoxicillin/clavulanic acid disk) with a proximity of 25 mm center to center, when the inhibition zone around any of the applied disks was enhanced towards amoxicillin/clavulanic acid disk, forming a characteristically shaped zone referred as a "keyhole," or "belling", and it was the indication of ESBLs.

Quality control organism

The following organisms were used as control strains in the study.

Escherichia coli ATCC 25922; and *Pseudomonas aeruginosa* ATCC 27853

Data analysis

Microsoft Excel & MS-Word were used for the graphical representation of data.

Results and Discussion

The current study was carried out at the Department of Microbiology, College of Medical Laboratory Technology, National Institute of Health Islamabad from January 2017 to February 2018 to look for the presence of ESBL producing aerobic Gram negative bacteria (AGNBs) isolated from post-operative infections in surgical wounds.

200 pus samples were collected from post-operative infections like appendectomy, cholecystectomy, laparotomy, trauma, mastectomy, thyroidectomy, etc. (Fig. 2). All surgical areas of hospital were included, i.e. surgical wards, outpatient departments (OPDs) and intensive care units (ICUs). Out of the 200 samples, 116 samples yielded AGNB. Thus, 150 organisms were isolated from 116 patients (Fig. 3, Table 2). Out of these 150 organisms, 101 were derived from males and 49 were from females (Fig. 4, Table 3). Out of the 150 organisms, 94 were from inpatients, 49 from outpatients and 7 organisms were isolated from the surgical intensive care unit (SICU) (Table 4).

Fig. 6 and Table 5 show the percentages of organisms. *E. coli* was the commonest organism, i.e. 43/150, followed by *Pseudomonas aeruginosa* 40/150, *Klebsiella* spp. 36/150, *Acinetobacter* spp. 17/150, and

Table 2 Sample distribution

Total samples	200
No growths	84
Growth positive patients	116
Total no of isolates	150

Table 3 Distribution of isolates according to gender

Gender	Total number of isolates
Male	101 (67%)
Female	49 (33%)
Total	150 (100%)

Table 4 Distribution of isolates according to patient location

Wards	Number of isolates (n = 150)
IPD	94 (62%)
OPD	49 (33%)
SICU	7 (5%)
Total	150 (100%)

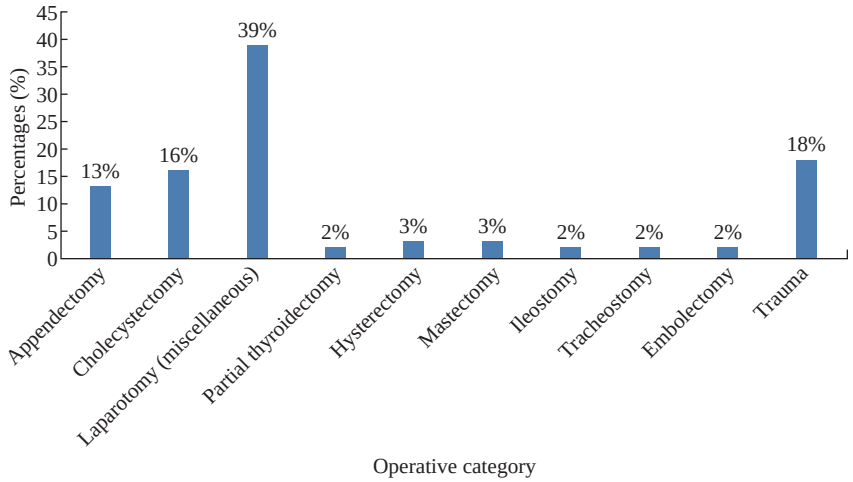

Fig. 2 Overall distribution of surgical procedures in patients from whom samples were collected (n = 116).

Table 5 Organism identified overall

Organisms	Number of isolates (n = 150)
Acinetobacter spp.	17 (11%)
E. coli	43 (29%)
Klebsiella pneumoniae	36 (24%)
Pseudomonas aeruginosa	40 (27%)
Proteus spp.	14 (09%)
Total	150 (100%)

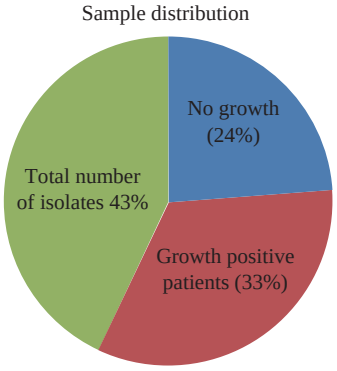

Fig. 3 Sample distribution. Total number of isolates: 43%; no growth: 24%; patients with positive results: 33%.

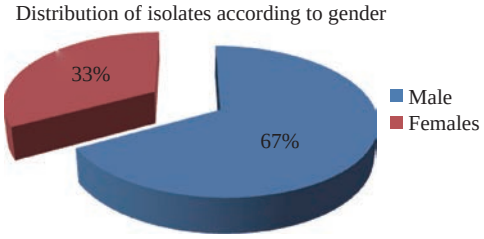

Fig. 4 Distribution of isolates according to gender. 67% patients were male and 33% were female from whom samples were collected.

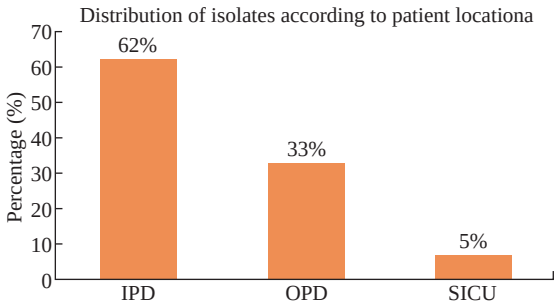

Fig. 5 Distribution of patients according to their location in different wards, including inpatient department (IPD), outpatient department (OPD), and surgical intensive care unit (SICU) department.

Proteus spp. 14/150. As already indicated, 84 samples did not yield any growth.

Fig. 7 and Table 6 show that out of the 150 organisms, the best sensitivity result of 81% was shown by imipenem, 78% sensitivity by polymyxin B, 74% by amikacin, and 46% by cefoperazone-

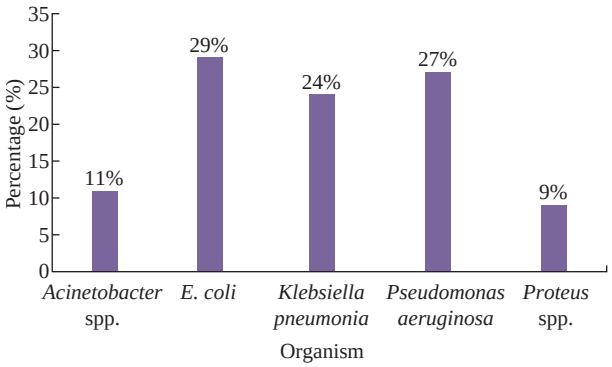

Fig. 6 Prevalence of different micro-organisms in isolates. *Acinetobacter* spp.: 11%, *E. coli*: 29%, *Pseudomonas aeruginosa*: 27%, *Klebsiella pneumonia*: 24%, and *Proteus* spp.: 9%.

sulbactam. All other isolates showed sensitivity of less than 36%. Only 7% sensitivity was shown by AMC. Individual details of the susceptibility pattern are also presented.

Table 7 shows that a large number of the 150 isolates were part of a polymicrobial growth. 87 samples yielded single growth, while 63 yielded polymicrobial growth. Among the polymicrobial growths, 25 samples showed 2 organisms, 3 samples showed 3 organisms, while 1 sample yielded four organisms.

Fig. 8 shows that out of the 29 samples yielding polymicrobial growths, 16 samples with double growth were isolated from inpatients, 11 from outpatients, and only 2 samples were from SICU. Overall location wise distribution of polymicrobial growths is simplified in Table 8.

Table 9 shows the complete data regarding polymicrobial growths, i.e. organisms isolated and ESBL production, according to patient location as given in Table 8. From this table, it could be seen that the commonest combination of organisms was *Pseudomonas aeruginosa* with *Klebsiella pneumoniae* 7 (24%); and *Klebsiella pneumoniae* with *E. coli* 7 (24%). Details of polymicrobial growth and ESBL production in polymicrobial growth are also presented.

The double disk synergy test (DDST) identified ESBL production in 49% of the total 150 isolates. Of these 74 ESBL producers, 64% were derived from male patients and 36% from female patients (Fig. 9, Table 10).

Fig. 10 and Table 11 show the distribution among the AGNBs isolated. 74 organisms (49%) were ESBL producers. The organisms included *Acinetobacter* spp., *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas*

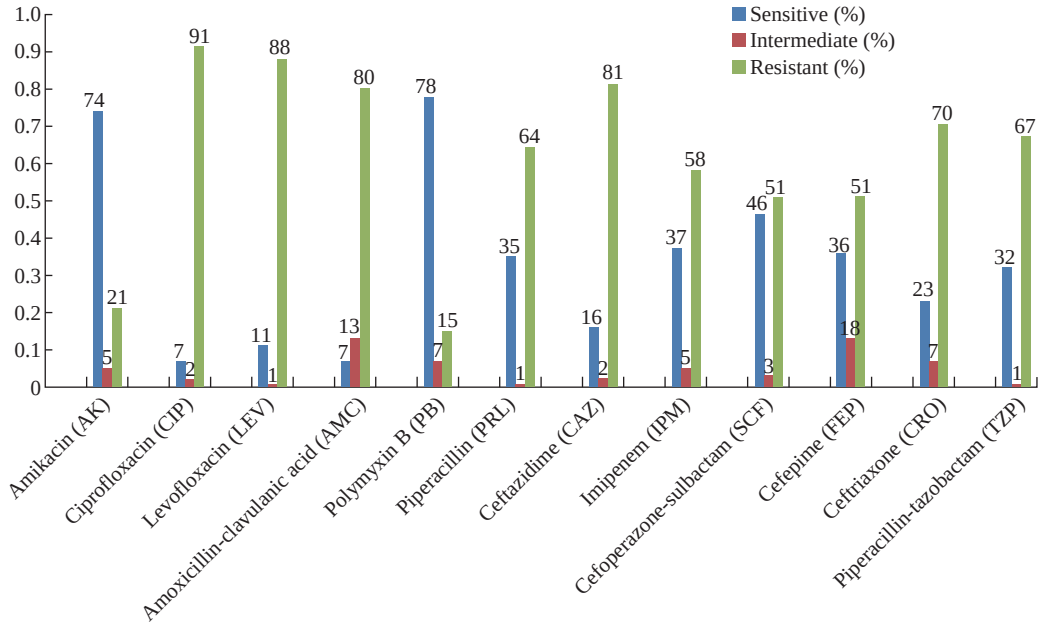

Fig. 7 Overall sensitivity pattern of different micro-organisms from different antimicrobial drugs. Ciprofloxacin was a highly resistant drug, and polymyxine B was a highly sensitive drug as compared to others.

Table 6 Overall sensitivity pattern

Antibiotics	Sensitive	Intermediate	Resistant
Amikacin (AK) (147)	(109) 74%	(7) 5%	(31) 21%
Amoxicillin-clavulanic acid (AMC) (114)	(8) 7%	(15) 13%	(91) 80%
Cefoperazone-sulbactam (SCF) (140)	(64) 46 %	(5) 3%	(71) 51%
Cefepime(FEP) (72)	(26) 36%	(9) 13%	(37) 51%
Ceftazidime (CAZ) (150)	(24) 16%	(4) 2%	(122) 81%
Ceftriaxone (CRO) (149)	(34) 23%	(10) 7%	(105) 70%
Ciprofloxacin (CIP) (83)	(6) 7%	(2) 2%	(75) 90%
Imipenem (IPM) (150)	(121) 81%	(6) 4%	(23) 15%
Levofloxacin (LEV) (131)	(15) 11%	(1) 1%	(115) 88%
Piperacillin (PRL) (91)	(32) 35%	(1) 1%	(58) 64%
Piperacillin-tazobactam (TZP) (115)	(37) 32%	(1) 1%	(77) 67%
Polymyxin B (PB) (88)	(69) 78 %	(6) 7%	(13) 15%

Table 7 Single versus polymicrobial growths

Organism isolated from 116 positive samples	150	
Single growths	87	
Polymicrobial growths	Number 29	Isolates 63
(i) Double growths	25	50
(ii) Triple growth	3	9
(iii) Four organisms	1	4

Table 8 Polymicrobial (double) growths according to location (n = 29)

Location	DG = IS	TG = IS	FO = IS
IPD	14 = 28	2 = 6	1 = 4
OPD	9 = 18	1 = 3	NIL
SICU	2 = 4	NIL	NIL
Total	25 = 50	03 = 09	1 = 4

Note: DG = double growth; IS = isolated; TG = triple growth; FO = four organisms; IPD = impatient department; OPD = outpatient department; SCIU = surgical intensive care unit.

aeruginosa, and *Proteous* spp. The highest ESBL production was shown by *Klebsiella pneumoniae* as of 34 (46%), and by *E. coli* as of 31(42%), respectively, followed by *Proteus* spp. (8%), *Acinetobacter* spp. (3%) and *Pseudomonas aeruginosa* (1%).

Table 12 shows that the maximum rate of ESBL positivity was seen in inpatients (57%) followed by the surgical OPD (39%). Only 4% ESBL production was seen in the SICU.

The most effective antimicrobial in ESBL producers

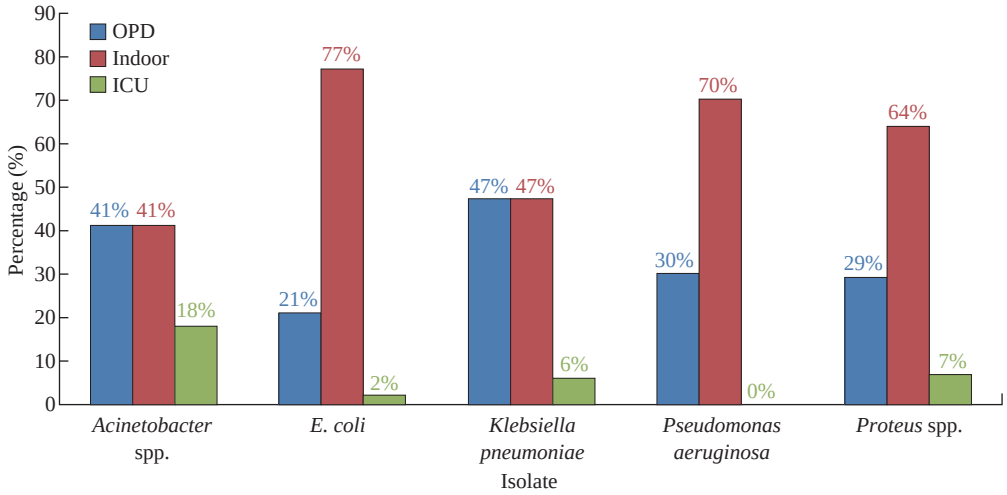

Fig. 8 Isolates distribution according to wards. *E. coli* showed the highest prevalence in inpatients and lower in intensive care unit (ICU), followed by *Pseudomonas* showing 70% prevalence in inpatients and not present in ICU patients, and *Proteus* showing 64% prevalence in inpatients and 7% in ICU patients.

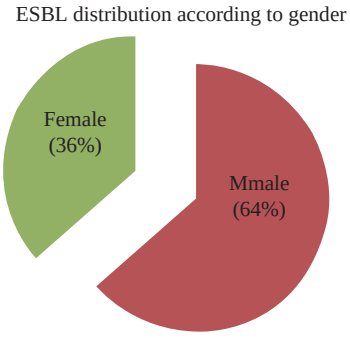

Fig. 9 Distribution of ESBL organisms in male and female. 64% male patients and 36% female patients were positive for ESBL.

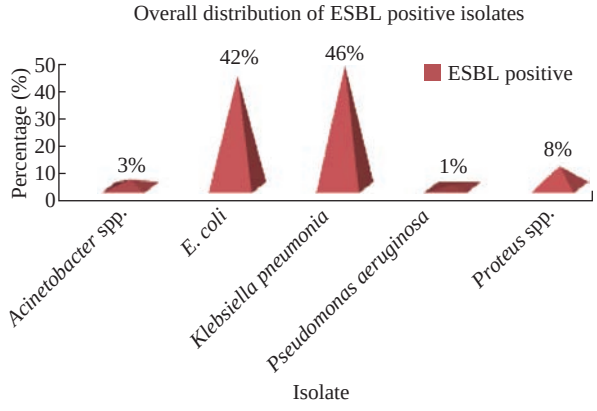

Fig. 10 Distribution of different micro-organisms that were ESBL producers. *Klebsiella*: 46%, *E. coli*: 42%, and *Proteus*: 8%.

was imipenem (82%) followed by amikacin (55%) and polymyxin B (50%), respectively. Piperacillin-tazobactam showed 47% of sensitivity, while cefoperazone-sulbactam showed 46%. Cefepime was least sensitive at 3% (Fig. 12, Table 13).

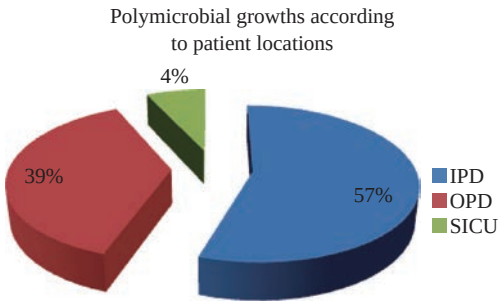

Fig. 11 Polymicrobial growth in different clinical isolates according to patients' locations in inpatient department, outdpatient department (OPD) and surgical intensive care unit (SICU).

Table 14 shows the complete susceptibility pattern of ESBL producers. In the present study, all 74 ESBL producers showed resistance to amoxicillin-clavulanic acid, ceftazidime and ceftriaxone. They showed variable resistance to ciprofloxacin, levofloxacin and amikacin.

The emergence of antibiotic resistance bacteria is threatening the effectiveness of many antimicrobial agents, which has increased in the hospitalized patients and in turn caused great increase in the economic burden. The current study was conducted in hospital situated in Islamabad, It is a 2000-bed tertiary care institute located in Islamabad. There are 6 surgical units taking care of 240 inpatients, and thus there is a large turnover rate. Post-operative wound infections are a common problem here as in any other large surgical departments. In this study, we found different types of AGNBs involved in post-operative surgical infections and their susceptibility patterns, and also determined

Table 9 Details of polymicrobial growths according to patient location and ESBL production

Sr. no.	Isolate no.	Isolate	ESBL production	location
1	3	PA + KP	-+	IPD
2	4	*E. coli* + Pr spp.	+--	IPD
3	5	Pr spp. + PA	+-	IPD
4	6	PA + KP + *E. coli*	-+++	IPD
5	8	PA+ KP	-++	IPD
6	12	*E. coli* + PA	+-	IPD
7	16	KP + Pr spp.	++	OPD
8	22	*E. coli* + PA	+-	IPD
9	23	Ab spp. + *E.coli*	--	IPD
10	28	*E. coli* + PA	--	IPD
11	32	PA + *E. coli* *E.coli*	-++	IPD
12	38	*E. coli* + KP	-+	OPD
13	40	Ab spp. + PA	--	OPD
14	45	*E. coli* + KP	-+	OPD
15	55	*E. coli* + Ab spp.	+-	IPD
16	61	KP + *E. coli*	++	OPD
17	62	*E. coli* + KP + PA	-+-	IPD
18	69	*E. coli* + PA	+-	IPD
19	80	KP + Ab spp.	+-	IPD
20	84	KP + Pr spp.	++	IPD
21	96	KP + Pr spp. + Ab spp. + PA	+++-	OPD
22	97	PA + Ab spp. + KP	--+	OPD
23	100	Pr spp. + KP	--	SICU
24	101	Ab spp. + KP	++	SICU
25	104	KP + Pr spp.	+-	IPD
26	106	Ab spp. + *E. coli*	-+	OPD
27	111	KP + PA	+-	OPD
28	113	*E. coli* + KP	++	OPD
29	118	*E. coli* + KP	++	OPD

Notes: + = positive; - = negative; PA = *Pseudomonas aeruginosa*; Ab spp = *Acinetobacter* spp.; Pr spp. = *Proteus* spp., KP = *Klebsiella pneumoniae*; *E. coli* = *Escherichia coli*

Table 10 ESBL distribution according to gender ($n = 74$)

Gender	Number of ESBL
Male	47 (64%)
Female	27 (36%)
Total	74 (100%)

Table 11 ESBL positive isolates ($n = 74$)

Isolates	ESBL positive
Acinetobacter spp. (17)	2 (3%)
E. coli	31 (42%)
Klebsiella pneumoniae	34 (46%)
Pseudomonas aeruginosa	1 (1%)
Proteus spp.	6 (8%)
Total	74 (100%)

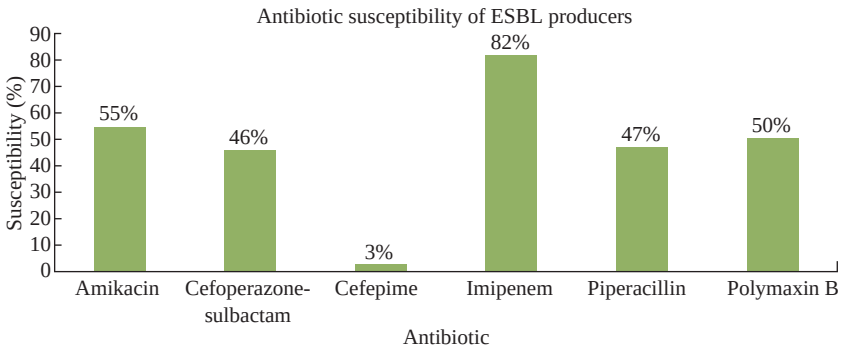

Fig. 12 Antibiotic susceptibility pattern of different ESBL producers. Imipenem: 82%, amikacin: 55%, polymaxin B: 50%, piperacillin: 47%, cefoperazone-sulbactam: 46%, and cefepime: 3%.

Table 12 Distribution of ESBL according to patient location in surgical area

Wards	ESBL positive	Age (%)
IPD	42	57
OPD	29	39
SICU	3	4
Total	74	100

Table 13 Effective antimicrobial in ESBL producers

Antibiotics	Frequency	Age sensitivity (%)
Amikacin	41	55
Cefoperazone-sulbactam	34	46
Cefepime	2	3
Imipenem	61	82
Piperacillin-tazobactam	35	47
Polymyxine B	37	50

the incidence of ESBL production in these organisms.

Patients with post-operative infections admitted or attending OPD during the period from January 2017 to February 2018 were included. In our study, the maximal number of patients was male (67%). The male predominance of infection in post-operative patients is also seen in studies conducted by many other authors. Accoring to Colodner et al. [14] and Motayo et al. [31], it may be due to the general finding that male patients have a greater susceptibility to infections. And apart from that, in our society, men are more involved in outdoor activities as compared to women; thus, chances of infections or injuries are more likely in male patients.

In our study, the maximal number of isolates was derived from inpatients (62%), which could be due to nosocomial infections that are very common in OPD and SICU. The patients on high dosage of antibiotics and have prosthetic implants are more susceptible to infections. In our study, this was acquired because there were fewer patients from SICU. A similar finding was demonstrated by to Colodner et al. [14] and Motayo et al. [31]. In our study, the commonest organism isolated was *E. coli* (29%), followed by *Pseudomonas aeruginosa* (27%), *Klebsiella* spp. (24%), *Acinetobacter* spp. (11%) and *Proteus* spp. (9%). Similar results were observed in developing countries by Anvikar et al. [5] who documented that *Klebsiella pneumoniae* was the commonest bacteria isolated from general surgical wounds. The difference could be due to different geographical distributions and climates. These isolates are normal flora in hospital environment, and the nosocomial spread might be due to poor adhering of aseptic procedures.

The high resistance to amoxicillin/clavulanic acid in our study (i.e 100%) was relatively higher than previous data from studies conducted by Blomberg et al. [7], Lyamuya et al. [25] and Moyo et al. [33]. Resistance to ciprofloxacin is an early warning sign since fluoroquinolones are effective agents for

Table 14 Antibiotic susceptibility of ESBL producers (n = 74)

Isolates	CAZ	AMC	CRO	AK	SCF	CIP	LEV	TZB	PRL	IPM	FEP	PB
	S	S	S	S	S	S	S	S	S	S	S	S
Acinetobacter spp. (2)	0	0	0	1	0	0	0	0	1	2	1	0
E. coli (31)	0	0	0	26	21	1	1	17	7	25	3	9
Klebsiella pneumoniae (34)	0	0	0	8	7	3	2	0	6	27	7	22
Pseudomonas aeruginosa (1)	0	0	0	1	0	0	0	1	1	1	1	1
Proteus spp. (6)	0	0	0	6	4	0	1	4	0	6	0	0

Notes: S = sensitivity; AMC = amoxicillin-clavulanic acid; AK = amikacin; CAZ = ceftazidime; CRO = ceftriaxone; CIP = ciprofloxacin; FEP = faropenem; LEV = levofloxacin; PB = polymyxin B; SCF = spectinomycin; PRL = piperacillin; TZB = tazobactam.

treatment of Gram negative bacterial infections. Therefore, the use of these drugs in treatment of surgical site infections should be closely monitored. The predominant ESBL producer in our case was *Klebsiella pneumoniae* followed by *E. coli*. This result is similar to the study done by Zaman et al. [44] and many others. In Pakistan, the highest frequency of ESBL production reported was by *Klebsiella* sp. followed by *E. coli*. The SENTRY surveillance programme form Asia Pacific and South Africa reported the commonest ESBL producer was *Klebsiella* spp. [15]. In our study, ESBL production was also seen in the case of *Pseudomonas aeruginosa*, but it was much lower as compared to *Enterobacteriaceae*. Similar findings were given by Nathisuwan et al. [34. It is an established fact that ESBL producers show cross resistance to other antimicrobial agents, and thus limit the therapeutic choice.

In our study, the highest sensitivity towards ESBL producers was imipenem (82%) followed by amikacin (55%) and polymyxin B (50%). Our result for imipenem is similar to other studies [15, 20, 22, 35, 41, 43]. But the poor performance as observed in the case of polymyxin B was surprising; better results might have been achieved if MIC testing should be conducted for polymyxin B.

The findings of the present study highlight the problem of ESBLs among post-operative infections. Major risk factors for the existence of ESBLs among post-operative infections at hospital include the use of unsterilized instruments, long term exposure to beta lactamase antibiotics especially the third and fourth generations of cephalosporins, and prolonged hospital stay.

Conclusions

In our set-up, 49% isolates were ESBL producers. The maximal number of patients was male (67%), and the maximal number of isolates was derived from inpatients (62%). In our study, the highest level of resistance was to amoxicillin/clavulanic acid, and the best sensitivity to ESBL producers was shown by imipenem. Imipenem is the drug of choice because it was sensitive to more than 80% *E. coli* strains.

Future recommendations include hand washing, never staying in hospital for extended period of time, removing catheter/needles as soon as possible, avoiding misuse and overuse of antibiotics, making policies for medication, and conducting seminars for awareness of staff and patients as well.

Conflict of Interests

The authors declare that no competing interest exists.

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