Therapeutic options for extended-spectrum β-lactamases (ESBLs), AmpC β-lactamases producing *Escherichia coli* and *Klebsiella* sp. isolated from various clinical samples

Vimal Kumar, Narinder Kaur*, Shubham Chauhan, Rosy Bala, Jyoti Chauhan, Harit Kumar, Shivani Devi

Department of Microbiology, Maharishi Markandeshwar Institute of Medical Science and Research, Maharishi Markandeshwar (Deemed to be) University, Mullana, Ambala, India

ABSTRACT

Escherichia coli and *Klebsiella* sp. are the predominant species isolated from clinical samples. Recent and proper understanding of the antibiotic susceptibility pattern of extended-spectrum β-lactamases (ESBL) and AmpC β-lactamases (AmpC) producing *E. coli* and *Klebsiella* sp. will prevent the distribution and future incidence of ESBL and AmpC. We designed this study to understand antibiotic susceptibility patterns of ESBL and AmpC producing *E. coli* and *Klebsiella* sp. isolated from a tertiary care hospital in North India. A cross-sectional study was conducted from March 2021 to February 2022. During this period, various clinical samples were collected and further tested for ESBL producing *E. coli* and *Klebsiella* sp. by using the Double disc Synergy test, whereas AmpC was detected by the Boronic acid disk potentiation method. Their antibiotic susceptibility patterns were noted. Various clinical specimens were collected, in which 37.95% were shown growth of bacteria. Among them, 46.67% of *E. coli* and 25.21% of *Klebsiella* sp. were identified by standard laboratory protocol. ESBL producing isolates were 44.37% and 34.20% in *E. coli* and *Klebsiella* sp., respectively. Whereas AmpC production was detected in 18.27% of *E. coli* and 29.36% of *Klebsiella* sp. ESBL and AmpC producing *E. coli* and *Klebsiella* sp. isolated from pus, blood, and sputum samples showed the highest sensitivity towards colistin, tigecycline, and imipenem while in urine samples imipenem, meropenem showed the highest sensitivity. Susceptibility patterns of ESBL and AmpC producing *E. coli* and *Klebsiella* sp. from various clinical specimens enhance hospital infection management and help clinicians to prescribe the appropriate antibiotics. The carbapenem, nitrofurantoin, colistin and tigecycline were showed highest susceptible against ESBL and AmpC producing *E. coli* and *Klebsiella* sp.

ABSTRAK

Escherichia coli dan *Klebsiella* sp. adalah spesies utama yang diisolasi dari sampel klinis. Pemahaman tentang pola kerentanan terhadap antibiotik dari *E. coli* dan *Klebsiella* sp. penghasil β-laktamase spektrum luas (*extended-spectrum β-lactamases*/ESBL) dan β-laktamase AmpC (AmpC *β-lactamases*/AmpC) akan mencegah distribusi dan munculnya ESBL dan AmpC ke depan. Penelitian ini dirancang untuk mengkaji pola kerentanan terhadap antibiotik dari *E. coli* dan *Klebsiella* sp. penghasil ESBL dan AmpC yang diisolasi dari rumah sakit perawatan tersier di India Utara. Penelitian potong lintang ini dilakukan dari Maret 2021 hingga Februari 2022. Selama periode ini, berbagai sampel klinis dikumpulkan dan diuji lebih lanjut untuk *E. coli* dan *Klebsiella* sp. penghasil ESBL menggunakan uji sinergi cakram ganda, sedangkan penghasil AmpC dideteksi dengan metode potensiasi cakram asam boronat. Pola kerentanan isolate terhadap antibiotik selanjutnya dicatat. Dari berbagai spesimen klinis yang dikumpulkan, sebanyak 37,95% menunjukkan pertumbuhan bakteri, dengan di antaranya, 46,67% *E. coli* dan 25,21% *Klebsiella* sp. diidentifikasi oleh protokol laboratorium standar. Isolat penghasil ESBL berturut-turut sebesar 44,37% dan 34,20% pada *E. coli* dan *Klebsiella* sp. Sedangkan produksi AmpC terdeteksi pada 18,27% *E. coli* dan 29,36% *Klebsiella* sp. *Escherichia coli* dan *Klebsiella* sp. penghasil ESBL dan AmC diisolasi dari sampel pus, darah,

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*corresponding author: docnarinder@gmail.com

dan sputum menunjukkan sensitivitas tertinggi terhadap colistin, tigesiklin, dan imipenem sedangkan pada sampel urin imipenem, meropenem menunjukkan sensitivitas tertinggi. Informasi tentang pola kerentanan *E. coli* dan *Klebsiella* sp. penghasil ESBL dan AmpC dari berbagai spesimen klinis dapat meningkatkan manajemen infeksi rumah sakit dan membantu dokter meresepkan antibiotik yang tepat. Karbapenem, nitrofurantoin, colistin dan tigesiklin menunjukkan kerentanan tertinggi terhadap *E. coli* dan *Klebsiella* sp. penghasil ESBL dan AmpC.

INTRODUCTION

The threat of antimicrobial resistance in humans is not new; around 700,000 people death annually around the world due to drug resistance.[1] Lack of accurate detection of bacterial resistance may increase mortality and morbidity, whereas knowledge of current trends of antibiotic sensitivity decreases the risk of bacterial resistance.[2] Since 1970, there has been a growing recognition and medical concern towards extended-spectrum β-lactamases (ESBL) and AmpC β-lactamases (AmpC) producing *E. coli* and *Klebsiella* sp. due to the overproduction of newer β-lactamase enzymes.[3] These enzymes are plasmid-mediated and can be transmitted from one bacterium to another. The ESBLs are enzymes that cause resistance to extended-spectrum cephalosporins (ESCs) such as cefotaxime, ceftriaxone, and ceftazidime, as well as the monobactam aztreonam.[2,3] The AmpC are enzymes that are responsible to cause bacterial resistance to penicillin, second and third generation cephalosporins, and cephamycins. According to Ambler's structural classification, AmpC belongs to the molecular C class while by the scheme of Bush they belong to group-1.[3] *Escherichia coli* and *Klebsiella* sp. are the major bacteria isolated from various community and hospital-acquired infections such as bloodstream infection, urinary tract infection, and meningitis.[4]

Various methods are available for the detection of ESBL and AmpC. Common methods available to detect ESBL are the double disc synergy test.[5] Other are combination disc method (the phenotypic confirmatory disc diffusion test).[6] Three dimensional test[7] such as broth dilution test,[6] ESBL E-Test[8] and VITEK-2.[9]

Escherichia coli and *Klebsiella species* produce ESBL and AmpC enhancing therapeutic problems and treatment failure. As a result detection of ESBL and AmpC producing *E.coli* and *Klebsiella* sp. is important for successful therapy as well as prevention of these resistant bacteria. Furthermore, the proper understanding of susceptible antibiotics in ESBLs and AmpC *E. coli* and *Klebsiella* sp. will prevent the distribution and future incidence of ESBLs and AmpC. The study provides the data on recent resistance patterns of ESBL and AmpC producing *E. coli* and *Klebsiella* sp. as well as their current treatment options. This study focused on the antibiotic sensitivity pattern of ESBL, AmpC producing *E. coli* and *Klebsiella* sp. isolated from various clinical specimens at a North Indian tertiary care hospital.

MATERIALS AND METHODS

Design of study

The study was conducted after the approval of the Ethical Committee with ethical letter no- IEC-2100. In this cross-sectional study, 5214 samples were collected from various clinical sites from blood, urine, pus, sputum, and swabs from the patients, who had septicemia, UTI, wounds infections, lower respiratory infections, and local infections respectively, after consent of the patient by nurses during January 2021 to February 2022. Then specimens were processed in the Microbiology Department of the Maharishi

Markandeshwar Institute of Medical Sciences & Research, Mullana, Ambala, India. All the specimens were inoculated on Blood agar and MacConkey Agar. Gram-positive bacteria were excluded and *E. coli and Klebsiella* sp. were only included in further processing identification and antibiotic sensitivity testing was done by Vitek-2 and confirmed by Kirby Bauer disc diffusion method as per CLSI 2021.[6,9] The bacteria control used in the study were *E. coli* ATCC 25922, and *K. pneumoniae* ATCC 700603.

Detection of ESBL

ESBL screening test

Disc Diffusion Test-Mueller Hinton Agar (MHA) was inoculated with the lawn culture of the test organism (0.5 McFarland`s turbidity). Disc of cefpodoxime (10μg), ceftazidime (30μg), cefotaxime (30μg) was applied on surface of MHA. The zones formed for each drug are as follows; cefpodoxime ≤17mm, ceftazidime ≤22mm, cefotaxime ≤27mm. The zones above indicated ESBL production.[6]

Confirmation of ESBL (combination disc method)

The test inoculum (0.5 MacFarland turbidity) was lawn onto the MHA by using a sterile (cotton swab) a ceftazidime disc (30μg) and a ceftazidime-clavulanic acid disc (20+10 μg) were placed at a distance of 20mm from each other, the plates were inoculated at 37⁰C for 18 to 24 h and the results was noted, ≥5mm size increased in the zone of inhibition observed in ceftazidime-clavulanic acid than ceftazidime consider as ESBL-producing organism.[6]

Detection of AmpC

Cefoxitin disc test

MHA plate was inoculated with the test organism (0.5 McFarland turbidity). A cefoxitin disc (30 μg) was placed in the center. The plates were incubated at 37ᵒC isolate that yielded a zone diameter of <18 mm and was accepted by AmpC enzyme producers.[10]

Boronic acid disk potentiation method

MHA plate was inoculated with a lawn culture of the test organism (0.5 McFarland turbidity). AmpC production was detected by using a disc of cefoxitin (30 μg) and another disc with boronic acid (20μL) on the culture plate at a distance of 20mm from the center of the disc. The overnight incubation was done at 37ᵒC. The organism was considered an AmpC producer if there was a ≥5mm increase in the zone of cefoxitin plus boronic acid disc as compared to cefoxitin disc.[11]

Preparation of disc containing boronic acid + cefoxitin

As much as 120 mg of phenylboronic acid was dissolved in 3 mL of dimethylsulphoxide and 3 mL of sterile distilled water was added to this solution. As much as 20μL of the stock solution was dispensed onto each disc of cefoxitin (30μg). Discs were allowed to dry for 30-60 minute and used immediately. A ≥ 5 mm increase in the zone diameter around the disc containing cefoxitin + boronic acid than around cefoxitin alone was considered positive for AmpC enzyme production.[11] Data was recorded and interpreted with excel and in the form of charts and TABLEs.

RESULTS

A total of 5214 samples were received in a laboratory during the study period, in which 62.05% samples were sterile and 37.95% were positive for bacterial growth, 41.18% were gram-positive bacteria, 53.91% were gram-negative bacteria, and 4.90% were *Candida* sp. The 59% samples were collected from females and 41% samples were collected

from males and the bacterial positivity rate was 54% in female and 45% in male patients. The age of the patients has also noted classified into different groups in which the highest number of samples i.e. 48% was collected from patients belongs the 21-40 years age group followed by the 41-60 age group (22%), 0-20 age group (19%) and 61-80 years old age group (11%) and the culture positivity was highest in patients belongs to 21-40 age group i.e. 36% followed by 41-60 age group (33%), 61-80 years age group

(22%) and lowest in 0-20 age group (5%). The majority of the samples were collected from IPD (71%) and OPD (29%) patients. the culture positivity rate was 58% from IPD patients while 42% from OPD patients.

In total Gram-negative isolates, *E. coli* (46.67%) was the predominant bacteria, followed by *Klebsiella* sp. (25.21)%, *Proteus* sp. (4%), *Pseudomonas* sp. (12.74%), *Acinetobacter* sp. (7.77%), *Providencia* sp. (2.%) and *Sphingomonas* sp. (2%) (FIGURE 1).
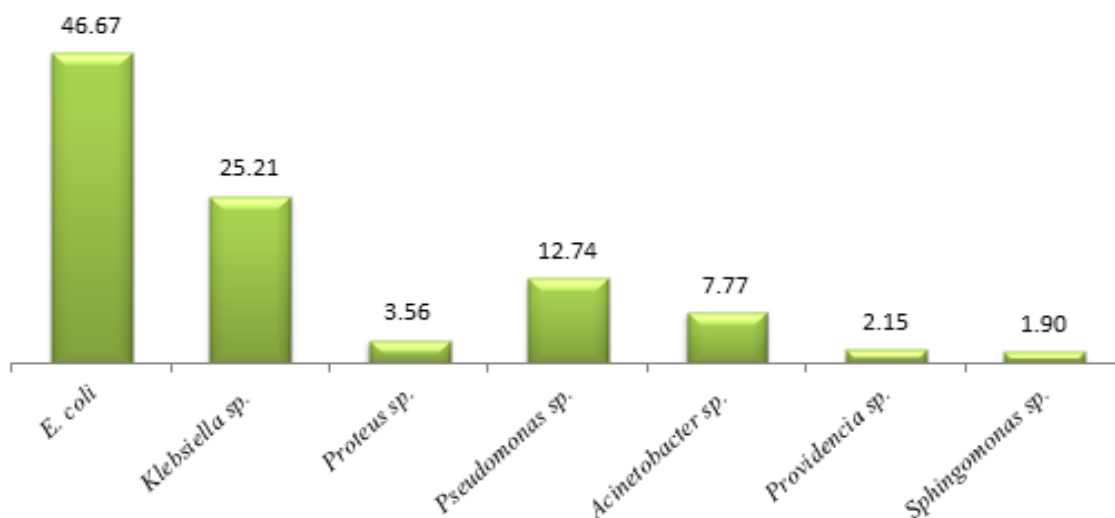

FIGURE 1. Frequency (%) of Gram-negative bacteria isolated in the study

Out of a total of 498 *E. coli*, 44% of *E. coli* were detected as ESBL producers, while 34% of *Klebsiella* sp. were identified as ESBL producers from a total of 269 *Klebsiella* sp. by using CLSI recommended phenotypic confirmatory disc diffusion

test (PCDDT). However, 18.27% of *E. coli* and 29.36% of *Klebsiella* sp. isolates were identified as AmpC producers by using a boronic acid test whereas 16% of isolates were found to be co-producers of ESBL and AmpC (TABLE 1).

TABLE 1. ESBL, AmpC and co-producer *E. coli* and *Klebsiella* sp.

Isolates (n=767)	ESBL producers [n (%)]	AmpC producers [n (%)]	Co-porducers [n (%)]
E. coli (n=498)	91 (18.27)	221 (44.37)	58 (11.64)
Klebsiella sp. (n=269)	79 (29.36)	92 (34.20)	39 (14.49)
Total (n=767)	170 (22.16)	313 (40.80)	97 (12.64)

The majority of ESBL producing *E. coli* was isolated from urine specimens i.e. 64.25% followed by pus (17.19%), sputum (2.71%), blood (4.97%), and the majority of ESBL producing *Klebsiella* sp. was isolated from urine specimens i.e. (28.57%), followed by pus (19.78%), sputum (3.29%), and blood (5.49%). Maximum AmpC producing *E. coli* was isolated from (59.34%) urine samples followed by pus (18.68%), sputum (3.29%), blood (5.49)% while in *Klebsiella* sp., maximum isolates were identified from urine samples i.e. 34.17%, followed by pus (18.98%), sputum (5..06%), and blood (15.18%). Whereas co-production of ESBL and AmpC in *E. coli* were maximum in urine samples (55%) followed by pus (22%), sputum (5%), blood (7%) while in *Klebsiella* sp. (23%) co-producers isolated from urine samples followed by pus (18%), sputum (5%), and blood (23%) (TABLE 2).

TABLE 2. Sample wise distribution of ESBL, AmpC, Co-producing *E. coli*, and *Klebsiella* sp. (Others swabs and tissues collected from the various department.)

Sample	*E. coli* [n (%)]			*Klebsiella* sp. [n (%)]		
	AmpC producers	ESBL producers	Co-porducers	AmpC producers	ESBL producers	Co-producers
Urine	54 (59.34)	142 (64.25)	32 (55)	27 (34.17)	26 (28.57)	9 (23)
Pus	17 (18.68)	38 (17.19)	13 (22)	15 (18.98)	18 (19.78)	7 (18)
Sputum	3 (3.29)	6 (2.71)	3 (5)	4 (5.06)	11(12.08)	2 (5)
Blood	5 (5.49)	11 (4.97)	4 (7)	12 (15.18)	12 (13.18)	9 (23)
Others	12 (13.18)	24 (10.85)	6 (10)	21(26.58)	24 (26.37)	12 (31)
Total	91 (100)	221 (100)	58 (100)	79 (100)	91 (100%)	39 (100)

The 55% of ESBL producers *E. coli* and *Klebsiella* sp. were isolated from females while 45% from male and AmpC were identified in 52% from females, while 47% from male patients. Co-producers of ESBL and AmpC in females were 53% and in males were 47%, respectively.

The ESBL producing *E. coli* and *Klebsiella* sp. were detected in 42% of IPD patients and 40% in opd patients while AmpC producing *E. coli* and *Klebsiella* sp. were 24% in IPD patients and 19% from OPD patients. Co-producers of ESBL and AmpC in OPD were 13% and IPD were 12%, respectively.

The antibiotic sensitivity pattern of ESBL producing *E. coli* and *Klebsiella* sp. isolated from various clinical samples showed 71.23% strains were susceptible to tigecycline, 66% to nitrofurantoin and 60% to amikacin and AmpC producing *E. coli* and *Klebsiella* sp. showed 80% susceptible to fosfomycin and 71% to nitrofurantoin (FIGURE 2).

The antibiotic sensitivity pattern of ESBL producing *E. coli* and *Klebsiella sp.* isolated from urine samples showed 95% strains were susceptible to imipenem, 96% to meropenem and 87% to fosfomycin and AmpC producing *E. coli* and *Klebsiella* sp. showed 96% susceptible to imipenem, 95% to meropenem and 84% to fosfomycin (FIGURE 3).
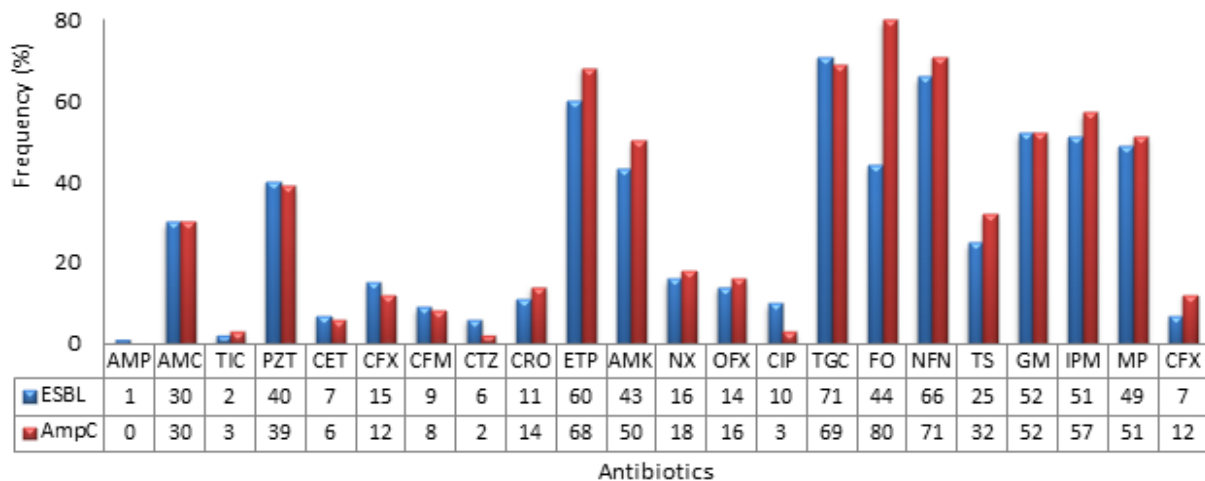
FIGURE 2. Susceptibility pattern of ESBL and AmpC producing *E. coli* and *Klebsiella* sp. isolated from various sample. Note: AMP (ampicilin); AMC (amoxicillin/ clavulanic acid); TIC (ticarcillin); PZT (piperacillin/tazobactam); CET (cefalotin); CFX (cefoxitin); CFM (cefixim); CTZ (ceftazidime); CRO (ceftriaxone); ETP (ertapenem); AMK (amikacin); NX (norfloxacin); OFX (ofloxacin); CIP (ciprofloxacin); TGC (tigecyclin); FO (fosfomycin); NFN (nitrofurantoin); TS (trimehoprim/sulfonamide); GM (gentamicin); IPM (imipenem); MP (meropenem); CFX (cefuroxime).
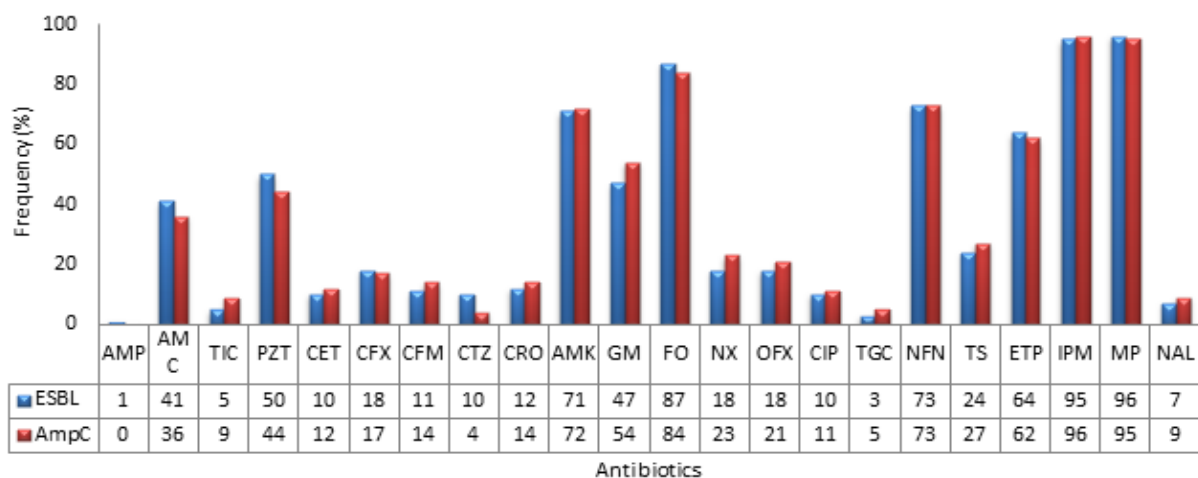

FIGURE 3. Susceptibility pattern of ESBL and AmpC producing *E. coli* and *Klebsiella* sp. isolated from urine specimens. Note: AMP (ampicilin); AMC (amoxicillin/ clavulanic acid); TIC (ticarcillin); PZT (piperacillin/tazobactam); CET (cefalotin); CFX (cefoxitin); CFM (cefixim); CRO (ceftriaxone); AMK (amikacin); GM (gentamicin); FO (fosfomycin); NX (norfloxacin); OFX (ofloxacin); CIP (ciprofloxacin); TGC (tigecyclin); NFN (nitrofurantoin); TS (trimehoprim/ sulfonamide); ETP (ertapenem); IPM (imipenem); MP (meropenem); NAL (nalidixic acid).

The antibiotic sensitivity pattern of ESBL producing E. coli and Klebsiella sp. isolated from pus samples showed 100% strains were susceptible to colistin, 80% to tigecycline and The antibiotic sensitivity pattern of ESBL producing

E. coli and Klebsiella sp. isolated from blood samples showed 100% strains were susceptible to colistin, 57% to tigecycline and 58% to amikacin change into The antibiotic sensitivity pattern of ESBL producing E. coli and Klebsiella

sp. isolated from pus samples showed 100% strains were susceptible to colistin, 80% to tigecycline and 58% amikacin. The antibiotic sensitivity pattern of ESBL producing E. coli and Klebsiella sp. isolated from blood samples showed 100% strains were susceptible to colistin, 57% to tigecycline and 52% to amikacin (FIGURE 4).

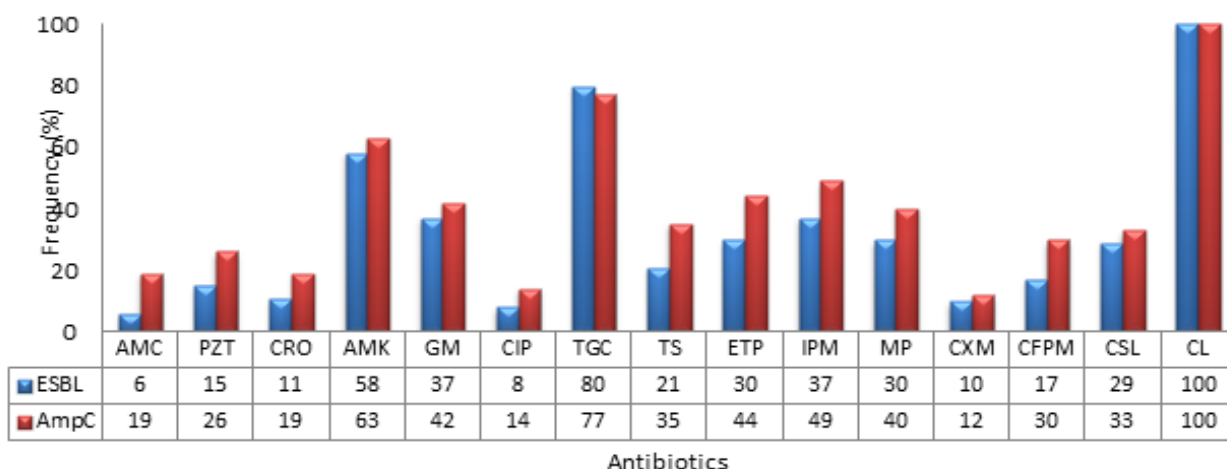|	AMC	PZT	CRO	AMK	GM	CIP	TGC	TS	ETP	IPM	MP	CXM	CFPM	CSL	CL
ESBL	6	15	11	58	37	8	80	21	30	37	30	10	17	29	100
AmpC	19	26	19	63	42	14	77	35	44	49	40	12	30	33	100

FIGURE 4. Susceptibility pattern of ESBL and AmpC-producing *E. coli* and *Klebsiella* sp. isolated from pus specimens. Note: AMC (amoxicillin/clavulanic acid); PZT (piperacillin/tazobactam); CRO (ceftriaxone); AMK (amikacin); GM (gentamicin); CIP (ciprofloxacin); TGC (tigecyclin); ETP (ertapenem); IPM (imipenem); MP (meropenem); CXM (cefuroxim); CFPM (cefepime); CSL (cefoperazone/sulbactam); CL (colistin).

52% to amikacin and AmpC producing E. coli and Klebsiella sp. showed 100% susceptible to colistin, 59% to tigecycline and 53% to amikacin change into AmpC producing E. coli and Klebsiella sp. showed 100% susceptible to colistin, 59% to tigecycline and 53% to amikacin (FIGURE 5).

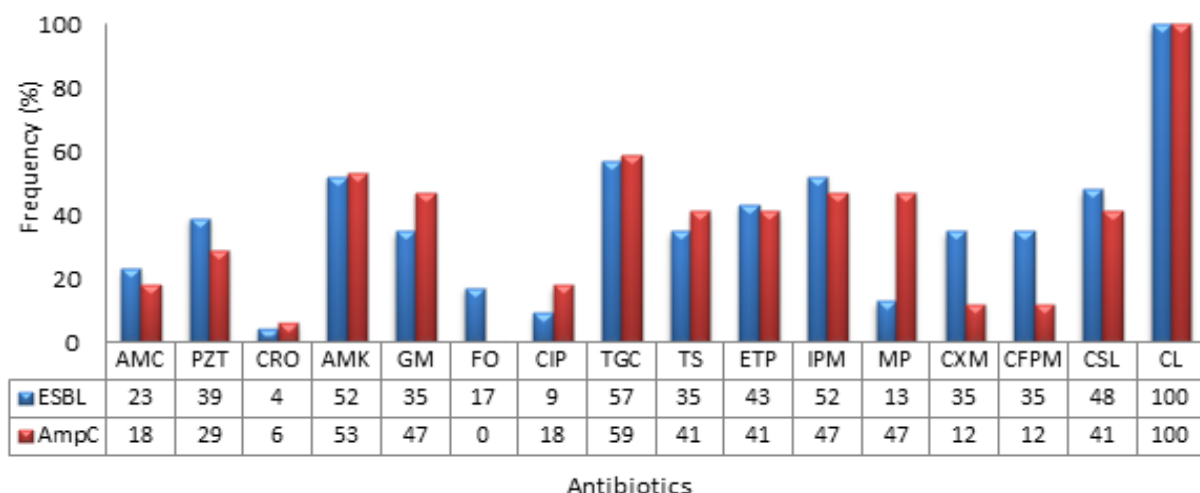|	AMC	PZT	CRO	AMK	GM	FO	CIP	TGC	TS	ETP	IPM	MP	CXM	CFPM	CSL	CL
ESBL	23	39	4	52	35	17	9	57	35	43	52	13	35	35	48	100
AmpC	18	29	6	53	47	0	18	59	41	41	47	47	12	12	41	100

FIGURE 5. Susceptibility pattern of ESBL and AmpC producing *E. coli* and *Klebsiella species* isolated from blood samples. Noted: AMC (amoxicillin/clavulanic acid); PZT (piperacillin/tazobactam); CRO (ceftriaxone); AMK (amikacin); GM (gentamicin); FO (fosfomycin); CIP (ciprofloxacin); TGC (tigecyclin); TS (trimehoprim/sulfonamide); ETP (ertapenem); IPM (imipenem); MP (meropenem); CXM (cefuroxim); CSL (cefoperazone/sulbactam); CL (colistin).

The antibiotic sensitivity pattern of ESBL producing *E. coli* and *Klebsiella* sp. isolated from sputum samples showed 100% strains were susceptible to colistin, 79% to tigecycline, 68% to imipenem, and AmpC producing *E. coli* and *Klebsiella* sp. showed 100% susceptible to colistin, 86% to tigecycline, and 86% to imipenem (FIGURE 6).
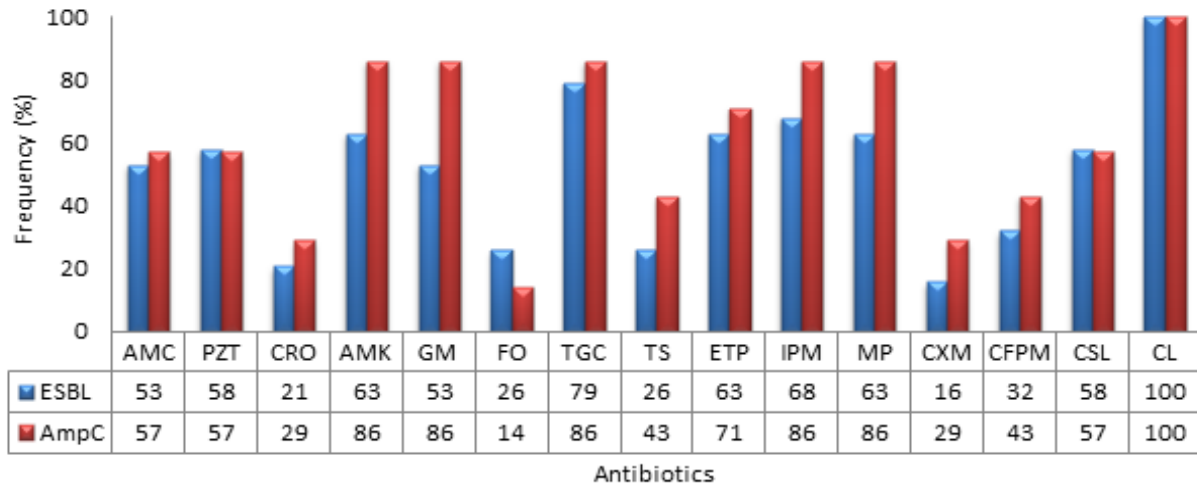

TABLE 6. Susceptibility pattern of ESBL and AmpC producing *E. coli* and *Klebsiella* sp. isolated from sputum samples. Note: AMC (amoxicillin/clavulanic acid); PZT (piperacillin/tazobactam); CRO (ceftriaxone); AMK (amikacin); GM (gentamicin); FO (fosfomycin); TGC (tigecyclin); TS (trimehoprim/ sulfonamide); ETP (ertapenem); IPM (imipenem); MP (meropenem); CXM (cefuroxim); CFPM (cefepime); CSL (cefoperazone/sulbactam); CL (colistin).

The antibiotic sensitivity pattern of ESBL producing *E. coli* and *Klebsiella* sp. isolated from other samples showed that 100% of strains were susceptible to colistin, 65% to tigecycline, 56% to amikacin, and AmpC producing *E. coli* and *Klebsiella* sp. showed 100% susceptible to colistin, 64% to tigecycline, and 67% to amikacin (FIGURE 7).
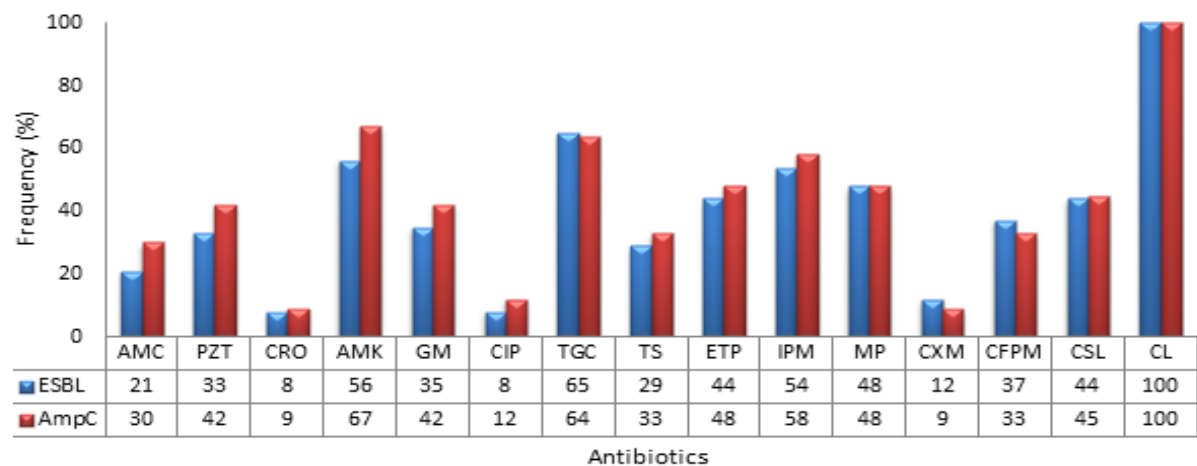

FIGURE 7. Susceptibility pattern of ESBL and AmpC producing *E. coli* and *Klebsiella* sp. isolated from other samples (i.e. swabs and tissues). Noted: AMC (amoxicillin/ clavulanic acid); PZT (piperacillin/tazobactam); CRO (ceftriaxone); AMK (amikacin); GM (gentamicin); CIP (ciprofloxacin); TGC (tigecyclin); TS (trimehoprim/sulfonamide); ETP (ertapenem); IPM (imipenem); MP (meropenem); CXM (cefuroxim); CFPM (cefepime); CSL (cefoperazone/ sulbactam); CL (colistin).

DISCUSSION

Escherichia coli and *Klebsiella* sp. are the predominant bacteria isolated from various community and hospital-acquired infections. *Escherichia coli* and *Klebsiella* sp. that produce ESBLs and AmpC enzymes will cause therapeutic problems and failure, including illness and death. As a result detection of ESBLs and AmpC is important for successful therapy as well as prevention of those resistant bacteria.[8] Precise detection of ESBL and AmpC producing *E. coli* and *Klebsiella* sp. would decrease the mortality and multidrug-resistant organisms. Proper understanding of susceptible antibiotics against ESBLs and AmpC producing *E. coli* and *Klebsiella* sp. can help in the treatment of these organisms. There is a need for susceptibility pattern for ESBLs and AmpC producing *E. coli* and *Klebsiella* sp. from various samples.

In the present study *E. coli* was showed 46.67% growth Rate While *Klebsiella* sp. showed 25.21% followed by *Proteus* sp. 3.56%, *Pseudomonas* sp. 12.74%, *Acinetobacter* sp. 7.77%, *Provedencia* sp. 2.15% and *Sphingomonas* sp. 1.90% (FIGURE 1). As reported by study of Sah *et al.*,[13] among 109 Gram negative bacteria isolates, 40.3% were *E. coli*, 30% *Klebsiella* sp. and 11% were *Acinetobacter* sp. As the similar study conducted by Nepal *et al.*,[14] stated that *E. coli and* Klebsiella sp. were 51.5% and 14.6%, respectively. As per the both studies *E. coli and Klebsiella* sp. were the predominenet gram negative bacteria isolates. The number of the bacteria might be different because of the locations of the study.

In this present study ESBL producer *E. couple* was 44.17%, whereas *Klebsiella* sp. were 18.27% and AmpC producers *E. coli* were 34.20%, *Klebsiella* sp. 29.36%. Co-producers of ESBL+AmpC also observed in 11.64% in *E. coli* and 14.49% in Klebsiella sp. (TABLE 1). Similar study conducted by Vijaya *et al.*,[15] found about 16% *E. coli*, 6% *Klebsiella* sp. were ESBL positive, 9% *E. coli*, and 3% *Klebsiella* sp. were AmpC producers. Co-production of ESBL+AmpC seen in approximately 15% of total isolates. A study conducted by Nasir *et al.*[16] showed that ESBL production was slightly higher as compared to AmpC in both *E. coli* and *Klebsiella* sp. 12 % *E. coli* and 10% *Klebsiella* sp. were Co-producers for ESBL + AmpC. The increasing number of ESBL and AmpC are the matter of concern that can be resolved by the proper understanding of the antibiotic sensitivity pattern of the samples before starting the empirical treatment or at least send the sample for antibiotic sensitivity testing before starting the empirical treatment.

ESBL-producing *E. coli* and *Klebsiella* sp. from urine samples were 64.25% and 28.57% respectively, followed by pus 17.19% and 19.78%, sputum 2.71% and 12.08%, blood 4.97% and 13.18% respectively. Out of 170 AmpC, 59.34% *E. coli* and 34.17% *Klebsiella* sp. were isolated from urine samples followed by 18.68% and 18.98% from pus, 5.49% and 15.18% in blood samples positively (TABLE 2). A study conducted by Yusuf *et al.*[17] reported that higher ESBL producers detection in *E. coli* and *Klebsiella* sp. was 22.2% in blood samples, followed by 17.6% in urine, 14.5% in urogenital swabs, and 13.6% in wound swab samples whereas AmpC detection in *E. coli* and *Klebsiella* sp. were 50% in urine specimens followed by 20% in catheter tip, 10% in ear swab and10% in wound swab. Saffar *et al.*[18] reported that a higher prevalence rate of ESBL and AmpC producing *E. coli* and *Klebsiella* sp. were seen in the wound and pus samples followed by respiratory samples, body fluid, and blood whereas in AmpC-producing *E. coli* and *Klebsiella* sp. higher rate came from body fluids 63% followed by blood 57%, urine 56%, and respiratory samples 28%. These studies showed that blood and body

fluid was the major source of ESBL and AmpC producing *E. coli* and *Klebsiella* sp. whereas we identified urine as a major source of ESBL and AmpC producing *E. coli* and *Klebsiella* sp. UTI patients should be assessed for antibiotic sensitivity testing as early possible as.

In the present study, 55% of ESBL producers *E. coli* and *Klebsiella* sp. were isolated from females while 45% were from males, and AmpC was identified in 52% from females, while 47% were from male patients. Co-producers of ESBL+AmpC in females were 53% and in males were 47% respectively. The ESBL-producing *E. coli* and *Klebsiella* sp. was detected in 42% IPD patients and 40% in opd patients while AmpC producing *E. coli* and *Klebsiella* sp. were 24% in IPD patients and 19% from opd patients. Co-producers of ESBL+AmpC in OPD were 13% and ipd were 12% respectively. Another study conducted by Yusuf *et al.*[17] showed that 52% of ESBL producers in *E. coli* and *Klebsiella* sp. were isolated from males while 48% from females and AmpC were identified in 60% from male, while 40% from female patients. Somily *et al.*[19] reported that the prevalence rate of ESBL and AmpC producers i.e. 32% of patients were female and 27% patients were male. The ESBL and AmpC producing *E. coli* and *Klebsiella* sp. were detected higher percentage in ipd patients as compared to opd patients. So as per our study females had a higher chance of getting infected with ESBL and AmpC producers *E. coli* and *Klebsiella* sp. We identifed more patients with UTIs and females had more chance of getting UTIs than males. Co-producers are a matter of concern in UTIs of females.

The treatment options against ESBL and AmpC producing *E. coli* and *Klebsiella* sp. depend on the antibiotic sensitivity pattern of the samples. It is very important that every hospital should have their local antibiotic surveillance systems or the hospital may use studies that provide the antibiotic sensitivity pattern on current basics. The sensitive antibiotics can be utilized for the treatment of ESBL and AmpC producing *E. coli* and *Klebsiella* sp.

Antibiotics susceptibility patterns of ESBL and AmpC producing *E. coli* and *Klebsiella* sp. were found to be variable. Most of the ESBL producing isolates were susceptible to tigecycline (71.23%), followed by nitrofurantoin (66%) and (60%) amikacin. Highest sensitivity in AmpC producing isolates was susceptible to fosfomycin (80%), nitrofurantoin (71%), tigecycline (69%), and amikacin (68%) (FIGURE 2). A similar study conducted by Nepal *et al.*[14] showed most of the ESBL producing bacteria were sensitive to imipenem followed by piperacillin/tazobactam, amikacin, and cefoperazone/sulbactam. This is in accordance with the study conducted by Sasirekha *et al.*[20] that reported the highest susceptibility seen in AmpC-producing isolates were imipenem (100%), and amikacin (93%). As per, Nepal *et al.*[14] and Sasirekha *et al.*[20], imipenem and meropenem were the highest sensitive antibiotics in our study imipenem and meropenem were lower than 50% resistant in case of ESBL and AmpC. Fosfomycin, nitrofurantoin, and tigecycline were the highest sensitive antibiotics.

Urine and their antibiotic susceptibility pattern of AmpC and ESBL producing *E. coli* and *Klebsiella* sp. showed high-level susceptibility to imipenem and meropenem 96% and 95% respectively, followed by fosfomycin (81%), nitrofurantoin (71%), and amikacin (70%) (FIGURE 3). Cho *et al.*[21] also showed the susceptibility against ESBL and AmpC producers where highly sensitive antibiotics were imipenem and meropenem (100%) respectively followed by Fosfomycin (96%), amikacin (91%), and nitrofurantoin (90%). A similar study conducted by Halabi *et al.*[22] showed that maximum sensitivity was observed in ertapenem and imipenem followed by

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fosfomycin, and amikacin. The resistance towards imipenem, meropenem, and nitrofurantoin have been increased over time. Even though their resistance to carbapenem was also noted and 30% resistance was seen in nitrofurantoin.

Pus samples and their Antibiotic sensitive pattern of ESBL and AmpC producing *E. coli* and *Klebsiella* sp. the highest susceptibility found in colistin (100%) followed by tigecycline (80%), amikacin (75%), and carbapenems (75%) (FIGURE 4). A similar study conducted by Hedaoo *et al.*[23] showed maximum susceptibility to ciprofloxacin followed by amikacin, and cefotaxime. In the pus samples, the colistin and tigecycline was the only option left for the treatment of wound infections, even imipenem was 30 % resistant.

The sensitive pattern of ESBL and AmpC producing *E. coli* and *Klebsiella* sp. from blood samples, the highest susceptibility seen in colistin (100%), tigecycline (58%), followed by amikacin (53%), and imipenem (50%) (FIGURE 5). This is in accordance with the study performed by Saikumar *et al.*[24] that reported colistin, polymyxin-B and carbapenems (100%) sensitive against ESBL producing Gram negative bacteria. The resistance was very high in blood samples. It was 50% for imipenem and colistin was the highest sensitive antibiotic. Susceptibility pattern of ESBL and AmpC producing *E. coli* and *Klebsiella* sp. from sputum showed colistin 100% sensitivity, followed by tigecycline (85%), and carbapenems (80%) (FIGURE 6). However, a similar study was conducted by Malik *et al.*[25] that reported amikacin (80%) and gentamycin (80%) were highly susceptible and followed by cotrimaxazole (69%) and imipenem (55%). In sputum samples, colistin, tigecycline, and carbapenems were the highest sensitive antibiotics. These are all costly antibiotics that increase the cost of treatment so early detection of antibiotic sensitivity may prevent the incident of ESBL and AmpC in patients.

The antibiotic sensitive pattern of ESBL producing *E. coli* and *Klebsiella* sp. from other samples (i.e. swabs and tissues) showed that colistin was higher sensitive (100%), followed by tigecycline (65%), and amikacin was (56%) (FIGURE 7). A similar study conducted by Tekele *et al.*[26] showed that ESBL and AmpC producing *E. coli* and *Klebsiella* sp. were highly sensitive to amikacin (100%), followed by imipenem (98%), and meropenem (96%). Other samples included swabs such as ear swabs, eye swabs, body fluids, and tissues imipenem and meropenem showed resistance in these samples. The condition of humanity is worrisome, especially in the case of antibiotics. Every patient should be assessed for ESBL and AmpC.

Some limitation of the study was observed. No molecular testing of antibiotic resistance in isolates was done, even though it's possible that isolates possess resistant genes but do not exhibit them phenotypically. As a result, they may be able to pass on their resistance to other bacteria. Our findings are highly exciting and therapeutically important since we evaluated the susceptibility pattern of ESBL and AmpC producing *E. coli* and *Klebsiella* sp. isolated from various sources. More in depth surveys with a larger sample size, as well as collaboration with other hospitals, might result in more enticing offers.

CONCLUSION

Multidrug-resistant Gram-negative bacteria (MDR-GNB) isolated from clinical samples are increasing day by day. Extended-extended-spectrum β-lactamase and AmpC enzymes play a major role to refurnish susceptible bacteria into MDR-GNB. The easy spread of these pathogens in hospitals is becoming a major public health issue. As a result, continual screening for resistance mechanisms in nosocomial

infections is essential. Susceptibility patterns of ESBL and AmpC-producing *E. coli* and *Klebsiella* sp. from various specimens enhance hospital infection management and help doctors prescribe the most sensitive antibiotic.

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