Original Research Article

Identification and antibiogram of Amp C β – lactamases present in gram negative bacilli – Has the trend changed?

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ABSTRACT

Background and Objectives: Resistance to therapeutic drugs poses problems in hospital settings as well as in the community since most of the bacteria exhibit multi drug resistance pattern. The resistance is mostly conferred by ESBL and Amp C β-lactamase production in gram negative bacilli. Thus, identification of Amp C is required to bring improvement in the management of patients suffering from infections in hospitals. There is lack of information on documentation of Amp C β-lactamases, thus the present retrospective study was undertaken to assess the method of identification and documentation Amp C β- lactamases among gram negative isolates and their antibiogram from hospitalized patients in BTGH, Gulbarga and also to analyse the changes in antiobiotic susceptibility trend over the years.

Materials and Methods: A total of 250 Gram negative consecutive and non-repetitive isolates obtained from various clinical samples were screened for Amp C β-lactamase and ESBL production by modified double disk approximation method (MDDM). The screen positive isolates were subjected to Amp C β-lactamase detection by modified 3 – dimensional extract test. All these isolates were also subjected to antibiotic sensitivity testing.

Results: Of all the gram negative isolates, 55.2% were found resistant to third generation cephalosporins, out of which 47.8% were ESBL producers. A total of 23.2% isolates were found to be Cefoxitin resistant, 60.3% of which were Amp C producers. Most of the Amp C producing organisms showed sensitivity to Imipenem, Amikacin and Ciprofloxacin.

Conclusion: In the present study, the AmpC type β – lactamase were seen to be produced mostly in Klebsiella pneumonia which can be attributed to a combined type of drug resistance mechanisms by AmpC and ESBL production prevalent in these organisms.

1. Introduction

Resistance to therapeutic drugs poses problems in hospital settings as well as in the community since most of the bacteria exhibit multi drug resistance pattern. It is increasing especially towards β-lactam group of antibiotics and is a major healthcare issue worldwide. Various mechanisms confer resistance by gram negative bacteria to antibiotics which include extended spectrum beta-lactamases (ESBL) production, Amp C β-lactamase production, efflux mechanism and porin deficiency. Among these, Amp C β-lactamases and ESBLs are the most commonly enzymes detected clinically.[1–4]

Amp C β- lactamases are cephalosporinases which belong to molecular class C as classified by Ambler in 1980 and group I under the classification of Bush et al. in 1995. They may confer resistance to narrow and broad spectrum β–lactam drugs when overexpressed.[5,6] They can be plasmid or chomosomally mediated and hydrolyse all β-lactam antibiotics except cefepime and carbapenems. AmpC is inducible in most genera of the family *Enterobacteriaceae*. Many plasmid-mediated Amp C enzymes, such as CMY-type-lactamases, have been found in bacterial species that naturally lack a chromosomal

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Amp C -lactamase, for e.g. *Klebsiella pneumoniae, Proteus mirabilis*, and *Salmonella* spp. [7–10]

According to a World Health Organization (WHO) fact sheet, of every 100 hospitalized patients at any given time, 7 in developed and 10 in developing countries will acquire at least one hospital acquired infection. [11] Supported by many stakeholders in the field of IPC, WHO has issued recommendations and specifications for effective IPC programmes. These are included in the evidence-based WHO Guidelines on core components of IPC programmes (2016) which have included hospital surveillance as an important aspect of IPC. Also, Centers for disease control and prevention (CDC) has stated strengthening surveillance and epidemiology as one of the five strategic areas that need to be focused on for a better global health.

Many groups in India, like the autonomous National Accreditation Board of Hospitals and the National Health Mission's National Health Systems Resource Centre have conveyed the importance of surveillance of healthcare associated infections through various programmes on infection prevention and control. [12,13] With the growing recognition of the need for policy and guidance documents, the Indian Council of Medical Research released guidelines on infection prevention and control in 2016. [14] Also, the National Health Mission launched *Kayakalp* (clean hospital initiative) with the focus laid on cleanliness, hygiene, and infection control practices in public healthcare facilities. [15] For these procedures to be implemented successfully in healthcare settings, standardised surveillance of healthcare associated infections need to be carried out and the data so obtained should be utilized to further promote infection control policies, interventions, and indicators as per need.

It has been observed from various epidemiological studies that AmpC enzyme producing bacteria are recovered from hospitalized patients usually after several days of admission to the hospital. A majority of patients are treated with β-lactam antibiotics including cefoxitin in hospitals. Many physicians and clinical laboratories are still unaware of the clinical importance of lactmases which results in the lactamase producing bacteria going unnoticed. This in turn accounts for these bacteria being responsible for nosocomial outbreaks in hospitals. A lack of accurate laboratory detection and reporting of such resistant phenotypes and strains producing plasmid-mediated Amp C, only a suboptimal treatment could be delivered for gram negative infections. [16,17]

Thus, identification of Amp C is required to bring improvement in the management of patients suffering from infections in hospitals as well as in assessment of its epidemiological status. However, currently, there are no Clinical and Laboratory Standards Institute (CLSI) guidelines for detection of resistance in gram negative clinical isolates mediated by Amp C. Most laboratories depend on phenotypic and polymerase chain reaction (PCR) methods to detect Amp C β-lactamases which are usually time consuming and costly. Also, problems are posed by misleading results especially so in cases of phenotypic tests. [18,19]

There is a paucity of information on the documentation of Amp C β-lactamases among Gram negative isolates in our region. This may partly be due to lack of standard guidelines for detecting Amp C-producing isolates and partly due to lack of awareness among physicians and laboratories. The present retrospective study was therefore undertaken to analyse the method of identification and documentation of Amp C β- lactamases in gram negative bacilli isolates particularly in *E.coli* and *Klebsiella* from hospitalized patients of HKE'S Basaveshwar Teaching and General Hospital, Gulbarga. This study also focuses on evaluation of changes in antiobiotic susceptibility trend, if any, in such AMP C lactamases over a period of 9 years. This study also aims to create awareness among the clinicians regarding the prevalence of Amp C β- lactamases in gram negative infections, to guide them for an appropriate use of antibiotics in such cases and to take appropriate infection control measures to prevent the further antibiotic resistance in gram negative bacilli.

2. Materials and Methods

The present study was carried out in the Department of Microbiology, M.R. Medical College, Gulbarga, Karnataka, from January 2011- December 2011. The patients of Basaveshwar Teaching and General Hospital Gulbarga contributed as the study population. A total of 250 Gram negative consecutive and non-repetitive isolates obtained from various clinical samples in the Microbiology laboratory during the study period constituted the material for the study.

2.1. Processing of samples

The samples for the study included sputum, urine, blood, exudates and pus. The specimens were processed in the laboratory within two hours of collection using the following methods:

1. Gram stain – to examine pus cells and gram negative bacteria
2. Culture
   a. For sputum, exudates and urine sample, nutrient agar, Mac Conkey agar and blood agar were used.
   b. For blood samples, brain heart infusion broth followed by nutrient agar, Mac Conkey agar and blood agar were used.

2.2. Biochemical tests

The biochemical tests that were carried out included – catalase test, oxidase test, indole test, methyl red test, Voges-Proskauer test, triple sugar iron agar (TSI), citrate utilization, urease test, lysine decarboxylase test, ornithine decarboxylase test and fermentation of sugars. The organism was identified from the specimens using the details given in Table 1.

Modified Double Disk Approximation method was used to screen the gram negative isolates for production of ESBL and Amp C. Double Disk Synergy Test was performed on screen positive isolates for confirmation of ESBL production. Modified Three Dimensional Test and Amp C disk test were performed for confirmation of Amp C production. The antibiotic susceptibility test of the isolates was also carried out. These tests were carried out as follows:

2.2.1. Modified double disk approximation method (MDDM):[21] Screening for ESBL and Amp C producing isolates simultaneously

Test isolate suspension equivalent to 0.5 McFarland Barium sulphate standard was prepared in 5 ml of Muller Hinton broth which was then swabbed on Muller Hinton Agar plates. Disks of cefotaxime (30 μg) and ceftazidime (30 μg) were placed adjacent to clavulunic acid (10 μg) and cefoxitin (30 μg) disk at a distance of 20mm from each other. An enhanced zone of inhibition between any of the disks (ceftazidime/ cefotaxime) and clavulunic acid was interpreted as presumptive evidence for the ESBL presence. Isolates that showed blunting of cefotaxime or ceftazidime zone of inhibition adjacent to cefoxitin disk or that showed reduced susceptibility to either of the drugs (ceftazidime or cefotaxime) and cefoxitin were considered as "screen positive" and selected for detection of Amp C ß lactamases.

2.2.2. Double disk synergy test (DDST:[22] Phenotypic confirmatory test for ESBL

The suspension for inoculum was prepared from 4-5 isolated colonies and turbidity was compared with 0.5 McFarland standard. Sterile cotton swab soaked in this suspension was used to make lawn culture on Mueller Hinton agar plates. Ceftazidime (30 μg) and ceftazidime + clavulunic acid (20 μg + 10 μg) were placed at the distance of 20mm from center to center. Plates were incubated at 37°C overnight. An enhanced zone of inhibition towards the ceftazidime + clavulunic acid disc was considered as a positive result.

2.2.3. Modified three dimensional test[23]

Fresh overnight growth from Muller Hinton Agar (MHA) was transferred to a micro centrifuge tube which was sterile and pre-weighed. To determine the weight of bacterial mass, the tube was weighed again. The bacterial mass was suspended in peptone water and pelleted by centrifugation at 3000rpm for 15 minutes. Repeated freeze thawing of bacterial pellet was performed for approximately 10 cycles to obtain crude enzyme extract. Lawn culture of *E.coli* ATCC 25922 was prepared on MHA plates with cefoxitin (30 μg) disk placed on the plates. Using sterile a surgical blade, linear cuts of 3cm were cut at a distance of 3mm away from cefoxitin disk. A small circular well was made at the other end of the slit where enzyme extract was loaded. The extract loaded in the well amounted to 30-40μl and was loaded at an increment of 10μl. The liquid was allowed to dry by positioning the plates upright for 5-10 minutes following which they were incubated at 37°C for 24 hrs. An enhanced growth of the surface organism at the point of slit insertion into the zone of inhibition of cefoxitin was recorded as positive test and interpreted as evidence for Amp C ß lactamases presence. Three types of results were recorded. Isolates exhibiting clear distortion of the zone of inhibition were taken as Amp C producers. Isolates exhibiting no distortion were taken as non- Amp C producers whereas isolates exhibitng minimal distortion were taken as indeterminate strains.

2.2.4. AMP C disk test[24]

In this test, a lawn culture of *E.coli* ATCC 25922 was prepared on MHA plate. Sterile disk (6mm) moistened with sterile saline (20μl) was inoculated with several colonies of the test organism. The disk was then placed beside a cefoxitin disk (almost touching) on the inoculated plate with the inoculated side facing the agar. The plates were incubated overnight at 37°C. A flattening or indentation of the cefoxitin zone of inhibition in the vicinity of the test disk was recorded as positive test whereas an undistorted zone was recorded as negative test.

2.3. Antibiotic susceptibility test

The antibiotic discs used in the study were ordered from HiMedia, Mumbai and are shown in Table 2. *K.pneumoniae* ATCC 700603 and *Escherichia coli* ATCC 25922 were used as positive and negative controls respectively.

Antimicrobial susceptibility of all the isolates was performed using Kirby-Bauer disc diffusion method as per NCCLS guidelines for ceftriaxone, ceftazidime, Imipenem, Amikacin, gentamycin, chloramphenicol and ciprofloxacin.[25]

The criteria shown in Table 3 was used for deciding an organism as an ESBL producer, inducible Amp C producer or a derepressed mutant.[26]

3. Results

The present study was carried out in 250 gram negative consecutive and non-repetitive isolates obtained from various clinical samples in the Microbiology laboratory of

Table 1: Identification of organisms from various biochemical tests [20]

Biochemical test	Escherichia	Edwardsiella	Citrobacter	Salmonella	Shigella	Klebsiella	Enterobacter	pseudomonas	Proteus	Morganella	Providencia
Motility	+	+	+	+	-	-	+	+	+	+	+
Gas from glucose	+	+	+	+	-	+	+	-	D	+	+
Acid from sucrose	D	-	D	-	-	+	+	-	D	-	D
Growth in KCN	-	-	+	D	-	+	+	-	+	+	+
Indole	+	+	D	-	D	-	-	-	D	+	+
MR	+	+	+	+	+	-	-	-	+	+	+
VP	-	-	-	-	-	+	+	-	-	-	-
Citrate	-	-	+	+	-	+	+	-	D	D	D
H2S	-	+	+	+	-	+	-	-	+	-	-
Urease	-	-	-	-	-	+	D	-	+	+	D
Phenylalanine deaminase (PPA)	-	-	-	-	-	-	-	-	+	+	+
Arginine dehydrolase	D	-	D	+	-	-	D	+	-	-	-
Lysine decarboxylase	+	+	-	+	-	D	D	-	-	-	-
Ornithine decarboxylase	D	+	D	+	D	-	+	-	D	+	-

*D implies that results varied in various species or strains Important exceptions: 1) S.typhi – no gas production from sugars; 2) Sh.sonnei – late fermentation of lactose and sucrose

Table 2: Various antibiotic discs used in the present study (Himedia, Mumbai)

S. No.	Antibiotic disc
1.	Cefotaxime - $30\mu g$
2.	Ceftazidime - $30\mu g$
3.	Cefoxitin - $30\mu g$
4.	Ceftriaxone - $30\mu g$
5.	Clavulunic acid - $10\mu g$
6.	Ceftazidime + clavulunic acid – $20/10\mu g$
7.	Co – trimoxazole – $1.25/23.75\ \mu g$
8.	Gentamycin - $10\mu g$
9.	Amikacin - $30\mu g$
10.	Ciprofloxacin - $5\mu g$
11.	Imipenem - $10\mu g$
12.	chloramphenicol - $30\mu g$

Table 3: Criteria for deciding the role of organism in production of lactamases

Category	Criteria
ESBL producer	1. zone diameters for various 3rd generation Cephalosporins as mentioned above 2. susceptibility to cefoxitin 3. increase in zone size with addition of an inhibitor by $>/= 5mm$
Inducible AmpC producer	1. blunting zone towards inducer 2. no increase in zone size with addition of an inhibitor 3. susceptible to Cefepime
Derepressed mutants	1. resistant to cefoxitin and cefotaxime 2. no increase in zone size with addition of an inhibitor
Multiple mechanisms	1. resistant to cefoxitin 2. blunting of zone towards inducers 3. Increase in zone size with addition of an inhibitor by $>/= 5mm$.

Table 4: Various clinical specimens included in the study

Clinical specimens	Total number	Percentage
Urine	124	49.6
Sputum	48	19.2
Exudate / pus / swab	40	16.0
Stool	26	10.4
Blood	12	4.8
Total	250	100

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Number of isolates of each clinical specimen collected is shown in Table 4. Out of all the specimens, 49.6% were from urine, and the rest 19.2%, 16.0%, 10.4%, 4.8% were from sputum, exudate / pus / swab, stool and blood respectively.

Table 5 shows the number of isolates that were resistant to third generation cephalosporins. A total of 138/250 (55.2%) of the isolates were found to be resistant with maximum resistance seen in 47/70 (67.1%) of *Klebsiella* followed by 46/76 (60.5%) of *E.coli.*

Table 6 shows the number of ESBL producers and non-producers among resistant isolates. A total of 66 (47.8%) of the 138 third generation cephalosporin resistant isolates were found to be ESBL producers. Out of which, 34/47 (72.3%) of *Klebsiella* and 25/46 (54.3%) of *E.coli* isolates resistant to third generation cephalosporins showed ESBL production.

Table 7 shows the number isolates that were resistant tocefoxitin. A total of 58 (23.2%) of the total 250 isolates

were found to be cefoxitin resistant and were considered as "SCREEN POSITIVE". These screen positive isolates were further subjected to detection of AmpC β-lactamases. These included mainly *E.coli* (36.8%) and *Klebsiella* (28.6%) isolates.

Table 8 shows the number of Amp C producers and non-producers among the screen positive isolates which were resistant tocefoxitin. Out of all the isolates, 60.3% of the screen positive cefoxitin resistant isolates were found to be Amp C producers by 3 – dimensional extract test and Amp C disk test. Both the tests showed concordant results. Only *E.coli* and *Klebsiella* were present among Amp C producing isolates.

Tables 9 and 10 shows the antibiotic sensitivity pattern of all the gram negative isolates obtained from the clinical samples. Of all the antibiotic groups, maximum sensitivity was observed for Imipenem followed by Amikacin and Ciprofloxacin. Among isolates, maximum sensitivity was shown by *E.coli* and *Klebsiella* isolates. Maximum resistance was shown towards Cotrimoxazole followed

Table 5: Isolates resistant to third generation cephalosporins

Organisms	Total number of isolates	Number resistant to third generation cephalosporins	Percentage
E.coli	76	46	60.5
Klebsiella	70	47	67.1
Pseudomonas	45	24	53.3
Proteus	12	6	50.0
Salmonella	10	4	40.0
Shigella	12	5	41.7
Enterobacter	4	1	25.0
Vibrio	9	0	0
Citrobacter	12	5	41.7
Total	250	138	55.2

Table 6: ESBL producers among those resistant to third generation cephalosporins

Organisms	ESBL producers	ESBL non producers
E.coli	25 (54.3%)	21 (45.7%)
Klebsiella	34 (72.3%)	13 (27.7%)
Pseudomonas	03 (12.5%)	21 (87.5%)
Proteus	01 (16.7%)	05 (83.3%)
Salmonella	01 (25%)	03 (75%)
Shigella	01 (20%)	04 (80%)
Enterobacter	00 (00%)	01 (100%)
Citrobacter	01 (20%)	04 (80%)
Total	66 (47.8%)	72 (52.2%)

Table 7: Cefoxitin resistance in various isolates

Organisms	Total screened	Cefoxitin resistant Screen positive	Percentage
E.coli	76	28	36.8
Klebsiella	70	20	28.6
Pseudomonas	45	06	13.3
Proteus	12	01	8.3
Salmonella	10	01	10.0
Shigella	12	01	8.3
Enterobacter	4	00	00
Vibrio	9	00	00
Citrobacter	12	01	8.3
Total	250	58	23.2

Table 8: Amp C producers in screen positive isolates

Organisms	Amp C producers	Amp C non producers
E.coli	20	8
Klebsiella	15	5
Pseudomonas	00	6
Proteus	00	1
Salmonella	00	1
Shigella	00	1
Citrobacter	00	1
Total	35 (60.3%)	23 (39.7%)

Table 9: Antibiotic sensitivity pattern

Antibiotic used	Number of sensitive organisms							
	E.coli	*Klebsiella*	*Pseudomonas*	*Shigella*	*Proteus*	*Citrobacter*	*Salmonella*	*Total*
Amikacin (30μg)	23	17	05	01	01	01	01	49 (84.5%)
Chloramphenicol (30μg)	12	02	02	00	00	00	00	16 (27.6%)
Imipenem (10μg)	28	20	00	01	00	01	01	51 (87.9%)
Cotrimoxazole (23.75μg)	02	01	01	00	00	01	00	5 (8.6%)
Gentamycin (10μg)	14	16	01	01	01	01	01	35 (60.3%)
Ciprofloxacin (5μg)	21	19	05	01	00	01	01	48 (82.8%)

Table 10: Antibiotic sensitivity pattern

	Number of resistant organisms							
	E.coli	*Klebsiella*	*Pseudomonas*	*Shigella*	*Proteus*	*Citrobacter*	*Salmonella*	*Total*
Amikacin (30μg)	05	03	01	00	00	00	00	9 (15.5%)
Chloramphenicol (30μg)	16	18	04	01	01	01	01	42 (72.4%)
Imipenem (10μg)	00	00	06	00	01	00	00	7 (12.1%)
Cotrimoxazole (23.75μg)	26	19	05	01	01	00	01	53 (91.4%)
Gentamycin (10μg)	14	04	05	00	00	00	00	23 (39.7%)
Ciprofloxacin (5μg)	07	01	01	00	01	00	00	10 (17.2%)

by Chloramphenicol. Among the isolates which exhibited maximum resistance were *E.coli* and *Klebsiella*.

4. Discussion

The present study was carried out in the Department of Microbiology, M. R. Medical College, Gulbarga during January 2011 to December 2011. In this study a total of 250 clinical isolates of gram negative bacilli were isolated from various clinical specimens and identified by standard methods.

Majority of the organisms 124 (49.6%) were isolated from urine followed by sputum 19.2%, exudates/pus/swab 16.0%, stool 10.4% and blood 4.8% in decreasing order. The most common organism isolated was *E.coli* 76 (30.4%) followed by *Klebsiella* 70 (28%). The other organisms isolated were Pseudomonas 45 (18%), Proteus 12 (4.8%), Salmonella 10 (4.0%), *Shigella* 12 (4.8%), *Enterobacter* 4 (1.6%), *Vibrio* 9 (3.6%) and *Citrobacter* 12 (4.8%).

In a study conducted by Motta RN et al.,the most frequently isolated pathogens were *K.pneumoniae* and *E.coli*. Another study conducted by Mathur P et al. had similar observations with *E.coli* and *K.pneumoniae* being

the most common organisms isolated.[27,28] In the present study also similar results were obtained with most common isolated species being the *E.coli* and *K.pneumoniae*.

All the isolates were subjected to Modified double disk approximation method for simultaneous screening of ESBL and Amp C with third generation cephalosporins (Ceftazidime, Cefotaxime), Cefoxitin and Clavulunic acid. A total of 55.2% of the isolates were found to be resistant to the third generation cephalosporins. Among the resistant isolates, maximum resistance was shown by *Klebsiella* (67.1%) followed by *E.coli* (60.5%). In a study conducted by Manchanda V et al., a total of 59% isolated were resistant to third generation cephalosporins which is similar to the observations of present study.[29] In other studies, higher resistance of 64.8% has been reported.[30]

Double Disk Synergy Test was performed to confirm ESBL production. Of all the cephalosporins resistant isolates, 66 (47.8%) isolates were positive for ESBL production. Among these, 34/47 (72.3%) of *Klebsiella* and 25/46 (54.3%) of *E.coli* isolates were ESBL producers. In a study conducted by Neelam et al., 51.2% *Klebsiella* and 40.2% *E.coli* isolates were reported to be ESBL producers, which is comparable to the observation in the present study.

Some studies such as those conducted by Ami. Y.V et al. showed a lower prevalence of 30% for ESBL producers whereas other studies as those conducted by Amita Jain et al., Singhal S et al. have shown incidence higher than 55%.[31–34]

In the present study, 23.2% of isolates were resistant to cefoxitin which is comparable to a study conducted by Singhal et al., where the resistance was reported to be 23%.[34] Another study reported by Ratna A. K. et al. reported a lower resistance of 6%.[35] In a study by Subha et al., a higher resistance was reported to cefoxitin similar to the present study.[36]

Among the resistant isolates 60.3% of the screen positive cefoxitin resistant isolates (14% of total isolates) were found to be Amp C producers by 3 – dimensional extract test and Amp C disk test, which included only *E.coli* and *Klebsiella* species. This result was similar to other studies by Lee SH et al.[37] Many studies have confirmed that Amp C β lactamases production in *Klebsiella* is plasmid mediated while in *E.coli* it may be plasmid or chromosomal mediated.[38,39]

All the cefoxitin resistant organisms were subjected to antibiotic susceptibility testing with Amikacin (30μg), Chloramphenicol (30μg), Imipenem (10μg), Cotrimoxazole (1.25/23.75μg), Gentamycin (10μg) and Ciprofloxacin (5μg). Maximum sensitivity was seen to Imipenem (87.9%) followed by Amikacin (84.5%) and Ciprofloxacin (82.8%) and maximum resistance was seen to Cotrimoxazole (91.4%) and Chloramphenicol (72.4%). A susceptibility of 100% to Carbapenems and 84.7% to Amikacin was reported in a survey conducted in Italy.[40] A study conducted by Amita Jain and Rajesh Mondal et al. reported a sensitivity of 100% to Imipenem, 45% to Chloramphenicol and 28% to Cotrimoxazole.[33]

Most of the investigators have shown 100% sensitivity to Imipenem where as in our study 7 (12.1%) organisms were found to be resistant to Imipenem which may be due to the presence of Metallo-β-lactamases along with other enzymes encoded by plasmids. Also, multiple mechanisms such as ESBL and AMP C production were prevalent in the isolates conferring resistance. Thus, an appropriate antibiotic protocol is the need of the hour in order to minimize further spread of the responsible organism.

The results of the current study and recent research articles on identification and detection of AMP C lactamases and their antibiogram were compared. It was done to analyse changes in antibiotic susceptibility trend, if any, among the AMP C lactamases over the years. In a study by Liu X (2016), a higher proportion of *Klebseilla* isolates were resistant to cefoxitin which was 47.7% compared to a 28.6% resistant isolates in our study. Among those resistant, a lower percentage of 10.8% were Amp C producers against a 21.4% in our study. Also, 85.7% were susceptible to imipenem which was lower to susceptibility of 100% observed in our study.[41] In a study by Kaur S et al. (2016), it

was observed that among the total GN isolates, 13.3% were AMP C producers which is comparable to 14% of AMP C producers in our study. Also, 14.4% of *K.penumoniae* and 7.8% of *E.coli* were AMP C producers which was much higher than 6% of *K.pneumoniae* AMP C producers and comparable to 8% of *E.coli* AMP C producers in our study. The resistance to imipenem was 10.1% which was lower than 12.1% resistance observed in our study.[42]

In a study by Koshesh M et al. (2017), it was observed that *E.coli* that were ESBL producers were 37.2% which was comparable to our study (32.9% ESBL producers) and AMP C producers were 2% which was much lower as compared to 26.3% AMP C producers in our study.[43] In a study by Ibrahim M.E. et al. (2019), it was observed that among total gram negative isolates, 27% were ESBL producers and 32.5% were AMP C producers which was comparable to 26.4% ESBL producers and lower than 39.7% AMP C producers in our study. Also, the high resistance to Cotrimoxazole observed was 90.5% which was comparable to 91.4% resistance in our study.[44]

It can be observed that more or less the trend regarding prevalence of gram negative isolates and AMP C producers in hospital based infections hasn't changed much over the years. The infections were predominated mainly by *E.coli* and *K.pneumoniae* then as well as in the current scenario. Thus, the risk of hospital acquired infections still remains a major area of concern. The antibiotic susceptibility has fluctuated little with slight decrease in susceptibility towards imipenem whereas towards other antibiotics, it hasn't changed much. Thus, there may be a need to procure newer antibiotics in future if the resistance towards current antibiotics keeps increasing and better treatment protocols need to be formulated to reduce excessive use of antibiotics and risk of hospital acquired infections.

5. Conclusion

The results of the present study showed that most commonly isolated gram negative bacilli from hospital infection samples are *E.coli* and *K.penumoniae.* Also, most of the resistance to third generation cephaloporins especially cefoxitin was seen among *E.coli* and *K.pneumoniae* isolates. The results have also shown an increasing trend of multiple drug resistance in gram negative bacilli specially extended spectrum β lactamases (ESBL's) type of resistance and also a novel type of resistance mechanism due to Amp C type of β lactamases. Some of the isolates have shown resistance to potent antibiotics like Imipenem and Meropenem.

The trend regarding prevalence of gram negative isolates and AMP C producers in hospital based infections hasn't changed much over the years, *E.coli* and *K.pneumoniae* still being most prevalent among the gram negative isolates. As the antibiotic susceptibility pattern is changing, newer antibiotics are required with an appropriate antibiotic treatment protocol in hospitals to reduce excessive use of

antibiotics and prevent spread of resistant organisms.

6. Source of Funding

None.

7. Conflict of Interest

None.

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Author biography

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