# Detection of Ampc B-Lactamase Producing Gram Negative Bacteria Isolated From Different Clinical Samples among Patients Visiting Bhaktapur Cancer Hospital

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## Abstract

The emergence and spread of antimicrobial resistance due to the production of AmpC β-lactamases by Gram negative bacteria represent clinical threat among the cancer patients undergoing chemotherapy. This cross sectional study was carried out with the aim of detecting the AmpC production by Gram negative bacteria isolated from different clinical specimens among the cancer patients during October 2015 to April 2016 at Bhaktapur Cancer Hospital. A total of 885 different clinical specimens were subjected to culture in appropriate microbiological media and incubated at appropriate temperature. The isolated Gram negative bacteria were identified by conventional biochemical tests and subjected to AmpC screening by using 30 µg Cefoxitin disc. AmpC production was confirmed phenotypically by disc inhibitory method using Phenylboronic acid supplemented Cefoxitin disc. The bacterial isolates were subjected to antibiotic susceptibility test by modified Kirby Bauer method by using CLSI guidelines. Out of 855 clinical specimens, (26.54%); 227/855, showed the bacterial growth, of which 54.6% (n=124) were Gram negative bacteria. Among Gram negative bacteria, Escherichia coli (62.1%) were the predominant organism causing infection of all types. Nitrofurantoin (74.4%) was the most sensitive followed by Imipenem (71.4%) and Amoxycillin was highly resistant (96.1%). Out of 124 Gram negative isolates, 80.6 % were MDR of which, 23 (18.5%) were AmpC producer. AmpC production was higher in females (73.91%) than in males and was statistically significant (p=0.021). AmpC production was found high among bacteria isolated from urine (87.0%) and was significantly associated (p=0.005). Within the organism type, AmpC was distributed high in E. coli (78.3%).AmpC production among Gram negative isolates among cancer patient was relatively high.

**Keywords:** Gram negative bacteria, Cancer patients, multidrug resistant, AmpC.

## Introduction

Infections due to Gram-negative bacilli are common in cancer patients during aggressive therapy. In recent years, there has been marked increase in the incidence of antibiotic resistance against Gram-negative bacilli (Eldomany and Abdelaziz 2011).AmpC β-lactamases are cephalosporinases that confer resistance to a wide variety of β-lactams including extended spectrum cephalosporins(ESCs) rum cephalosporinsand advanced spect (ASCs) thereby creating serious

therapeutic problems. AmpCβ-lactamases are produced to a greater or lesser degree by almost all Gram negative bacteria including clinically important isolates of *Citrobacter freundii, Enterobacter saerogene, E. cloccae, Morganella morganii,* and *Pseudomonas aeruginosa* and *Salmonella* with *Serratia marcescens* being the exceptions *Klebsiella*( Bradford et al1997 ; Stapletonet al1999 ).AmpC overproduction in addition to porin mutations of the outer membrane can reduce susceptibility to carbapenems, in particular in plasmid-mediated AmpC producers (Mammeri et al., 2010; Oteo et al., 2008).

To counteract the challenge of beta lactamases, β- lactams with greaterβ-lactamase stability ,including cephalosporins ,carbapenems ,and monobactams were introduced in early 1980s )Ruppeet al2006 ). The most important risk factors for resistance acquisition can be due to previous exposure to broad-spectrum antibiotics, especially the third-generation cephalosporins and carbapenems, previous use of fluoroquinolone prophylaxis, serious illness (e.g. end-stage disease, sepsis, pneumonia), nosocomial infection, prolonged hospital stay and/or repeated hospitalizations, urinary catheters, older age, intensive care unit stay. Knowledge of these risk factors and early identification in the susceptible population may help optimize empirical antibiotic therapy from the beginning (Gudiol & Carratalà 2014).

In a study carried out by Rai et al 2017, the AmpC production among Gram negative isolates was relatively low (2.7%),however in a study carried out by Baral et al 2013, AmpC production among Gram negative bacteria was found to be 27.8%.

AmpC betalactamase production isone of the major cause of antimicrobial reistance and hence it is required for continual surveillance of resistance, rapid identification of such organisms as they emerge using reliable methods, assess their potential impact on health, measure their prevalence in the hospital and community.Hence, this study was conducted with a aim to determine prevalence and the resistance pattern of clinically relevant bacteria producing AmpC β-lactamases among the cancer patients.Because there has not been extensive studies among the cancer patients.Cancer patients receives chemotherapy and thus may be predisposed to resistance.

**Methodology**

The research was hospital based cross-sectional study and conducted from October 2015 to April 2016 at Bhaktapur Cancer Hospital. Samples were collected from inpatients (admitted to different wards) and outpatients of the hospital undergoing chemotherapy. A total of855 different samples including urine(578) , pus )110(, blood(93) ,body fluids )28(, sputum(27) ,drain fluid )6(, stool(6) , vaginal swab )3(, central venous pressure catheter(CVP) tip(2) ,peripherally inserted central catheter (PICC) l ine(1) , foley's tip)1 (were processed during the study periodMid stream urine was . collected in a clean and sterile vial, pus imens collected from various sites spec following standard methods, blood samples were collected and processed by following standard methods, body fluids were collected by expert physicians and

processed accordingly, other specimens were collected( Cheesbrough2006.(

All the identified Gram negative isolates were subjected to in-vitro antibiotic susceptibility test by modified Kirby-Bauer disc diffusion method as recommended by CLSI guidelines 2009 and tested for sensitivity against Amoxycillin (10µg), Cefexime (5µg), Cefoxitin (30µg), Ciprofloxacin (5µg), Co-Trimoxazole (25µg), Imipenem (10 µg), Levofloxacin (5µg), Nitrofurantion (10µg), Norfloxacin (10 µg), Ofloxacin (5µg) and Piperacillin/Tazobactam (100/10 µg) (Cheesbrough 2006). Organisms that showed resistance to more than two classes of antibiotics were considered multi drug resistance (MDR) and further processed (CLSI, 2014).

Phenotypic detection of AmpC production was performed according to (Tan et al., 2008) by disk based inhibitory assay. AmpC producers were screened by testing the inoculated pure inoculum with the 30 µg cefoxitin disk. The zone of inhibition of ≤ 18mm around the test inoculum was considered for the AmpC production. Those test organisms screened positive were tested for confirmation of the AmpC production by utilizing Cefoxitin (30µg) against the Cefoxitin ((30µg) disk supplemented with 400 µg of phenyl boronic acid. The increased in zone of inhibition of ≥ 5 mm in boronic acid supplemented disc against cefoxitin only was detected as AmpC production.

**Results**

Two hundred and twenty seven(26. 54%) of the855 specimens showed siginificant growth, 124(54.62%) were Gram negative isolates. The highest isolation rate was on pus samples (63.63%), then on urine (23.87%). Out of 138 growths in urine, 59.42% was Gram negative organism, 48.57% of the pus infections were caused by Gram negative bacteria, 57.14% of the sputum infection, 100% of drain infections, 42.85 % of blood stream infections were caused by Gram negative bacteria. (Table1).

Table 1: Bacterial Growth in Total Clinical Samples and the Organism Pattern

| Specimen | Total no. | Growth No. (%) | Gram Negative isolates No. (%) |
|---|---|---|---|
| Urine | 578 | 138(23.87%) | 82/138(59.42%) |
| Pus | 110 | 70(63.63%) | 34/70(48.57%) |
| Sputum | 27 | 7(25.92%) | 4/7(57.14%) |
| Drain fluid | 6 | 1(16.66%) | 1/1(100%) |
| Blood | 93 | 7(7.52%) | 3/7(42.85%) |
| Body fluid | 28 | 3(10.71%) | 0(0.0%) |
| Miscellaneous | 13 | 1(7.90%) | 0(0.0%) |
| Total | 855 | 227/855(26.54%) | 124/227(54.62%) |

7 different Gram negative organism were isolated of which *Escherichia coli* (62.1%) was predominating, other organisms isolated were *K.pneumoniae* (8.1%), *K.oxytoca* (8.1%), *P.mirabilis* (5.0%), *P.vulgaris* (3.2%), *Pseudomonas* spp (4.8%), *C.freundii* (4.0%) and *C.koseri* (3.2%). *E.coli* was predominant isolate in urine and pus samples with the rate of 70.7% and 55.9% respectively, while *Klebsiella pneumoniae* (50%) was predominant in sputum specimen, *Pseusomonas* spp (100%) in drain fluid whereas *K.oxytoca*, *Proteus mirabilis* and *Citrobacter koseri* each were dominant organisms in blood specimen with the rate of 33.3% each (Table 2)

Table 2: Distribution of Gram Negative Bacteria among Different Clinical Specimens

| Organism | Specimens | | | | | | p-value |
|---|---|---|---|---|---|---|---|
| | Urine No. % | Pus No. % | Sputum No. % | Drain fluid No. % | Blood No. % | Total No. % | |
| *E coli* | 58 (70.7%) | 19 (55.9%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 77 (62.1%) | |
| *Klebsiella oxytoca* | 8 (9.8%) | 1 (2.9%) | 0 (0.0%) | 0 (0.0%) | 1 (33.3%) | 10 (8.1%) | 0.007* |
| *Klebsiella pneumoniae* | 6 (7.3%) | 2 (5.9%) | 2 (50.0%) | 0 (0.0%) | 0 (0.0%) | 10 (8.1%) | |
| *Proteus mirabilis* | 2 (2.4%) | 4 (11.8%) | 1 (25.0%) | 0 (0.0%) | 1 (33.3%) | 8 (6.5%) | |
| *Proteus vulgaris* | 4 (4.9%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 4 (3.2%) | |
| *Pseudomonas spp* | 1 (1.2%) | 4 (11.8%) | 0 (0.0%) | 1 (100.0%) | 0 (0.0%) | 6 (4.8%) | |
| *Citrobacter freundii* | 1 (1.2%) | 3 (8.8%) | 1 (25.0%) | 0 (0.0%) | 0 (0.0%) | 5 (4.0%) | |
| *Citrobacter koseri* | 2 (2.4%) | 1 (2.9%) | 0 (0.0%) | 0 (0.0%) | 1 (33.3%) | 4 (3.2%) | |
| Total | 82 (66.1%) | 34 (27.4%) | 4 (3.2%) | 1 (0.8%) | 3 (2.4%) | 124 (100%) | |

Gram negative isolates presented highest sensitive rates with Nitrofurantoin (74.4%), Imipenem (71.4%), Piperacillin/tazobactam (40.7%) and were highly resistant rate was against Amoxycillin (96%), Cefoxitin (79.8%), Cefixime (75.8%) (Table 3).

Table 3: Antibiotic Sensitivity Pattern of Gram Negative Isolates from Different Clinical Specimens

| Antibiotic used | Sensitive | | Intermediate | | Resistant | | Total |
|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % | |
| Amoxycillin(10µg) | 5 | 4.0 | 0 | 0.0 | 119 | 96 | 124 |
| Co-Trimoxazole(25µg) | 34 | 27.4 | 3 | 2.4 | 87 | 70.2 | 124 |
| Ciprofloxacin(5 µg) | 42 | 33.9 | 0 | 0.0 | 82 | 66.1 | 124 |
| Ofloxacin**)** 5µg) | 37 | 29.8 | 0 | 0.0 | 87 | 70.2 | 124 |
| Norfloxacin**)** 10µg) | 29 | 35.4 | 0 | 0.0 | 53 | 64.6 | 82 |
| Nitrofurantoin(300 µg) | 61 | 74.4 | 3 | 3.7 | 18 | 22.0 | 82 |
| Cefoxitin (30 µg) | 23 | 18.5 | 2 | 1.6 | 99 | 79.8 | 124 |
| Levofloxacin (5 µg) | 8 | 23.5 | 1 | 2.9 | 25 | 73.5 | 110 |
| Cefixime (30 µg) | 30 | 24.2 | 0 | 0.0 | 94 | 75.8 | 124 |
| Imipenem(10 µg) | 50 | 71.4 | 0 | 0.0 | 20 | 28.6 | 70 |
| Piperacillin/ tazobactam(10/100 µg) | 50 | 40.7 | 6 | 4.9 | 67 | 54.5 | 123 |

Out of 124 gram negative bacterial isolates, 80.6%isolates were MDR. The highest rate of MDR was seen in *Pseudomonas* spp (100%), *P. mirabilis* (100%) and *Citrobacter koseri* (100%) (Table 4)

Table 4: MDR Strains among Different Gram Negative Isolates

| Gram negative isolates | MDR | | Non-MDR | | Total | p-value |
|---|---|---|---|---|---|---|
| | No. | % | No | % | | |
| *Escherichia coli* | 63 | 81.8 | 14 | 17.5 | 77 | |
| *Klebsiella oxytoca* | 8 | 80.0 | 2 | 20.0 | 10 | |
| *Klebsiella pneumoniae* | 6 | 60.0 | 4 | 40.0 | 10 | |
| *Proteus mirabilis* | 8 | 100 | 0 | 0.0 | 8 | 0.070* |
| *Proteus vulgaris* | 2 | 50.0 | 2 | 50.0 | 4 | |
| *Pseudomonas* spp | 6 | 100 | 0 | 0.0 | 6 | |
| *Citrobacter freundii* | 3 | 60.0 | 2 | 40.0 | 5 | |
| *Citrobacter koseri* | 4 | 100 | 0 | 0.0 | 4 | |
| Total | 100 | 80.6 | 24 | 19.4 | 124 | |

The highest prevalence of AmpC was evaluated in *P.mirabilis* (25%), followed by *E.coli* (23.4%) among the Gram negative isolates in our study site. Twenty three of 124 (18.5%) of the total Gram negative isolates were AmpC producers. Among 23 different AmpC producing Gram negative isolates, production rate was higher in *E.coli* (n=18, 78.3%), followed by *K.oxytoca* (n=2.8.7%), *P.mirabilis* (n=2, 8.7*%), Pseudomonas* (n=1, 4.3%). (Table 5).

Table 5: Distribution of AmpC in Various Gram Negative Organisms

| Organisms | Ampc Screen | AmpC | | Total | p-value |
|---|---|---|---|---|---|
| | | Present | Absent | | |
| | No. (%) | No. (%) | No. (%) | | |
| *E.coli* | 63(81.9%) | 18(23.4%) | 59(76.6%) | 77 | |
| *K.pneumoniae* | 9(90.0%) | 0(0.0%) | 10(100%) | 10 | |
| *K .oxytoca* | 7(70.0%) | 2(20.0%) | 8(80.0%) | 10 | |
| *P.mirabilis* | 8(100%) | 2(25.0%) | 6(75.0%) | 8 | |
| *P.vulgaris* | 1(25.0%) | 0(0.0%) | 4(100.0%) | 4 | 0.370* |
| *Pseudomonas* spp | 6(100%) | 1(16.7%) | 5(82.3%) | 6 | |
| *C.freundii* | 4(80%) | 0(0.0%) | 5(100%) | 5 | |
| *C.koseri* | 3(75%) | 0(0.0%) | 4(100%) | 4 | |
| Total | 99(79.8%) | 23(18.5%) | 101(81.5%) | 124 | |

**Discussion and Conclusions**

ut ofO855 clinical samples, only227 )26.54 (%showed significant growth of organisms .A similar study by Panta et al**.,** )2013 (showed the low culture positivity of 16.3% among various samples. Out of 227 growths124 )54.62 (%were Gram negative organisms .A similar study at Tumar ,India showed 64.9% prevalance of Gram negative bacteria. Among various samples82 )66.1 (%was isolated from urine sample ,21 .8 %isolated from pus , 5.6 %from wound swab, 3.2% from sputum, 2.4% from blood, 0.8% from drain fluid. The similar study shows that the isolation of Gram negative bacteria among different specimens was27.30%, distribution of the Gram negative isolates were68.18 %from urine ,23.48from % pus, 7.95% from sputum, 2.03% from blood and0.37 %from miscelleous specimens

Sankarankutty and Kaup)2014 .(Our study was in consistant to the previous study carried at Andra Pradesh ,India ) John et al 2015).

The Gram negative organism in urine samples were *Escherichia coli* (70.7%). Our results were similar to those carried out by Sankarankutty and Kaup(2014)**,** showing the higher prevalance of *Escherichia coli* )69.44(%. *E.coli* (55.9%) infections was most predominant in pus specimens. In sputum specimen *Klebsiella pneumoniae* (50%) was isolated most. *Pseusomonas* spp (100%) was the only organism isolated from drain fluid. Only three blood specimens showed positive growth during our study, each of the isolate of *K.oxytoca* (33.33%), *P.mirabilis* and *C.koseri* was isolated. Our study was in consistence to previous studies carried out by Baral(2013) ,Dahal )1999(, Hadifar et al (2016), John et al., (2015), Lamichanne (2014), Mathaiet al) 2001(, Panta et al., (2013) and Shrestha (2011).

Imipenem with the and Nitrofurantion more than seventy susceptibility of percent. ound to be the most were fThey active drug against gram negative isolates. Among others antibiotics Piperacillin/Tazobactam,Norfloxacin, Ciprofloxacin, Ofloxacin, Co-trimoxazole and Levofloxacin were found comparatively more effective than other ticscommonly used antibio. Amoxycillin , least Cefoxitin and Cefexime with effective drugs and less suceptibility. As these antibiotics are the first line drugs which are easily hydrolysed by the bacterial enzymes and offer less in the treatment of Gram-negative bacterial ctionsinfe. Our findings was consistent to the previous studies where, Nitroflurantion was found to be the most effective drug against gram negative isolates in the study of Baral et al(2013) ,Kehl and Dowzicky (2015), Oteoet al) 2008(.

Eighty point six of the total Gram negative isolates were MDR and *Pseudomonas* spp , *Proteus mirabilis* and *Citrobacter koseri* all three were 100% MDR. 63/77 (81.8%) *E.coli* was MDR, 80% of *K.oxytoca*, 60% of *K.pneumoniae* & *C.freundii* was MDR, and 50% of *Proteus vulgaris* were MDR strains. In a study at Nepal Police Hospital, Kathmandu by Panta et al (2013), the total MDR rate was 52.20%, 60.2% of *E.coli*, 100% of *Klebsiella* spp and 33.3% of *Proteus* spp were MDR. However our study revealed higher MDR Gram negative organisms isolated from cancer patients. A similar study showed higher multidrug

resistance rate among the cancer and the machanisms of resistance (Baguley, 2010).

Our study showed *Proteus mirabilis* (2/8, 25.0%) as AmpC producer, followed by *E.coli* (18/77, 23.4%), *K.oxytoca* (2/10, 20%), and *Pseudomona*s spp (1/6, 16.7%). Among all 23 isolates producing AmpC *E.coli* (78.26%) was predominant, *K.oxytoca (*8.26%), *P.mirabilis* (8.26%) and *Pseudomonas* (4.34%). The overall prevalence of AmpC β- lactamase production among the Gram negative isolates from different clinical samples in Cancer patients was 18.5%. AmpC screening using a Cephalosporin; Cefoxitin (30 µg) showed high rate of screening but confirmatory tests showed low detection in most of the clinical isolates. The use of cefoxitin resistance as a screening agent/marker for AmpC production was quite reliable with a good negative predictive value. But some of the studies have shown that cefoxitin is a poor screening agent for AmpC production because mechanisms other than AmpC such as porin channel mutation may lead to cefoxitin resistance leading to false positive interpretation (Kaur et al., 2015). The overall AmpC production was 27.8% in a study carried by Baral et al. (2013) among Enterobacteriacea. However our study revealed lower (18.5%) AmpC production.

We can conclude that *E.coli* was predominate organism isolated in different types of clinical specimens included in our study, Gram negative isolates were highly sensitive to Nitrofurantoin followed by Imipenem and were highly resistant to Amoxycillin.Altogether, two third prevalence of MDR Gram negative bacteria were detected in clinical specimens among the cancer patients.

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