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ORIGINAL ARTICLE

Prevalence of Extended spectrum β-lactamase, AmpC β-lactamase and Metallo-β-lactamases producing multi drug resistant Gram negative bacterial isolates from wound infection

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ABSTRACT
Wound infection is one of the major hospital-acquired infection that causes a problem in the healing of wound. The present study was carried out to determine the prevalence and antimicrobial resistant pattern of gram-negative-isolates from wound infection as well as the prevalence of Extended spectrum β-lactamase(ESBL), AmpC β-lactamase and Metallo-β-lactamases (MBL) producing gram-negative isolates. A total of 98 randomly selected isolates were obtained from pus samples. 53 samples are of E.coli, 32 Citrobacter species, 13 Klebsiella species. After characterization, antimicrobial susceptibility test was performed. ESBL and MBL were detected by double disk synergy test and modified hodge test respectively. AmpC β-lactamase producing isolates were screened by cefoxitin disk diffusion method. Surgery ward have highest (89.7%) infection rate. E.coli showed highest resistance to cefoperazone and ceftriaxone (92.4%). Citrobacter spp. had 93.7% resistance to cefoperazone, ceftriaxone, levofloxacin and gentamicin. Klebsiella spp. demonstrated to have 100% resistance to cefoperazone, levofloxacin Incidence of Multi Drug Resistance was found to be highest in Citrobacter spp. (56.2%) followed by Klebsiella spp.(53.8%) and E.coli (26.4%). E.coli had 14(26.4%) ESBLs, 10(18.8%) MBLs and 4(7.5%) AmpC β lactamase producing isolates. In Citrobacter spp. 1(3.1%), 8(25%) and 10(31%) were ESBLs, MBLs and AmpC β lactamase producing isolates respectively. While Klebsiella spp showed 1(7.6%) ESBLs, 5(38.4%) MBLs and 2(15.3%) AmpC β lactamases. A total of 39.8% MDR isolates were identified. Of the total 98 isolates 16 (16.3%) were ESBL, 23(23.4%) were MBL and 16(16.3%) were AmpC BL. The high prevalence of the multidrug-resistant and β- lactamase producing isolates in the hospitals emphasizes the need for strict guidelines for the antibiotic therapy to reduce the increasing burden of antibiotic resistance.

Keywords: *Antimicrobial resistance, AmpC β-lactamases (AmpC BL), Extended spectrum β-lactamase (ESBL), Metallo-β-lactamases (MBL) and Multidrug resistance (MDR).*

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INTRODUCTION

Wound infection, through which wound healing is delayed, causes herination, wound dehiscence and wound breakdown [1]. Infection occurs when one or more contaminants evade the host defenses, multiply and damage the host's tissues [2]. The most common organisms are *Staphylococcus aureus,*, *Pseudomonas aeruginosa, Escherichia coli, Klebsiella* species and *Acinetobacter* species [3,4]. Due to the problem of drug resistance, wound infections have not utterly stop [5]. Uses of antibiotics on large scale, together with the length of time, have leads to major problems for drug resistance organisms and causing morbidity and mortality [6]. Many bacteria produced beta-lactamase enzymes, also called as penicillinase which confers resistance to β-lactam antibiotics such as carbapenems (ertapenem), penicillins and cephamycins. Gram negative organisms secrete β-lactamase enzymes, when antibiotics are present in the environment [7]. ESBL producing bacteria inactivate narrow and extended spectrum of cephalosporins, penicillin and aztreonam. It is an important mechanism of resistance in Enterobacteriacaeae [8]. Metallo-β-lactamases capable to hydrolyze a wide variety of β-lactam antibiotics, eg. carbapenems, penicillins and

cephalosporins [9]. The world wide spread of acquired MBL producers results a major problems in *Pseudomonas* spp., *Acinetobacter* spp. and members of Enterobacteriaceae [10]. AmpC type beta-lactamases are mostly occurs in gram-ve bacteria and are resistant to penicillins, extended-spectrum cephalosporins, cephamycins and monobactams [11].The present study was carried out with an aim to determine the prevalence and antimicrobial resistant pattern of gram negative isolates from wound infection in a tertiary care hospital and to determine the multidrug resistant in gram negative isolates of wound infection. These MDR positive gram negative isolates were further detected for ESBL, AmpC BL and MBL producers.

MATERIAL AND METHODS

Clinical isolates: This study was carried out in the Department of Microbiology, J.N. Medical College, Aligarh Muslim University, Aligarh. A total of 98 isolates were separated from randomly selected pus of patients during a period of 4 months from January- May 2016. These isolates were identified by biochemical test.

Antimicrobial susceptibility test (AST) was performed by Kirby-Bauer disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines [12]. Antibiotic disks were used from Hi Media Laboratories Pvt. Ltd, India. Amikacin (30µg) cefepime (30µg), cefoperazone (75µg), cefixime (5µg), cefoperazone sulbactam (75/30µg), ceftriaxone (30µg), piperacillin tazobactam(100/10µg), levofloxacin (5µg), gentamicin (10µg), piperacillin (100µg), imipenem (10µg), tigecycline (15µg) ,tobramycin(10 µg) and chloramphenicol(30 µg) antibiotic disks were used for *Escherichia coli, Klebsiella* spp., and *Citobacter* spp.

Detection of ESBL producers: Phenotypic test by double disk synergy test (DDST) was used by piperacillin/tazobactam disks [13]. In this method inoculums containing test organism of 0.5 Mc Farland turbidity, were streaked onto a Muller-Hinton agar plate with the help of swab sticks. Piperacillin/tazobactam disk (100/10 µg) was placed in the centre of the plate whereas disks containing cefotaxime (30µg) and ceftazidime (30µg) were placed 20 mm away from central disk of piperacillin-tazobactam and incubated overnight at 37ºC. If the zone size around the test antibiotic disk increases towards the piperacillin-tazobactam disk then the test organism is considered to be ESBL producers.

Detection of MBL producers: Modified Hodge test (MHT) was used to detect the MBL producers [14]. Inoculum of *Escherichia coli* (ATCC 25922),equivalent to the 0.5 McFarland turbidity standard, was used to inoculate the surface of a Muller-Hinton Agar plate, by swabbing with a sterile cotton swab and the test strain was heavily streaked from centre to periphery. After the 15min incubation at room temperature, a 10µg imipenem disk was placed at the centre, and the plate was incubated overnight. The presence of distorted inhibition zone (cloverleaf) was interpreted as positive results for carbapenem hydrolysis and the strains were phenotypically confirmed for MBL production.

Screening for AmpC βLactamase producers: In this method inoculums containing test organism, of 0.5 Mc Farland turbidity were streaked onto a Muller-Hinton agar plate with the help of swab sticks. Cefoxitin (30µg) disk was placed in the centre of the plate and plates were incubated overnight at 37ºC. The test organism is considered resistant to cefoxitin disk.

RESULTS

A total of 98 pus isolates received by bacteriology lab of which *E.coli* was 54% followed by *Citrobacter* spp. 32.6 % and *Klebsiella* spp. 13.2%**.** Out of 98 isolates 89.7% received from surgery ward followed by 9.1% from gynecology and 1% from medicine. *E.coli* showed highly resistance to cefoperazone, ceftriaxone (92.4%) followed by levofloxacin (90.5%), cefixime (86.7%), cefepime (84.9%). *Citrobacter* spp. showed highly resistance to cefoperazone, ceftriaxone and levofloxacin, gentamicin (93.7%) followed by cefepime (87.5%). *Klebsiella* spp. showed highly resistance to cefoperazone and levofloxacin (100%) followed by amikacin, cefepime, cefoperazone/sulbactam, ceftriaxone (92.3%) (Table: 1). Multidrug resistance observed in *E.coli, Citrobacter spp., Klebsiella spp. was 2*6.4%, 56.2%, 53.8% respectively (Table: 2). *E.coli, Citrobacter* spp., *Klebsiella* spp. isolates, which were resistant to all the antibiotics used in table-1 were tested against tigecycline, tobramycin, chloramphenicol, piperacillin, imipenem antibiotic in repeat for further antimicrobial susceptibility testing. Out of the 14 MDR isolates of *E.coli,* highest resistance to tobramycin and piperacillin (92.8%) followed by imipenem (71.4%). 18 MDR isolates of *Citrobacter* spp. showed highly resistance to piperacillin (100%) followed by tobramycin (94.4%), chloramphenicol (83.3%). 7 MDR isolated of *Klebsiella* spp. had highest resistance to tobramycin and piperacillin (100%) followed by imipenem (71.4%) (Table: 3).

24 isolates of *E.coli*, 3 of *Citrobacter* spp. and 1 isolates of *Klebsiella* spp. were resistant to cefoperazone and sensitive to cefoperazone/sulbactam, and these were interpreted as ESBL producers and further

tested by DDST for confirmation. In DDST piperacillin/tazobactam disk were used for ESBL detection, where as tazobactam acted as an inhibitor. On either side of piperacillin/tazobactam disk, ceftazidime and cefotaxime were used to detected synergy. Out of suspected 28 ESBL producing isolates, 16 were tested positive for ESBL production (Table: 4)**.** Thus a total of 16.3% (16 isolates) from total 98 originally obtained isolates were confirmed ESBL producers (Table: 5). Out of these 16 ESBL producers, 14 isolates from *E.coli*, 1 from *Citrobacter* spp and 1 from *Klebsiella* spp.

Out of 39 MDR isolates, 23(58.9%) imipenem resistant isolates were found. These isolates were interpreted as MBL producers. Out of these 23 isolates 10 were of *E.coli,* 8 of *Citrobacter* spp. and 5 of *Klebsiella* spp. These isolates were then confirmed by Modified Hodge test using imipenem disk and all the 23 isolates were confirmed positive by Modified Hodge test. (Table: 4). Thus a total of 23(23.5%) isolates from total 98 isolates were MBL producers (Table: 5).

Imipenem sensitive isolates were the suspected AmpC β lactamase producers. Out of 39 MDR isolates 16 were imipenem sensitive thus interpreted as AmpC β lactamase producers. 4 isolates of *E.coli* (28.5%), 10(55.5%) isolates of *Citrobacter* spp. and 2(28.5%) isolates of *Klebsiella* spp. were sensitive to imipenem and these were and then confirmed by using cefoxitin disk. All the 16(41%) isolates out of 39 isolates were found to be cefoxitin resistant, thus were confirmed AmpC producers (Table: 4). A total of 16(16.3%) from 98 isolates were AmpC producers (Table: 5).

DISCUSSION

In our study *E.coli* (54%) was most commonly identified organism from wounds followed by *Citrobacter* spp. (32.6%) and *Klebsiella* spp. (13.2%) similar to Patel et al[15] who also found that *Escherichia coli* as most offending pathogen. In our study, overall surgical wound infection rate was 89.7%. The incidence in this study is much higher than in AIIMS (24.8%)[16].Our findings are similar to Mengeesha et al[17] who reported a 75% post operative wound infection. In the present study *E.coli* were 92.4% resistant to cefoperazone, 66% to piperacillin/ tazobactam and 58.4% to amikacin. *Kebsiella* spp. were found highly resistant to cefoperazone and levofloxacin (100%), 92.3% resistant to amikacin, cefepime, cefoperazone/ sulbactam, and 84.6% to piperacillin/tazobactam. While the *Citrobacter* spp. were found 93.7% resistant to cefoperazone, ceftriaxone, levofloxin and gentamicin , 84.3% resistant to amikacin, 75% resistant to piperacillin/tazobactam. But Neelima et al. [18] found that most of the Enterobacteriaceae members were susceptible to amikacin, 3rd generation cephalosporins and pipercillin with tazobactam. In our study total 39/98 (39.8%) multidrug resistant isolates were identified which were very less as reported by Mengesha *et al*[17]. The incidence of MDR was found to be highest in *citrobacter* spp. (56.2%) followed by *Klebsiella* spp.(53.8%) and *E.coli* (26.4%) but 55.3% *E.coli* was found to be MDR according to Banjara et al[19]. In our study prevalence of ESBL producers was 16/98 (16.3%) but 2.6 % was reported in Zenica-Doboj Canton, Bosnia and Herzegovina[20]. In our study analysis ESBL's were predominantly present among *E.coli* 14/53(26.4%) then *Klebsiella* spp. 1/13(7.6%) and 1/32(3.1%) in *Citrobacter* spp. It is similar to the finding of Kumar *et. al.*[21] where *E.coli* 63.7% predominantly has ESBL producers. The study was contradictory to the Kamberovic and Sestic[20] who reported *Klebsiella* spp as a predominating ESBL producing micro-organism.

In the present study 39 MDR isolates were tested for MBL production. 23/39 (58.9%) were impenem resistant and suspected to be MBL producers. These isolated were further confirmed by modified hodge test and all the 23 impenem resistant isolates were found positive as MBL producers. Out of the three organisms, *Klebsiella* spp. 5/13(38.4%) was present in highest percentage*, Citrobacter* spp. 8/32(25%), *E.coli* 10/53(18.8%) were MBL producers which is similar to the finding of Swetha *et al.*[22] who reported 37% *Klebsiella pneumonia* in highest rate when compared with 32% in *E.coli* and 7% in *Citrobacter frundii*. A total of 23out of the 98 isolates were MBL's 23/98 (23.4%) which is slightly higher than the finding of Loveena et al[23] in the intensive care unit of Panjab, India.

In this study, of 39 MDR isolates, 16/39 (41%) were imipenem sensitive thus suspected for AmpC β lactamase producing isolate and further tested with cefoxitin disk and all were found positive. *Citrobacter* spp. 10/32 (31.2%), *Klebsiella* spp.2/13 (15.3%) and *E. coli4/53* (7.5%) resistant to cefoxitin and were AmpC β- lactamase producers. But 24.1% of *Klebsiella* spp and 37.5% of *E.coli* were found to be AmpC β -lactamase producer isolates have been reported from Chennai[24]. From the total 98 isolates 16 /98 (16.3%) were confirmed AmpC β-lactamase producers (Table:5). Singhal et al.[25] found 22 isolates (8%) positive for AmpC β-lactamases production of the 272 total isolates. In another screening report from Kolkata hospital also found (8%) AmpC β-lactamase producers i.e. 23/284 nonclinical isolates[26].

The high prevalence of β-lactamase producing organisms emphasizing the need for early detection of antibiotic sensitivity against the isolated organism which helps them in providing the appropriate antimicrobial therapy and avoiding the use of inappropriate antimicrobial drugs as a trial. This will

prevent the development of multidrug resistant strains. It leads to cost effective treatment and reduces the adverse consequences of the antimicrobial use.

Table: 1 Antimicrobial resistant pattern of the *E.coli, Citrobacter* species, *Klebsiella* species

Antibiotics	*E.coli* (n=53)	*Citrobacter specie.* (n=32)	*Klebsiella species* (n=13)
Amikacin	31(58.4%)	27(84.3%)	12(92.3%)
Cefepime	45(84.9%)	28(87.5%)	12(92.3%)
Cefoperazone	49(92.4%)	30(93.7%)	13(100%)
Cefixime	46(86.7%)	26(81.2%)	11(84.6%)
Cefoperazone/ Sulbactam	25(47.1%)	27(84.3%)	12(92.3%)
Ceftriaxone	49(92.4%)	30(93.7%)	12(92.3%)
Piperacillin/Tazobactam	35(66%)	24(75%)	11(84.6%)
Levofloxacin	48(90.5%)	30(93.7%)	13(100%)
Gentamicin	38(71.6%)	30(93.7%)	11(84.6%)

Table: 2 Multidrug resistant isolates in *E.coli, Citrobacter* species & *Klebsiella* species

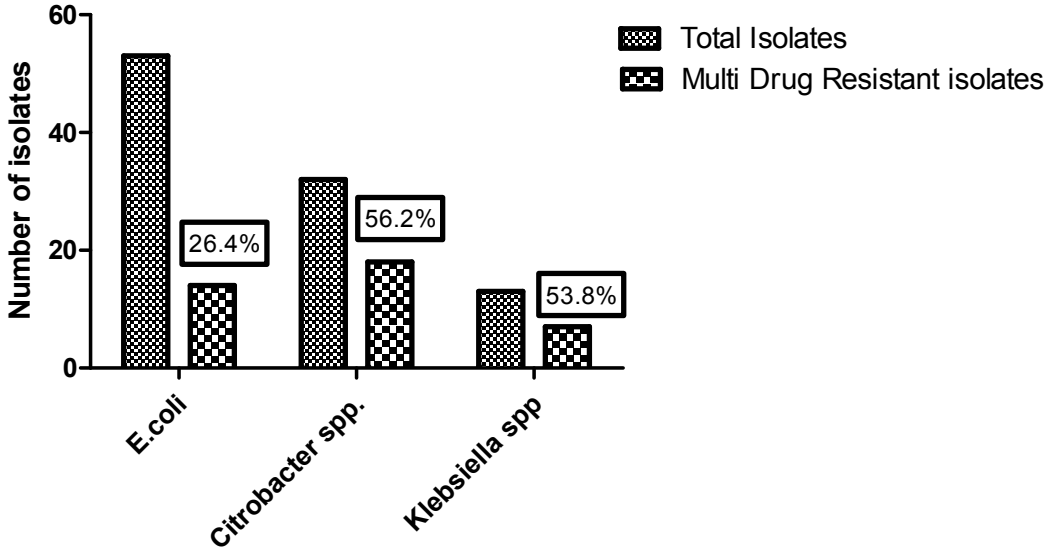


Table: 3 Resistance pattern of MDR isolates of *E.coli, Citrobacter* species, *Klebsiella* species.

Antibiotics	***E.coli* (n =14)**	***Citrobacter species* (n=18)**	***Klebsiella species* (n=7)**
Tigecycline	2(14.2%)	6(33.3%)	1(14.2%)
Tobramycin	13(92.8%)	17(94.4%)	7(100%)
Chloramphenicol	5(35.7%)	15(83.3%)	4(57.1%)
Piperacillin	13(92.8%)	18(100%)	7(100%)
Imipenem	10(71.4%)	8(44.4%)	5(71.4%)

Table: 4 Detection of ESBL producers (DDST), MBLproducers (MGT) and AmpC BL producers (CDT)

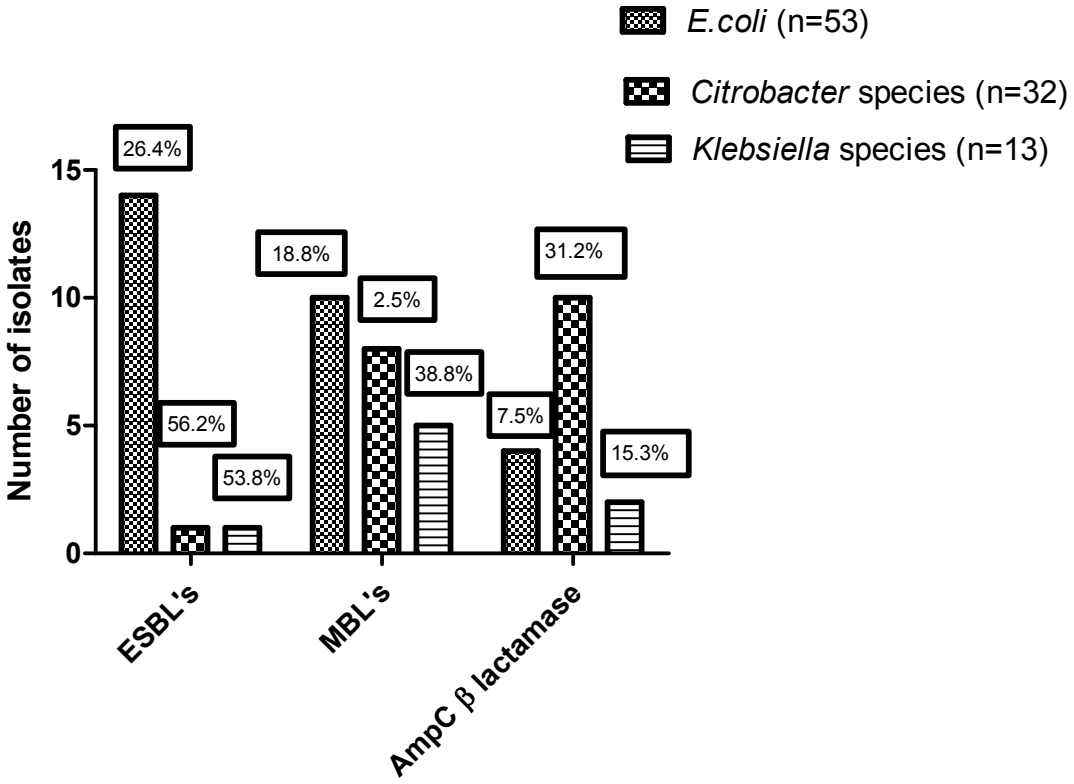
Name of Micro-organism	**Double Disk Synergy test Positive (ESBL producers)**	**Name of Micro-organism**	**Modified Hodge test Positive (MBL producers)**	**Cefoxitin disk Positive (AmpC BL producers)**
***E.coli* (n=24)**	14(58.3%)	***E.coli* (n=14)**	10(71.4%)	4(28.5%)
***Citrobacter* species (n=3)**	1(33.3%)	***Citrobacter* species (n=18)**	8(44.4%)	10(55.5%)
***Klebsiella* species (n=1)**	1(100%)	***Klebsiella* species (n=7)**	5(71.4%)	2(28.5%)
Total (n=28)	16(57.1%)	**Total (n=39)**	23(58.9%)	16(41%)

Table: 5 Distribution of various types of β lactamase producing isolates

(A)

Name of Microorganism	ESBL's	MBL's	AmpC β lactamase
***E.coli* (n=53)**	14(26.4%)	10(18.8%)	4(7.5%)
***Citrobacter* species (n=32)**	1(3.1%)	8(25%)	10(31.2%)
***Klebsiella* species (n=13)**	1(7.6%)	5(38.4%)	2(15.3%)
Total (n=98)	16(16.3%)	23(23.4%)	16(16.3%)

(B)



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CONFLICTS OF INTEREST

Authors have no conflict of interest.

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