Research Article

Microbiological profile of infection in intensive care unit and their antimicrobial susceptibility pattern with special reference to metallo β-lactamases and AmpC

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ABSTRACT

Background: Throughout the world multidrug-resistant healthcare-associated infections (HAI) are one of the leading causes of morbidity and mortality among hospitalized patients, leading to a major burden on public health system of any country. An intensive care unit (ICU) patient has five- to seven-folds higher risk of HAI and ICU infections contribute to 20–25% of all HAI. **Objective:** This prospective study was designed to isolate and identify the bacterial etiology, their antimicrobial susceptibility pattern and to detect the production of the metallo β-lactamases (MBL) and AmpC-β-lactamases in multidrug-resistant gram negative isolates. **Material and Methods:** The study was conducted for the period of 1 year. During the period a total 196 samples were collected and processed as per Clinical and Laboratory Standards Institute guidelines. **Result:** The majority of bacterial isolates causing HAI were found to be Gram-negative bacilli (73.68%). *Acinetobacter* species followed by *Pseudomonas aeruginosa*, *Escherichia coli, Citrobacter* species, *Klebsiella* species, and *Enterobacter* species, respectively. Gram-positive cocci were accounted 26.31% isolates. All isolates were multidrug-resistant. Among Gram negative bacilli, 38.10% AmpC producer, 35.71% MBL, and 16.67% were coproducer of AmpC and MBL. **Conclusion:** Microbiological profile of infection in ICU of our institute was multidrug-resistant, and in many isolates, drug resistance was due to the production of MBL and AmpC-β-lactamases, which represents basic information for future monitoring of HAI and could be repeated periodically. Thus, the future prevention program should focus on patients with a longer length of stay and those with invasive devices.

KEY WORDS: Healthcare-Associated Infections; metallo β-lactamases; AmpC-β-lactamases; ICU

INTRODUCTION

Throughout the world multidrug-resistant healthcare-associated infections (HAI) are one of the leading causes of morbidity and mortality among hospitalized patients, leading to a major burden on public health system of any country.[1-4] An intensive care unit (ICU) patient has five- to seven-folds higher risk of HAI and ICU infections contributes to 20% to 25% of all HAI in a hospital. Factors like increasing use of invasive devices, immunosuppressive drugs as well as the irrational use of antibiotic therapy in ICUs all are contributing for the same.[1,3] The center for disease control and Prevention, Atlanta, USA defines ICU associated infections as those that occur after 48 h of ICU admission or within 48 h after transfer from an ICU. Each HAI adds on an average extra 5–10 days to the affected patient's time in the hospital.[5,6] Antibiotic overuse and misuse partly due to incorrect diagnosis; as well as irrational and counterfeit antibiotic market combinations; and irregular consumption due to either wrong prescription or poor compliance; all contributes to the widespread drug resistance among the hospital-acquired organisms.[1,4] In

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particular, drug-resistant pathogens are a major concern, as they lead to higher morbidity and mortality and are more difficult to identify by routine laboratory assays, which can lead to a delay in diagnosis and institution of appropriate antimicrobial therapy.[7]

Among the β-lactamases, the carbapenemases especially transferrable metallo- β-lactamases (MBLs) are the most feared because of their ability to hydrolyze virtually all drugs in that class, including the carbapenems.[7] In addition to their resistance to all β-lactams, the MBL producing strains are frequently resistant to aminoglycosides and fluoroquinolones.[8] AmpC β-lactamases are well-defined enzymes with broad substrate specificity and classified as Class C according to Ambler and Group 1 by Bush-Jacoby-Medeiros.[9,10] All types of AmpC producers are equally significant and may lead to therapy failure in critically ill patients.[9,10]

The aim of present study was to identify the prevalence of predominantly isolated bacteria and their antimicrobial drug susceptibility patterns with special reference to MBL and AmpC for the patients admitted in ICU.

MATERIALS AND METHODS

The present prospective study was conducted in the Department of Microbiology, Gajra Raja Medical College. Depending on the clinical suspicion laboratory samples such as urine, sputum, pus, swab, and body fluids ascetic fluid, and pleural fluid, and blood were collected from the patients admitted for more than 48 h were included in our study. Detailed history including the name, age, sex, underlying clinical condition, date of admission to the ICU, date of indoor admission, any history of previous antibiotic intake, the treatment being administered in the ICU, and clinical outcome of each patient was noted.

Samples were collected from all patients admitted in the ICU for more than 48 h. Patients showing clinical signs of infection on or before admission or transfer to the ICUs were not included in the study.[1,3] All specimen were collected as per standard aseptic protocol and were transported to the laboratory as early possible. Gram stain preparations were made from all specimens and examined to determine the presence, type of cells, relative number of microorganisms and their morphologies.[11] All the samples were inoculated on blood agar, MacConkey agar, and chocolate agar.[12] The MacConkey plates were incubated at 37°C while blood agar and chocolate agar were incubated at 37°C in the presence of 5–10% CO_2 (carbon dioxide). Growth of 10^4–10^5 or more CFU/ml was taken as the cut-off threshold.[13,14] Samples are showing growth less than these thresholds were assumed to be due to colonization or contamination. In case of significant growth, the isolated colonies were subjected to gram stain and biochemical tests for identification.[15] Identification was carried out as per Clinical and Laboratory Standards Institute guidelines.[16]

Amp C β-lactamases detection was done by AmpC disk method [Figure 1]. A positive test appeared as flattening or indentation of the cefoxitin inhibition zone in the vicinity of the test disk.[17] MBL detection was done by imipenem-EDTA combined disk method [Figure 2]. If the increase in inhibition zone with the imipenem and EDTA disk was ≥7 mm than the imipenem disk alone, it was considered as MBL positive.[18] Methicillin-resistant *Staphylococcus aureus* (MRSA) detection was done by the cefoxitin disk diffusion method. If the inhibition zone around the cefoxitin disk was >22 mm then the isolate was considered MSSA and if the zone was <21 mm then it was considered as MRSA.[16]

RESULTS

During the period of 1 year, a total of 196 specimens includes 87 urine, 68 blood, 30 sputum and tracheal aspirate, 4 pus, 3

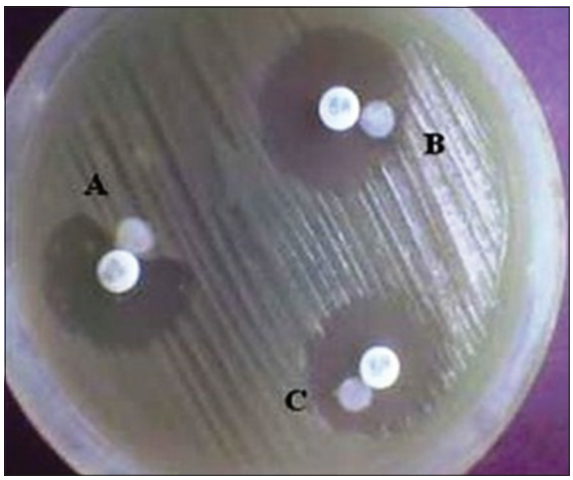


Figure 1: AmpC disk test with three isolates being tested on a 90-mm plate. A - positive test appeared as flattening or indentation of the cefoxitin inhibition zone in the vicinity of the test disc, (B and C) - negative test

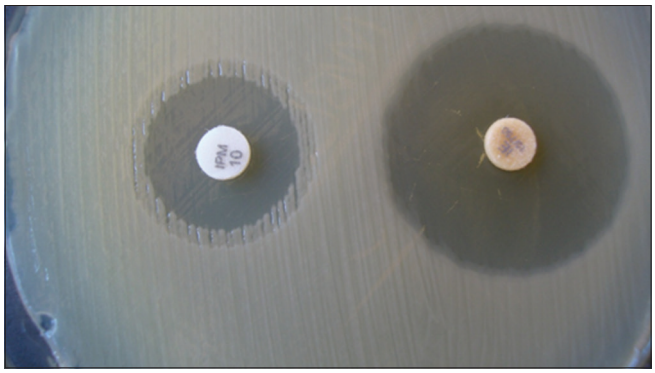


Figure 2: Metallo β-lactamases detection by imipenem-EDTA combined disk method

ascitic fluid, 2 pleural fluid, and 2 liver abscess were processed. Out of these, culture was positive in 57 cases [Table 1]. The incidence of HAI in ICU in our study was 29.08%. The most frequent culture positive in relation to age was noted in the age group of 21–30 years followed by 31–40, 51–60, 41–50, 61–70, 71–80, 11–20, and 81–90 years, respectively. The mean age of the culture positivity was 42.33 years. We also found the relationship between culture positivity and sex. The prevalence rate was higher in male (59.18%) patients compared with females (40.82%) [Table 2]. Overall the ratio of male to female was found to be 2.35:1 whereas specimen wise ratio was 2:1, 2.17:1, and 2.25:1 in sputum and tracheal aspirate in combination, blood, and urine culture, respectively [Table 3].

The higher rate of HAI was 39 (68.42 %), which could be attributed to the use of invasive devices such as urinary catheter, intravenous cannula, central venous pressure catheter, and mechanical ventilator support.

Specimen wise distribution of the identified blood culture isolates were found to be *Citrobacter koseri* (5), *Staphylococcus aureus* (3), *Pseudomonas aeruginosa* (3), and isolate of *Staphylococcus saprophyticus* (1), coagulase negative staphylococcus (1), *Acinetobacter baumannii* (1), *Escherichia coli*(1), *Klebsiella pneumoniae* (1), *Klebsiella oxytoca* (1), *Enterobacter aerogenes* (1), and *Citrobacter freundii* (1) respectively. In the urine culture isolates most frequent isolate were found to be *Escherichia coli* (5) followed by *Staphylococcus aureus* (4), *Acinetobacter baumannii*(3), *Acinetobacter lwoffii* (2), coagulase negative *staphylococcus* (2), *Staphylococcus saprphyticus* (1), *Pseudomonas aeruginosa* (1), *Klebsiella oxytoca* (1), Enterobacter aerogenes (1) and *Citrobacter freundii* (1) respectively. In the sputum culture, *Pseudomonas aeruginosa* (3) followed by *Acinetobacter baumannii* (3), *Staphylococcus aureus* (3), *Escherichia coli* (1) and *Enterobacter aerogenes* (1) respectively. In pus and liver abscess *Staphylococcus aureus* (1) followed by Pseudomonas aeruginosa (1), *Acinetobacter baumannii* (1) and *Escherichia coli* (1) respectively, which is depicted in Table 4.

The antimicrobial susceptibility testing, AmpC and MBL detection were performed for all identified isolates asper CLSI guideline. In the antimicrobial susceptibility testing of Gram-negative bacteria, highest resistance observed against ampicillin, cefotaxime, cefoxitin, and norfloxacin. Among Gram-negative bacteria, doxycycline and nitrofurantoin were found to be most effective antibiotics. Among Gram-positive organisms, cephalexin showed 100% resistance followed by amoxicillin/clavulanic acid combination and cefoxitin. The most effective antibiotic was found to be norfloxacin, vancomycin, and doxycycline.

Three strain of *Staphylococcus aureus* showed resistance against oxacillin (1µg disc) indicating presence of MRSA, and one of the isolate showed resistance against vancomycin (30 µg disc) indicating it was VRSA strain. Out of the 57 isolates, 20(35.09%) isolates were produced AmpC-β-lactamases. The highest prevalence rate of AmpC production was detected among *Acinetobacter baumannii* 7(87.5%), followed by *Klebsiella pneumoniae*, *Enterobacter aerogenes*, and *Escherichia coli* respectively. A total of 15(26.32%) isolates were metallo-β-lactamases producer. *Pseudomonas aeruginosa* 7 (77.78%) showed higher rate of MBL production, followed by *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *Acinetobacter lwoffii* respectively. A total of 50% of *Acinetobacter*

Table 1: Culture positivity rate among various specimens

Type of specimen	*n* (%)	
	No. of specimen	Culture positive
Blood	68 (34.69)	19 (27.94)
Urine	87 (44.39)	21 (24.14)
Sputum and tracheal aspirate	30 (15.31)	13 (43.33)
Pus	4 (2.04)	3 (75)
Ascitic fluid	3 (1.53)	0
Pleural fluid	2 (1.02)	0
Liver abscess	2 (1.02)	1 (50)
Total	196	57 (29.08)

Table: 2. Age and sex wise distribution of specimens

Age groups (in year)	*n* (%)		Total number of patient
	Male	Female	
11–20	8 (66.67)	4 (33.33)	12
21–30	27 (47.37)	30 (52.63)	57
31–40	17 (50)	17 (50)	34
41–50	17 (60.71)	11 (39.29)	28
51–60	19 (65.52)	10 (34.48)	29
61–70	14 (77.78)	4 (22.22)	18
71–80	11 (73.33)	4 (26.67)	15
81–90	3 (100)	0	3
Total	116 (59.18)	80 (40.82)	196

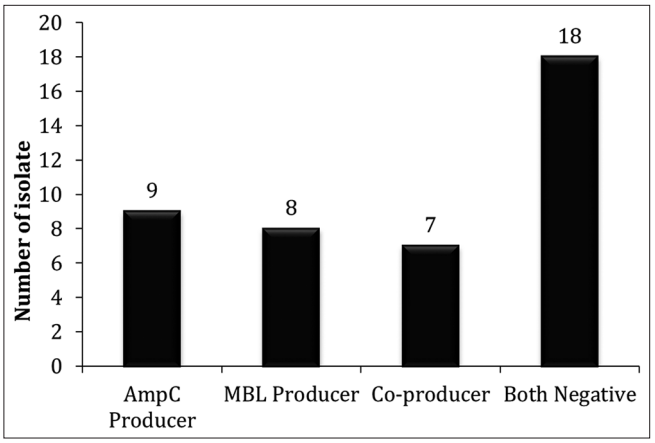
Table: 3. Incidence of HAI in males and female

Age group (in year)	*n* (%)		Total No.
	Male	Female	
11–20	3 (100)	0	3
21–30	11 (50)	11 (50)	22
31–40	9 (90)	1 (10)	10
41–50	5 (71.43)	2 (28.57)	7
51–60	3 (60)	2 (40)	5
61–70	3 (75)	1 (25)	4
71–80	5 (100)	0	5
81–90	1 (100)	0	1
Total	40 (70.18)	17 (29.82)	57

Table: 4. Distribution of the specimen and identified isolates

Isolate	*n* (%)				
	Blood	Urine	Sputum and tracheal aspirate	Pus	Liver abscess
Staphylococcus aureus	3 (15.78)	4 (19.05)	1 (7.69)	1 (33.33)	0
Staphylococcus epidermidis	0	0	1 (7.69)	0	0
Staphylococcus saprophyticus	1 (5.26)	1 (4.76)	0	0	0
Coagulase negative Staphylococcus	1 (5.26)	2 (9.52)	0	0	0
Pseudomonas aeruginosa	3 (15.78)	1 (4.76)	4 (30.77)	1 (33.33)	0
Acinetobacter baumannii	1 (5.26)	3 (14.29)	3 (23.08)	1 (33.33)	0
Acinetobacter lwoffii	0	2 (9.52)	0	0	0
Escherichia coli	1 (5.26)	5 (23.81)	1 (7.69)	0	1 (100)
Klebsiella pneumonia	1 (5.26)	0	2 (15.38)	0	0
Klebsiella oxytoca	1 (5.26)	1 (4.76)	0	0	0
Enterobacter aerogenes	1 (5.26)	1 (4.76)	1 (7.69)	0	0
Citrobacter freundii	1 (5.26)	1 (4.76)	0	0	0
Citrobacter koseri	5 (26.32)	0	0	0	0
Total (57)	19 (33.33)	21 (36.84)	13 (22.81)	3 (5.26)	1 (1.75)

S. aureus: Staphylococcus aureus, S. epidermidis: Staphylococcus epidermidis, S. saprophyticus: Staphylococcus saprophyticus, P. aeruginosa: Pseudomonas aeruginosa, A. baumannii: Acinetobacter baumannii, A. lwoffii: Acinetobacter lwoffii, E. coli: Escherichia coli, K. pneumonia: Klebsiella pneumonia, K. oxytoca: Klebsiella oxytoca, E. aerogenes: Enterobacter aerogenes, C. freundii: Citrobacter freundii, C. koseri: Citrobacter koseri, CONS*:* Coagulase-negative *Staphylococcus*



Graph 1: Distribution of AmpC, MBL, and AmpC/MBL coproducer (*n*=42)

baumannii, 33.33% of *Klebsiella pneumoniae* and 22.22% of *Pseudomonas aeruginosa* were reported as co-producer of AmpC and MBL [Graph 1].

DISCUSSION

HAI are one of the leading causes of morbidity and mortality among hospitalized patients, leading to a major burden on public health system of any country.[1-4] In our study, the incidence of HAIs in medical ICU was found to be 29.08%. The rate of HAIs nearly similar to our study was reported in a study by Deep A and Ghildiyal R (2004) from Hoshiarpur, Punjab. The author had been reported 27.38% incidence rate of HAIs. The most common organism isolated was *Klebsiella* spp. (33.33%) followed by *E. coli* (16.7%).[5] Bhandari *et al.* from Kathmandu, Nepal, had 60.88% of HAIs rate in ICU patients at National Institute of Neurological and Allied Sciences, Nepal.[19] In a study by Zaveri *et al*. the infection rate among ICU patients due to various organism was 31.33%, the predominantly isolated organism was *E. coli* (25%), coagulase-negative *Staphylococci* (16.4%), *Acinetobacter* spp. (15.62%), *Klebsiella* spp. (14.06%), and *Pseudomonas* spp. 13.28%.[1]

In our study, predominantly isolated organism in a specimen of medical ICU patients was *Acinetobacter* spp. 17.54% (*A. baumannii* - 14.04% and *A. lwoffii* - 3.51%) followed by *Staphylococcus aureus* and *Pseudomonas aeruginosa* 15.79% each, *Escherichia coli* 14.04%, *Citrobacter* spp. 12.28% (*C. freundii*-3.51% and *C. koseri*-8.77%), CONS 10.53%, *Klebsiella* spp. 8.77%, and *Enterobacter* spp. 5.26%. In our study, it was observed that distribution of HAIs in MICU was urinary tract infections (36.84 %), bloodstream infections (33.33 %), pneumonia (22.81 %), and other (7.02 %). Our study was nearly in concordance with the similar study by Mythri and Kashinath.[20] Raheja *et al.*[21] had reported 8% of nosocomial bloodstream infection at PGIMS Rohtak. Similarly, Pratham *et al*.[22] reported 10.93% of incidence, and higher incidence of 28% was quoted in a study by Ginawi *et al*.[23] Garg *et al.*[24] reported 20% of urinary tract infection among patient admitted in ICU. In similar such study by Al-Jebouri[25] quoted 28.1% and Patel and Garala[26] quoted 34.14% of nosocomial UTI. Ranjan *et al.*[27] reported 57.14% of device-related nosocomial pneumonia, in a similar study by Bhandari *et al.*[19] it was 74.3%. A similar study by Deep and Ghildiyal *et al.* cited male to female ratio of the incidence of nosocomial infection as 3:2.[5]

In the present study, a total of 196 patients admitted in the medical ICU, belongs to age between 16 and 83 years, mean

age of 42.34 years, out of 196, 57 were bacteriological proven case of HAIs, among them 40 were male and 17 were female, giving male to female ratio of 2.35:1. On Chi-square analysis male to female ratio was to be significantly associated *(P < 0.05*). Among both sexes, maximum cases were found to be in the age group of 21–30 years. Out of 57, 73.68% were Gram-negative and 26.31% were Gram-positive bacteria. In a study by Mustafa and Ahmed,[28] the antibiotic sensitivity pattern was cited that among the gram negative bacteria includes *Escherichia coli* (14), *Klebsiella pneumoniae* (22) and *Pseudomonas aeruginosa* (4), many of them were resistant to ampicillin (7.5%), gentamicin (45%), third-generation cephalosporins (ceftazidime-55%, cefotaxime-50%, and ceftriaxone-45%), and piperacillin (45%). They were moderately susceptible to Amikacin (68%) and ciprofloxacin (70%), but highly susceptible to Colistin (100%) and Meropenem (100%).

In our study, it was observed that *E. coli* shows maximum 87.5% resistance against ampicillin, ciprofloxacin, ceftazidime, norfloxacin, and ticarcillin/clavulanic acid combination, followed by 75% resistance to cefoxitin. Least resistant observed against amikacin and nitrofurantoin, and they were found to be a most effective antibiotic. Among *Klebsiella* spp., ampicillin, cefotaxime, and cefoxitin were found 100% resistant, up to 50–60% resistance observed against amikacin, ciprofloxacin, gentamicin, levofloxacin, piperacillin/tazobactam combination, antibiotics which were least resistant and most effective against *Klebsiella* spp. were doxycycline and nitrofurantoin.

Deep and Ghildiyal *et al*.[5] had reported both *Klebsiella* spp. and *Escherichia coli* were most sensitive to amikacin. It was found that *Staphylococcus aureus* was most sensitive to vancomycin (100%) and amoxicillin/clavulanic acid (76%) whereas CONS was found to be sensitive to amoxicillin/clavulanic acid combination (75%). *Pseudomonas* had maximum sensitivity to ticarcillin (52.6%) followed by amikacin and ciprofloxacin (47.3% each). *Citrobacter* spp. showed highest resistance (>70%) against ticarcillin/clavulanic acid combination, ampicillin, cefotaxime, cefoxitin, and norfloxacin. Moderate resistance (between 40 and 70%) observed against amikacin and gentamicin. Least resistance (<40%) showed against doxycycline, levofloxacin, nitrofurantoin, and amikacin. It was probably because of low use of these antibiotics.

It was observed that *P. aeruginosa* and *Acinetobacter* spp. were 100% resistant to norfloxacin and ampicillin, another antibiotic which showed a high rate of resistance against them was cefoxitin, cefotaxime, ticarcillin/clavulanic acid combination, ceftazidime, gentamicin, piperacillin/tazobactam, and cefoperazone. Least resistance showed against amikacin, doxycycline, and nitrofurantoin. Mustafa M and Ahmed SL (2014)[28] revealed that the antibiotic sensitivity pattern of Gram-positive bacteria *Staphylococcus aureus* (n=15) and CONS (n=7) showed high resistance to ampicillin (13.6%) and considerable resistance toward gentamicin (45%), they showed moderate susceptibility to third-generation cephalosporin cefotaxime 73%, ceftriaxone 68%, and cefoperazone (63%), amikacin (68%), and ciprofloxacin (63%), and they were highly susceptible to linezolid (100%) and vancomycin (95 %).

In the present study, *Staphylococcus aureus* showed maximum resistance 88.89% to azithromycin, ciprofloxacin, and linezolid. Minimum resistance was showed against vancomycin 11.11% and was indicating the presence of VRSA strain. Oxacillin showed 33.33% resistance, indicating the presence of MRSA strain in the ICU. CONS was 100% resistant to cephalexin followed by amoxicillin/clavulanic acid combination, cefoxitin, gentamicin, and azithromycin, while vancomycin and norfloxacin were found to be the most sensitive antibiotics.

Out of 42 Gram-negative isolates, 16 (38.1 %) were showed positive results by AmpC-disc test as *Amp*C-β-lactamases producer. Indentation indicating strong AmpC producer was observed in 10 isolates whereas flattening indicated weak AmpC was observed in 6 isolates. High prevalence of overall weak and strong *Amp*C-β- lactamase produced was detected in *Acinetobacter baumannii* 87.5%, followed by *Klebsiella pneumonia* 66.67%, *Escherichia coli* 37.5%, *Enterobacter aerogenes* 33.33%, and *Pseudomonas aeruginosa* 33.33%. Bhandari *et al.*[19] reported 31.28 % of isolate were AmpC producers. Moreover, the high prevalence of AmpC*β*-lactamase was detected in *Acinetobacter* spp. (29.4%) followed by *Staphylococcus aureus* (21.5%) and *K. oxytoca* (15.6%). High level of AmpC production is typically associated with the resistance to all *β*-lactam antibiotics except carbapenems and limits the therapeutic use. Bhandari *et al.*[19] cited 64.7% of prevalence of MBL producer organism. Moreover, among them 63.63 % were *Acinetobacter* spp. and the rest *Klebsiella* spp.

In a study by Ranjan N and Ranjan K P *et al* (2014)[27] observed that 66.67% *Citrobacter freundii*, 50% *Acinetobacter* spp., 33.33% *Enterobacter* spp., 26.7% *Klebsiella pneumoniae* and 22.2% *Pseudomonas aeruginosa* were the AmpC producer organism. 27.2% of *Pseudomonas aeruginosa*, 20.8% *Acinetobacter* spp. and 6.7% *Klebsiella pneumoniae* were found positive for MBL production. Out of 42 Gram-negative isolates, 15 (35.71 %) were showed positive for MBL production by imipenem-EDTA combined disc test. Increase in the zone of inhibition with the imipenem and EDTA disc was ≥ 7 mm than the imipenem disc alone, was considered as MBL producer organism. *Pseudomonas aeruginosa* 77.78% was predominantly produces MBL, followed by *Klebsiella pneumonia* 66.67%, *Acinetobacter baumannii* 62.5% and *Acinetobacter lwoffii* 50%. In our study, 50% of *Acinetobacter baumannii* was the most common coproducer of AmpC and MBL, followed by *Klebsiella pneumonia* 33.33% and *Pseudomonas aeruginosa* 22.22%.

CONCLUSION

This study represents basic information for future monitoring of HAI and should be repeated periodically. Thus, the future prevention program should focus on patients with a

longer length of stay and those with invasive devices. At the institutional level, it is urgent to establish HAI prevention programs. Elsewhere, prospective studies are desirable to describe more accurately HAI incidence as well as risk factors.

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