Prevalence of nosocomial lower respiratory tract infections caused by Multi- drug resistance pathologens

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Abstract

Introduction: Nosocomial infections caused by multi-drug resistant pathogens are major threat to the hospitalized patients. Extended spectrum beta-lactamase (ESBL) and metallo-beta-lactamase (MBL) producing bacterial strains causing hospital acquired lower respiratory tract infection are increasing in numbers. Only a limited number of studies related to MBL producers have been done in Nepal.

Objective: The goal of this study was to determine the etiology of nosocomial lower respiratory tract infections and to assess the current levels of antimicrobial resistance with special reference to ESBL and MBL producing bacterial strains.

Methods: A total of 100 specimens including sputum and endotracheal secretion from patients diagnosed of nosocomial lower respiratory tract infection were collected and processed according to the standard methodology. Combination disk method was done for the detection of ESBL and MBL producing isolates.

Results: Out of total 100 specimens, 87% was monomicrobial while the rest were polymicrobial. 96.5 % were gram negative while 3.5% were gram positive. All *E.coli*, *Klebsiella* spp and *S. aureus* were found to be MDR followed by *Acinetobacter* spp (97.2%) and *P. aeruginosa* (76.2%)

About 28.6 % of *E. coli*, 8.33% of *Klebsiella* spp and 2.4 % of *Pseudomonas aeruginosa* were ESBL producers. *Acinetobacter* spp. was not found to produce ESBL during the study. MBL was present in 17.4% of the gram negative isolates.

Conclusion: We found a high prevalence of MDR strains as a cause of nosocomial LRTI including significant proportions of ESBL and MBL producers. The rate of *Acinetobacter spp.*, including MBL producers, in our hospital setting was alarmingly high which prompts a special attention for the management of such patients as well as urgent need for implementation of infection control strategies.

Key words: MDR, LRTI, ESBL, MBL, nosocomial infection

Introduction

Nosocomial respiratory tract infections are major cause of excessive morbidity and mortality. Patients with serious underlying diseases have an especially high risk of acquiring these infections and that risk is magnified by exposure to respiratory therapy. Until recently, contaminated respiratory care devices were a major cause of infection, but procedures for the management of these devices have decreased their role substantially. Now aspiration of oropharyngeal flora appears to be responsible for most cases of bacterial respiratory infections. Therefore the techniques to alter the flora of the oropharynx and to diminish the risk of aspiration are important priorities for infection control. Exposure to intensive care units (ICUs) is also a major risk factor for nosocomial pulmonary infection and person to person spread of microorganisms within ICUs seems to be responsible for some of these infections[1] .

Nosocomial pneumonia is the second most common infection after urinary tract infection and has the highest mortality rate amongst nosocomial infections. Nosocomial pneumonia accounts for 15% of all nosocomial infections and affects 0.5- 2.0% of hospitalized patient. The highest incidence rate was seen in ICU (15-20%) particularly in intubated patients on mechanical ventilation [2] .

Almost three quarters of all antibiotic consumptions are for respiratory tract infections [3]. Beta-lactams remain a cornerstone for antimicrobial chemotherapy of a large number of bacterial infections, but their efficacy has been increasingly thwarted by dissemination of acquired resistance determinants among pathogenic bacteria [4]. The exposure of bacterial strains to a multitude of β-lactams has induced a dynamic and continuous production and mutation of β-lactamase in many bacteria, expanding their activity even against later

generation cephalosporins [5] and carbapenems by the production of extended-spectrum beta-lactamase (ESBL) and metallo-beta-lactamase (MBL) respectively. Since the genes that code for the production of ESBL are often linked to other resistance genes causing extended spectrum of drug resistance, this will result into fewer therapeutic alternatives [6]. According to Clinical and Laboratory Standards Institute (CLSI), once an ESBL producing strain is detected, the laboratory should report it as resistant to all penicillins, cephalosporins and monobactam, even if they test as susceptible in vitro [5]. For improving therapeutic outcomes, reducing the resistance, emergence or prevalence and minimizing costs by limiting and optimizing therapy, respiratory infections are clearly an appropriate area for action.

Carbapenem group of antibiotics play a vital role in the management of hospital acquired gram-negative infections, because of their broad spectrum activity and stability to hydrolysis by most of the β-lactamases, including extended-spectrum β-lactamases (ESBLs). Nosocomial outbreaks of carbapenem-resistant *Pseudomonas aeruginosa* and *Acinetobacter* spp. due to metallo β-lactamase production have been reported from different regions [7, 8, 9]. The emergence of these MBLs in gram negative bacilli is becoming a therapeutic challenge as these enzymes possess high hydrolytic activity that leads to degradation of higher generation cephalosporins.

New Delhi strain of MBL producer (NDM-1) gram negatives have become one of the major emerging global threats in recent years [10]. The NDM-1 gene, first identified in Sweden in 2008 in *Klebsiella pneumoniae* from a patient hospitalized in New Delhi, encodes a metallo-ß-lactamase that inactivates all ß-lactams except aztreonam. This bla (NDM-1) gene has been identified in hospital-acquired bacterial species, such as *K. pneumoniae*, but also in the typical community-acquired species, *Escherichia coli*. This gene has been identified in strains that possess other resistance mechanisms contributing to their multidrug resistance patterns. It has been recently extensively reported from the UK, India and Pakistan and, albeit to a lesser extent, from a number of other countries worldwide. In most of the cases a link with the Indian subcontinent has also been established [11].

This underlines the importance of identification of such resistance strains to prevent their dissemination. The resistance mechanisms like ESBL and MBL are already

disseminating on a worldwide scale. In recent years MBL genes have spread from *Pseudomonas aeruginosa* to *Enterobacteriaceae* and a clinical scenario appears to be developing that could simulate the global spread of ESBLs. We have also found many carbapenem resistant isolates in the sputum and endotracheal secretion specimens from patients admitted to TUTH.

Due to the lack of antibiotics and disinfecting policies as well as regular monitoring of microbial contamination label among the hospital patients, MDR organisms are believed to have been increased among the hospitalized patients. On the other hand, due to advancement of medical technology, defense mechanisms of the patient's body are bypassed particularly in the patients admitted in the ICU and CCU. This group of patients especially has been found to be victimized by MDR as a result of the immune status of their body. Keeping these in view this study has been subjected to address the issues regarding the burden of MDR as well as ESBL and MBL producing hospital generated lower respiratory tract infection.

Materials and Methods

One hundred nosocomial lower respiratory tract samples including sputum and endotracheal secretion were collected from March 2010 to August 2010, were prospectively studied in Department of Microbiology, Tribhuvan University Teaching Hospital (TUTH), Kathmandu, Nepal. Specimen collection, culture, identification tests were done according to the guidelines given by American Society for Microbiology. The antibiotic sensitivity test was done by using Mueller-Hinton agar by the standard disk diffusion technique of Kirby- Bauer method as recommended by Clinical and Laboratory Standards Institute (CLSI).

Isolates were labeled as MDR if they were resistant to at least two classes of first line agents including ampicillin, trimethroprim- sulfamethaoxazole, floroquinolones (ciprofloxacin and ofloxacin), Gentamycin and cephalosporins (cefotaxime, ceftriaxone and ceftazidime)[12]. Screening test for the production of ESBL was performed by using ceftazidime (CAZ) (30mg) and cefotaxime (CTX) (30mg) disks. If the zone of inhibition was between ≤22 mm for ceftazidime and between ≤ 27 mm for cefotaxime, the isolate was considered as a potential ESBL producer as recommended by CLSI. The confirmation of ESBL was done by Combination disk method in which CAZ and CTX

alone and in combination with clavulanic acid (CA) (10µg) was used. An increase ZOI of ≥ 5 mm for either antimicrobial agent in combination with CA versus its zone when tested alone confirmed ESBL [13]. *E. coli* ATCC 25922 and *K. pneumoniae* ATCC 700603 were used as negative controls respectively.

Screening for MBL detection was done for the isolates which were resistant to imipenem (IPM 10ug) and meropenem (MEM10ug). The zone of inhibition of 13mm is taken as resistant and 16 mm was taken as sensitive as recommended by CLSI Confirmation was done by combination disk method where two IPM disks (10µg), one containing 10µl of 0.1M (292µg) anhydrous EDTA, were placed 25mm apart from centre to centre. An increase in zone diameter of > 4mm around the IMP-EDTA disk compared to that of the IPM disk alone was considered positive for MBL. For MBL test standardization, *P. aeruginosa* ATCC 27853 and *P. aeruginosa* PA 105663 were used as negative and positive controls respectively.

Results

A total of 100 specimens including sputum and ET secretion collected from patients diagnosed of nosocomial lower respiratory tract infection were processed in the Department of Microbiology, TUTH, Kathmandu, Nepal.

Distribution of microbial isolates

Among the total bacterial isolates (n=113), 109 were gram negative and 4 were gram positive.

Distribution of different bacterial isolates

Among the 113 bacterial isolates, majority were *Pseudomonas aeruginosa* (37.2%) followed by *Acinetobacter* spp (31.9 %), *Klebsiella* spp (21.2%), *E. coli* (6.2 %) and *S. aureus* (3.5 %) which is shown in the Fig. 1.

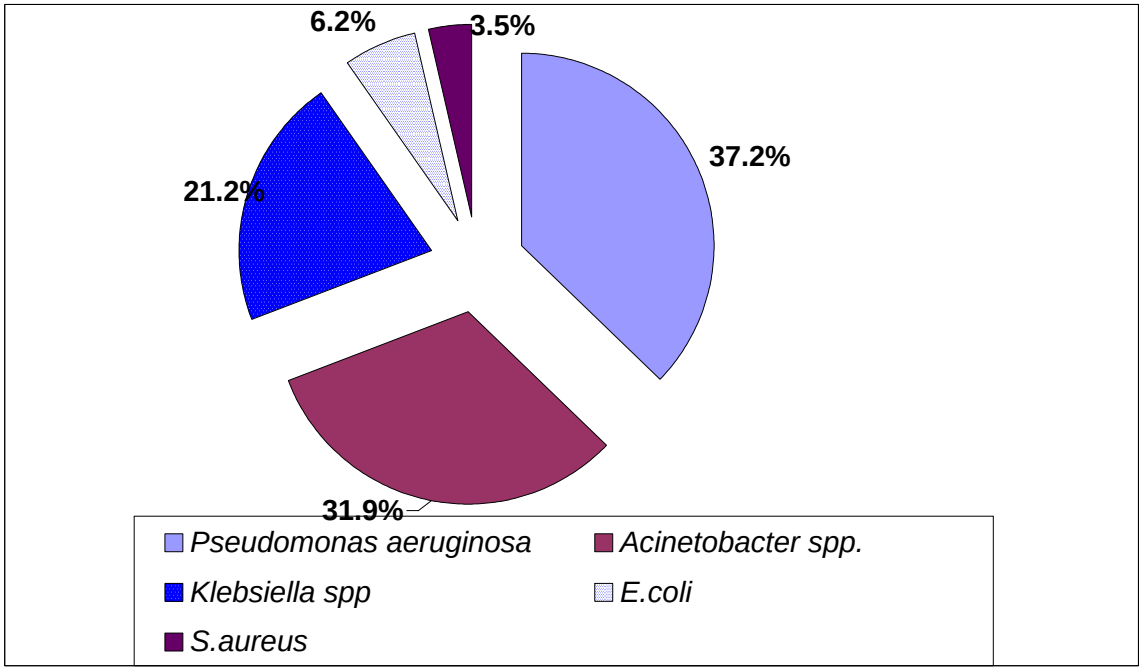

Fig.1. Distribution of different bacterial isolates (n=113)

MDR, ESBL and MBL production in *Pseudomonas aeruginosa*

Out of total 42 *Pseudomonas* isolates, 32 (76.2%) were MDR while equal number was (2.4%) ESBL and MBL producers. All the ESBL and MBL producing isolates were MDR. (Fig. 2)

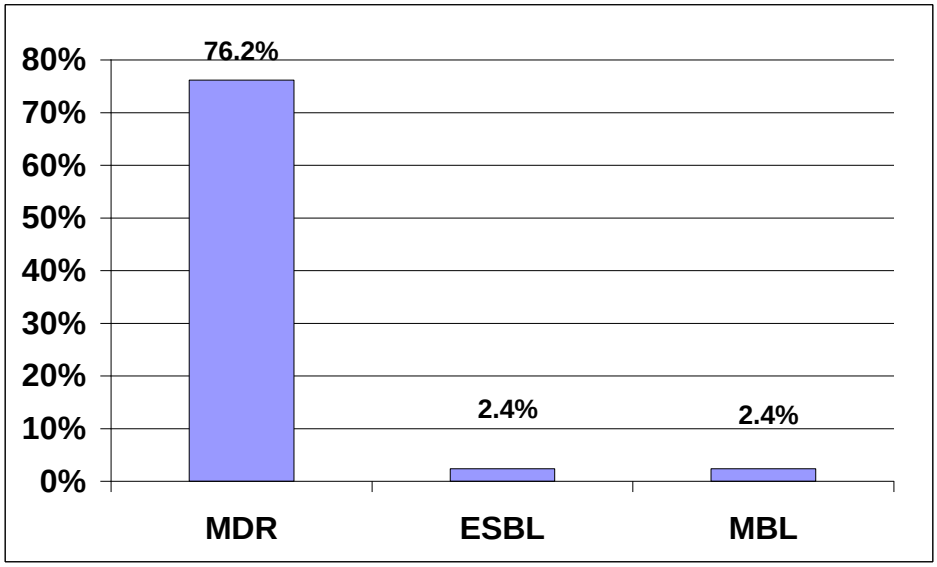

Fig.2. MDR, ESBL, MBL production in *Pseudomonas aeruginosa* (n=42)

MDR, ESBL and MBL production in *Acinetobacter* spp.

Around 97% of *Acinetobacter* isolates were MDR while 47.2% were MBL producers and none were found to produce ESBL which is shown in the fig. 3.

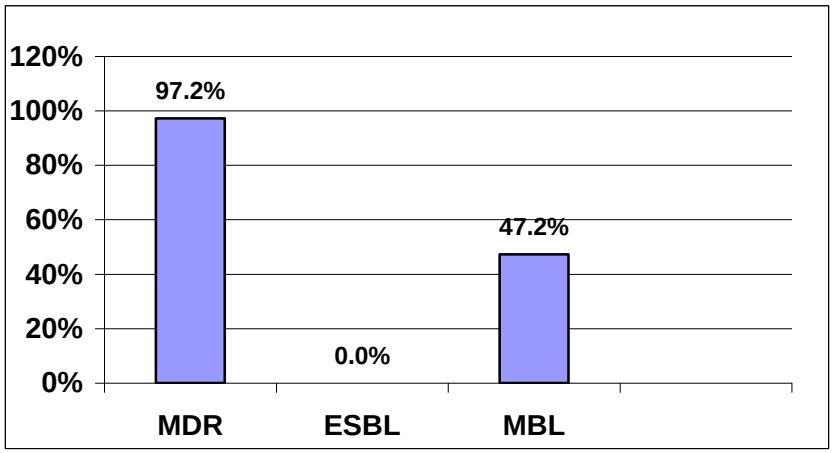

Fig. 3 MDR, ESBL and MBL production in *Acinetobacter* spp.

MDR, ESBL and MBL production in *Klebsiella* spp.

Fig. 4 shows that all *Klebsiella* spp were MDR, 8.3% were ESBL producers and 4.2% were MBL producers.

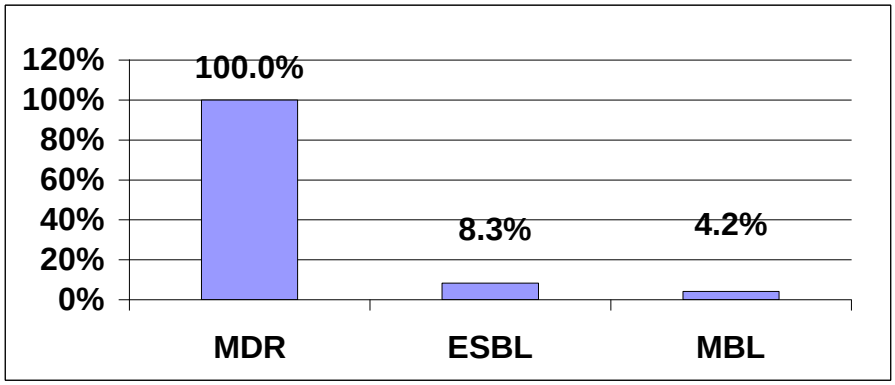

Fig. 4. MDR, ESBL, MBL production in *Klebsiella* spp.

MDR, ESBL and MBL production in *E. coli*

Out of 7 isolates of *E. coli*, all *E. coli* isolates were MDR, 28.6% were ESBL producers, and none were MBL producers.

MRSA

All 4 isolates of *S. aureus* were MRSA and they were sensitive to Vancomycin.

Comparison of MDR, ESBL and MBL production in gram negative isolates.

As shown in figure 5, highest number of MDR and MBL production was in *Acinetobacter* spp. while it was not found to produce ESBL.

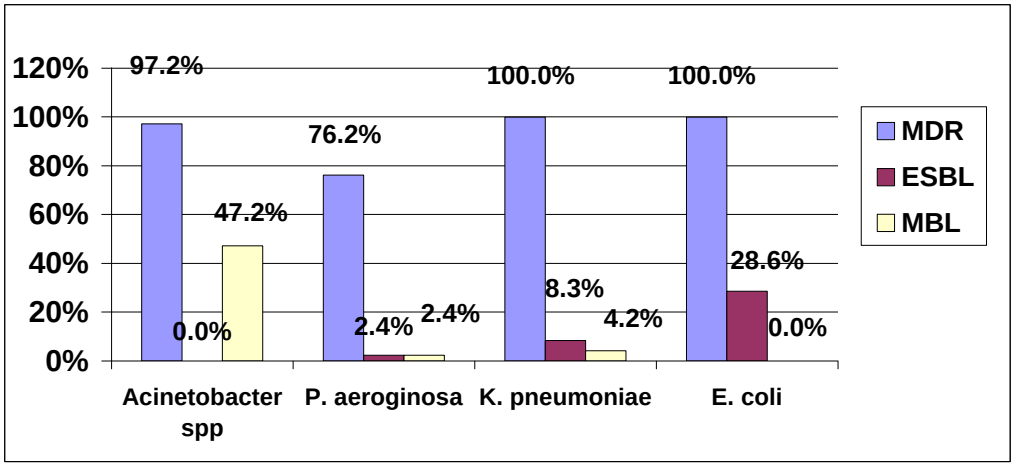

Fig.5. Comparison of MDR, ESBL and MBL production in gram negative bacterial isolates.

Ward wise distribution of MDR, ESBL, MBL and MRSA

Isolates from Intensive care unit (ICU) patient's specimens were found to carry relatively higher frequency of MDR and MBL property. It is followed by Medical Ward (MW), Cardiac care unit (CCU) (Fig. 6).

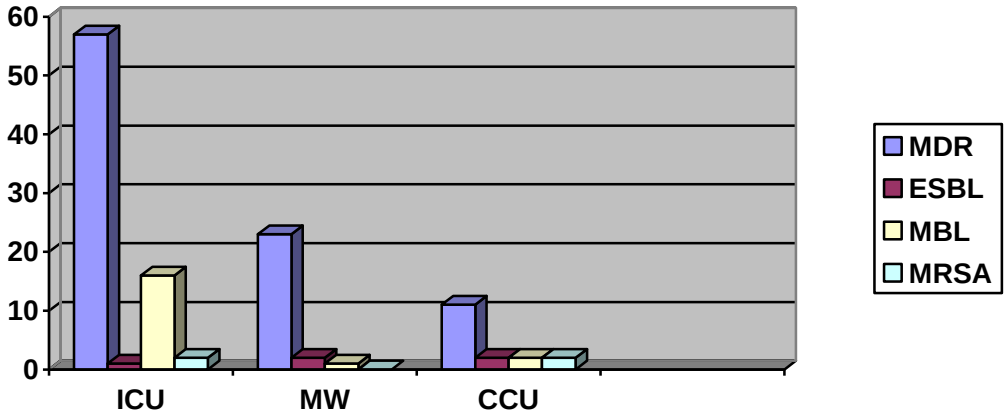

Fig. 6. Ward wise distribution of MDR, ESBL, MBL and MRSA

Discussion

In this study, gram negative bacteria accounted for 96.5% of the total isolates while gram positive bacterial growth was in 3.5% ($P < 0.01$). The most common isolates were found

to be *P. aeruginosa* (37.2 %) which has also been found in other studies such as a study by Kampf G et al in Germany [14]. According to Nidhi Goel et al 95.6 % of the isolates were Gram negative and 2.4 % were gram positive cocci. The most common gram negative in order of frequency were *P. aeruginosa* (35%), *Acinetobacter* (23.6%) and *Klebsiella* spp (13.6%) [15]. This correlates with our study which reveals the growth of *P. aeruginosa* (37.1%), *Acinetobacter* spp (31.9 %), *Klebsiella* spp (21.2%) and *E. coli* (6.2%).

Among the bacterial isolates, higher percentage of MDR strains belonged to *Klebisella* spp (100%) and *E. coli* (100%) followed by *Acinetobacter* and *Pseudomonas.* These pathogens are more common in hospital settings and are mainly accountable for nosocomial infections. Besides, infection by these bacteria is frequently difficult to treat because of both their intrinsic and acquired resistance to multiple groups of antimicrobial agents. Apart from *Klebsiella* spp, MDR isolates were widely present among other genera of *Enterobacteriaceae*. All *E. coli* were MDR. The emergence and increasing trend of MDR among *E. coli* has been reported by others too[16].

According to this study, 12.9% of *Enterobacteriaceae* and 1.3 % of non-fermenters were ESBL producing. Thus ESBL producing isolates are more prevalent among members of *Enterobacteriaceae* ($p < 0.01$). ESBL production is coded by genes that are prevalently located on large conjugative plasmids of 80-160 kb in size [17].Since these plasmids are easily transmitted among different members of the *Enterobacteriaceae*, accumulation of resistant genes results in strains that contain multi resistant plasmids. So ESBL producing isolates are resistant to a variety of classes of antibiotics. This study showed that all ESBL producers were MDR. Therefore, this study highlights the emergence of ESBL producing strains endowed with extremely wide spectrum of antibiotic resistance.

The decreased susceptibility of gram negative isolates towards the third generation generation cephalosporins- cefotaxime, ceftriaxone and ceftazidime (5-40%) could be attributed to ESBL or Amp C β-lactamase producers or some other relevant underlying mechanisms. This study showed 4.6 % of the gram negative isolates were ESBL producers. ESBL production was most common among *E. coli* (28.6%) followed by *Klebsiella* spp (8.3%) and *Pseudomonas aeruginosa* (2.4%). *Acinetobacter* spp. was not found to produce ESBL. A study by Pokhrel et al in 2004 at TUTH found 24.3% of the

total isolates were ESBL producers [18]. They found 55.0% of *K. pneumoniae*, 50% of *E coli* and 20.7% of *Pseudomonas* spp. were ESBL producing. In some hospitals sporadic nosocomial outbreaks due to strains producing ESBLs seem to lead to an endemic problem. Selection pressure from widespread hospital use of later generation cephalosporins apparently enhances colonization of the respiratory tract of patients and infection follows [19, 20].

In this study, out of total 113 isolates, 36 were *Acinetobacter* spp. Out of 36 *Acinetobacter* spp. 17 (47.2%) were found to produce MBL. Globally, there has been increasing concern regarding the rise of *Acinetobacter* infection. The infection caused by *Acinetobacter* most frequently involve respiratory tract of intubated patients and *Acinetobacter* pneumonia has been more common in critically ill patients in Asia ranging from 4-44% and European hospitals 0- 35%, however it is low in United states Hospitals (6-11%) [21] . Out of 36 *Acinetobacter* isolates, 35 (97.2%) were MDR, 16 were from ICU. The startling rate of MDR *Acinetobacter* underscores the need for cogent step in the treatment option. In the last few years, resistance to antibacterial drugs has been increasing in *Acinetobacter* spp which will likely become a substantial treatment challenge in the future [22]. Carbapenems have potent activity against multidrug resistant *Acinetobacter* isolates. *Acinetobacter* may develop resistance to carbapenem through various mechanisms including class B and D carbapenemase production, decreased permeability, altered penicillin binding proteins and rarely over expression of efflux pumps [23, 24].

Among the patients under study, 2 were from Medical Ward and another two from CCU that produces ESBL.. There was only one ESBL producer in ICU. These wards comprise the major domicile of ESBL producers. Third generation cephalosporins such as ceftriaxone, cefotaxime and ceftazidime are extremely used in ICUs even in our setting. Therefore the resistance observed here may have appeared under the selective influence of extensive usage of these antibiotics. Moreover, the specific risk factors that apply to ICU patients include the length of hospital stay, the severity of illness, the length of time spent in ICU as well as mechanical ventilation.

In any nosocomial settings, carbapenems are used as the last resort for the treatment of MDR gram negative bacterial infection. However, since last 15 years, acquired resistance

to this life saving antimicrobial has been increasingly reported not only in *Pseudomonas* and *Acinetobacter* spp but also among members of *Enterobacteriaceae* [25]. Out of total 109 gram negative bacteria, 17 *Acinetobacter*, 1 *Pseudomonas* and 1 *Klebsiella* spp were found to produce MBL. It consisted of 47.2 % of *Acinetobacter* and 2.4 % of *Pseudomonas* and 4.2 % of *Klebsiella* spp isolates. Out of 19 MBL detected, 16 (84.2%) were from ICU. This is an alarming situation. The MBL producing *P. aeruginosa* in this setting was found to be lower in number than in Bangalore, India (12.0%) [26].

For *Acinetobacter* spp., all the MBL producers were resistant to all the tested first and second line antibiotics except one isolates showed the sensitivity to cotrimoxazole.

Prompt detection of MBL producing isolates is necessary to prevent their dissemination. Carbapenem group of antibiotics play a vital role in the management of hospital acquired gram negative infection, because of their broad spectrum activity and stability to hydrolysis by most of the β-lactamase including extended spectrum β-lactamases (ESBL). Nosocomial outbreaks of carbapenem resistant *Pseudomonas aeruginosa* and *Acinetobacter* spp due to metallo β- lactamas (MBLs) production have been reported from different regions [7, 8 ,9]. The emergence of these MBLs in gram negative bacilli is becoming a therapeutic challenge as these enzymes possess high hydrolytic activity that leads to degradation of higher generation cephalosporins. Moreover, the treatment alternatives are unavailable or expensive/ toxic with poor outcome [27]. Plasmid mediated MBL genes spread rapidly to other species of gram negative bacilli [28.] Therefore rapid detection of MBL production is necessary to modify therapy and to initiate effective infection control to prevent their dissemination.

New Delhi strain of MBL producer (NDM-1) gram negatives have become one of the major emerging global threats in recent years [10]. Five multidrug-resistant non-clonally related Enterobacteriaceae isolates were recovered in Belgium in 2010 from 3 patients who had been hospitalised in Pakistan, Montenegro and Serbia/Kosovo. NDM-1 was detected in each of the isolates in addition to several extended-spectrum ß-lactamases (CTX-M-15, SHV-12)[29]. Four *A. baumannii* isolates with bla(NDM-1) were identified in four different provinces in China: no positive isolates were detected among *E. coli, K. pneumoniae* and *P. aeruginosa.* These bla(NDM-1)-positive *A. baumannii* were resistant to all carbapenems and cephalosporins, and three remained susceptible to

fluoroquinolones, aminoglycosides and colistin[30]. However, we did not test for specific types of MBL.

Emergence of MBLs producing *Acinetobacter* spp in our clinical strains is alarming and reflects excessive use of carbapenem. Therefore, early detection and prompt instillation of infection control measures is important to prevent further spread of MBLs to other gram negative rods. Additionally, it is also important to follow the antibiotic restriction policies to avoid the excessive use of carbapenem and other broad spectrum antibiotics. To understand the epidemiology, there is a need of genetic analysis and also the typing of metallo- β- lactamases.

In this study there was one *Klebsiella* spp (4.2%) producing MBL from ICU. The proportion of imipenem resistant *Klebsiella* spp. has increased from less than 1% in 2001, to 20% in isolates from hospital wards in Greece and to 50% in isolates from ICUs in 2006 [31] . This situation seems to be due to the spread of the blaVIM-1 cassette among the rapidly evolving multiresistant plasmids and multiresistant or even pan-resistant strains of mainly *K. pneumoniae* and also other enterobacterial species. However the exact biological basis of this phenomenon and the risk factors that facilitates it is not yet fully understood.

Among the MBL producing cases, 84.2% were present in ICU isolates. It has been proved elsewhere that MBL producing *P. aeruginosa* isolates have been reported to be important causes of nosocomial infections associated with clonal spread [32]. The genes for MBL are inserted in integrons and some of these integrons are located on conjugative plasmids. Because of their ability to spread, carbapenem resistance related to MBL production has become a serious concern [33].

Conclusion

In our study, *P. aeruginosa* was found to be the most predominant isolates as a cause of nosocomial lower respiratory tract infections followed by *Acinetobacter* spp, *Klebsiella* spp., *E. coli* and *S. aureus*. This study also showed a very high prevalence of MDR gram negatives among organisms causing nosocomial LRTI. All *Klebsiella* spp and *E. coli* were found to be MDR. Carbapenems and amikacin were found to be the most effective antibiotics for these MDR gram negative bacilli. *Acinetobacter* spp was not found to produce ESBL while they had a high prevalence of MBL. Prevalence of ESBL in *E. coli*

was found to be higher, however, no MBL was detected in *E. coli*. MBL producing isolates were more common in ICU.

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