The Bacterial Colonization with Extended Spectrum β -Lactamase- and Metallo- β -Lactamase Producing Gram-Negative Bacteria at Intensive Care Unit Patients

Tawfik Abd Motaleb[1], Mansour I Sayed[2], Mohamed H Attia[3], Amal Sharnooby[4], Mohamed M Farag[5], Maha Sabaawy[5]

Departments of Microbiology & Immunology[1], National Liver Institute; Microbiology[2], Faculty of Medicine, Al Azhar University; Internal Medicine[3], Faculty of Medicine Ain Shams University; Clinical Pathology[4] & Hepatology[5], National Liver Institute-Menoufyia University
ahmed_tawfik113@yahoo.com

Abstract: Metallo- β-Lactamase (MBL) and Extended spectrum β-Lactamase (ESBL) have been increasingly emerging as pathogens involved in serious nosocomial infection. ESBL producing strains colonize critically ill patients who serve as a reservoir for epidemic outbreak. The aim of this study was to detect the rate of colonization by ESBL and MBL producing Gram negative bacteria in ICU patients on admission. The study was conducted on 87 patients on admission to ICU; nasal, oral and rectal swabs were taken from each patient. Bacterial culture and strain identification were carried out using Standard microbiological methods, as well as antibiotic susceptibility test using the disc diffusion method. Gram negative isolates were tested for ESBL and MBL production by disc diffusion, double disc synergy test and E-test. PCR was done for MBL phenotypes. Evaluation of some risk factors for colonization includes: age, hospitalization before ICU admission, antibiotics intake and usage of ventilator. The study revealed that 46 (52.9%) out of overall 87 studied patients were colonized by Gram negative bacteria, ESBL production was detected in 43/46 (93.4%), the commonest of which was *E. coli* 22/46 (47.8%), MBL production was detected in 37/46 (80.4%) of them, the commonest of which was *Psudomonas aeruginosa* 16/46 (34.8%). The blaIMP was the commonest gene detected among MBL phenotype, it was detected in 48(45.7%) of overall 105 isolates, while blaGIM was the lowest genotype, it was detected only in 3/105 (2.9%) isolates. The study revealed that: age, hospitalization, antibiotic intake, use of mechanical ventilator is all risk factors for colonization by MBL and ESBL producing bacteria in ICU patients. We concluded that most critically ill patients may be colonized by ESBL and MBL producing Gram negative bacteria, therefore ICU patients must be examined for it, to be treated properly to avoid occurrence and spread of infection in ICU.

[Tawfik Abd Motaleb, Mansour I Sayed, Amal Sharnooby, Mohamed H Attia, Mohamed M Farag, Maha Sabaawy. **The Bacterial Colonization with Extended Spectrum β -Lactamase- and Metallo- β -Lactamase Producing Gram-Negative Bacteria at Intensive Care Unit Patients.** *Life Sci J* 2013;10(1):1907-1914] (ISSN:1097-8135). http://www.lifesciencesite.com. 274

Key wards: Nosocomial infections, Extended-Spectrum β-lactamase (ESBL), Metallo- β-Lactamase, *Pseudomonas aeruginosa*, ICU

1. Introduction

The incidence of nosocomial infections in critically ill patients is much higher than in general ward patients despite the immense advancement in therapeutic technologies (Brawley *et al.,* 1989; Bryan-Brown, 1992). Severe nosocomial infections contribute to prolonged intensive care unit (ICU) stay, increased morbidity and mortality, and increased resource utilization (Heyland et al., 1999; Inan et al., 2005).

The ability to produce β-lactamases enzymes is the major cause of resistance of bacteria to β-lactam antibiotics. Numerous β-lactamases are encoded either by bacterial chromosomal genes or transferable genes located on plasmids or transposons (Mary et al., 2005). Based on amino acid and nucleotide sequence studies, four distinct classes of β-lactamases have been defined. Class A (Extended spectrum β-lactamases) class B (Metallo β-lactamases), class C (AmpC β-lactamases) and class D (Cloxacillin hydrolysing β-lactamases) (Bush e*t al*., 1995; Jacoby, 1997).

Carbapenems are the drugs of choice in the treatment of infections caused by extended-spectrum β-lactamase (ESBL)-positive strains which are resistant to all penicillins, cephalosporins and monobactams (Nordmann and Poirel, 2002; Walsh *et al*., 2005). One of the most important mechanisms of microbial resistance to β-lactam antibiotics is hydrolysis by β-lactamases. Genes coding for β-lactamase enzymes mutate continuously in response to the heavy pressure of antibiotic use leading to development of newer β-lactamases with a broad spectrum of activity. Among the β-lactamases, the carbapenemases especially transferrable MBLs are the most feared because of their ability to hydrolyze virtually all drugs in that class, including the carbapenems. There are several mechanisms for carbapenem resistance such as the lack of drug

penetration due to mutation in pores, loss of certain outer membrane proteins and efflux mechanisms (Walsh *et al*., 2002).

ESBL-producing strains causing hospital-acquired infections are a serious therapeutical and epidemiological problem, especially in ICU patients who are of high risk for infections caused by multidrug-resistant bacterial strains (Livermore, 2008). *Klebsiella pneumoniae* is one of the most frequently isolated pathogens from ICU patients (Rudnicka et al., 2005), and one of the most frequent ESBL producers (Empel *et al*., 2008). A more disturbing problem has emerged, which is the growing number of reports on MBL- positive *Enterobacteriaceae* strains, which follow the Gram-negative non-fermenters (Giakkoupi et al., 2003; Luzzaro et al., 2004; Pournaras *et al*., 2005). MBLs hydrolyze penicillins, cephalosporins and carbapenems and are not inhibited by beta-lactamase inhibitors (Walsh et al., 2005; Queenan and Bush, 2007).

The acquired metallo– β -lactamases (MBLs) represent an emerging threat in the field of infectious diseases because of their rapid spread, increasing diversity, and broad hydrolytic spectrum, which includes the carbapenems. Five types of acquired MBLs have been identified: VIM, IMP, SPM, GIM, and SIM-type enzymes. Since the first description of VIM-2 in a *Pseudomonas aeruginosa* strain isolated in Portugal in 1995 and of VIM-1 in a *P. aeruginosa* strain isolated in Verona, Italy, in 1997 (Yong *et. al*.,2002), the VIM-type MBLs remain the most prevalent in Europe. *P. aeruginosa* represents the most important known reservoir of the VIM-type enzymes, but some studies have reported the dissemination of these enzymes in members of the family *enterobacteriaceae* (Shibata *et. al*., 2003).

The routine susceptibility tests done by clinical laboratories fail to detect β-lactamases positive strains and can detect isolates erroneously sensitive to any of the broad-spectrum cephalosporin like cefotaxime, ceftazidime, ceftriaxone and for imipenem or meropenem (Mathur et al., 2002; Chaudhary & Aggarwal, 2004). Hence, it is necessary to know the prevalence of β-lactamase positive strains in the ICU so as to formulate a policy of empirical therapy in these high risk units where infections due to resistant organisms are much higher.

The aim of this study was to determine the rate of occurrence of colonization by ESBL and MBL producing Gram negative - Bacteria among patients admitted to ICU, to study the role of some risk factors that may predispose to bacterial colonization and to optimize the therapeutic use of antimicrobials for treating these microbial infections with to formulation of antibiotic policy accordingly.

2. Patients and methods:

The present study was carried out from January 2011 to August 2012 in the National Liver Institute Menoufiya University, Al Azhar and Ain Shams University Hospitals. The studied patients were 87 ICU patients (57 males and 30 females), their mean age was 59.3±10.2 years. Full history was taken from each patient. Patients who had taken antibiotic in the previous week were excluded from the study.A written consent was taken from each patient .

Samples & microbiological examination:

Nasal, oral and rectal swabs were taken from each patient on admission to the ICU and sent immediately to the microbiology laboratory for processing, culture and sensitivity for detection of ESBL and MBL isolates, Genotype analysis for suspected strain were done.

1- Culture:

Samples were inoculated on Nutrient agar, MacConkey's agar, and mannitol salt agar, isolated bacteria were identified to the species level by the standard laboratory techniques (Baird,1996) including colony morphology, pigment production, Gram staining, and biochemical reactions; automated identification was carried out, for non-fermenters Gram negative bacilli by using the Vitek2C2 system **(USA).**

2- Antimicrobial susceptibility testing:

Antimicrobial sensitivity was performed on Mueller Hinton agar plates by Kirby-Bauer disk diffusion method according to CLSI full guidelines (Clinical and Lab Standards Institute (CLSI), 2006) for detection of ESBL and MBL. The Antibiotic discs used for detection of ESBL and BML were (Augmentin, cefotaxime, cefodexine, cefoxtine, ciprofloxacin, amikacin, gentamycin, piperacillin, imipenem, and meropenem).

a) Detection of ESBL:

All positive isolates of Gram negative bacteria including *Enterobactericea*, *Pseudomonas earuginosa* and *Acinetobacter* spp were screened for ESBL production by inoculation of a suspension of the growing colonies making a turbidity equal to 0.5 Macferlands opacity standard on a Muller Hinton agar plates and placing the AB discs of ceftazidime, cefotaxime, ceftrioxone and cefpodoxime at a distance of 15 mm apart, after incubation at 37ºC for 16-18 hours. Resistance to these antibiotics indicated a suspicion of ESBL production (Paterson et.al., 2005) confirmed by the combined disc test which was done by the inoculation of a suspension of the growing colonies making a turbidity equal to 0.5 Macferlands opacity standard on a Muller Hinton agar plate, putting a disc of ceftazidime (30µg) and ceftazidime plus clavulanic acid disc (30/10 µg) on the Mueller Hinton agar plate and incubated it at 37c for 18h.

Organism was considered as ESBL producer if there was a ≥ 5mm increase in diameter of ceftazidime plus clavulenic disc than that of ceftazidime disc alone (Bhattacharya, 2006).

b) MBL screening:

The Gram-negative isolates (including members of the family *Entero-bacteriaceae, Pseudomonas spp. and Acinetobacter spp.)* that were non-susceptible to imipenem (10 mg disc) and/or meropenem (10 mg disc) indicated suspicion of MBL production, confirmed by confirmatory phenotypic tests:

1- Double Disc Synergy Test (DDST):

The test was formed as described by (Yong *et al*., **2002**) to differentiate metalloenzymes from other carbapenemases. Ceftazidime disc (30µg) was placed on MH agar medium inoculated with test organism and 10 mm apart, a blank filter study disc impregnated with 10µl of 750µg EDTA solution was added, another ceftazidime disc was placed on the far side of the medium. The zone of inhibition around ceftazidime disc that expanded towards EDTA disc, compared to ceftazidime disc placed on the far side of the medium, was indicative of a positive result. The same test was conducted with imipenem disc with10µl of 750µg EDTA solution as well.

2-Combined-disk method

Ethylenediaminetetraacetic acid (EDTA):

Half milliter EDTA (Sigma Chemicals, St. Louis, MO) solution was prepared by dissolving 18.6 gram of disodium EDTA.2H2O in 100 ml of distilled water and its pH was adjusted to 7.8 by using NaOH. The mixture was then sterilized by filtration through a .45µm pore size filter. EDTA sol (4.5 µl) was poured on imipenem disc of 10µg. Antibiotic disks were dried immediately in an incubator and stored at -20°C in airtight vials without desiccant until used. An overnight broth culture of test strain (turbidity adjusted to 0.5 McFarland opacity standards) was inoculated on a plate of Mueller Hinton agar. One 10 µg imipenem discs with and without EDTA were placed onto the agar surface. The inhibition zones of imipenem and imipenem-EDTA discs were compared after 16-18 hours of incubation at 37ºC. Organisms were considered MBL producers if the zone around the IMP-EDTA disc is ≥8mm compared to the zone around the IMP disc alone (Petropoulou et al., 2006)

***3- E-test*t metallo-beta-lactamase strips** (Ab Biodisk Solna, Sweden) consist of a double sided seven dilution range of imipenem IP (4 to 256 µg/ml) and IP (1 to 64 µg/ml) overlaid with a constant gradient of EDTA (320 mg/L), inoculation and incubation were performed according to the manufacturer's instructions. Individual colonies were picked from overnight agar plates and suspended in 0.85% saline to a turbidity of 0.5% McFarland's standard. A sterile cotton swab was dipped into the inoculum's suspension and inoculated onto the Mueller-Hinton agar. Imipenem E -test strip was laid on the surface of Mueller-Hinton agar plate in a position that the whole length of strip be in complete contact with the agar surface. Plates were incubated for 16 to 18 h at 37°C. The MIC end points were read where the inhibition ellipses intersected the strip. Isolate was considered as MBL producer when the MIC ratio of IP/IPI is ≥8 or ≥3 log dilutions. Additionally, a strain was considered MBL producer if a phantom zone or deformation of the ellipse is observed regardless of IP/IPI ratio (Walsh et al., 2002).

c- Genotypic detection of MBL genes by PCR (Ellington et al., 2007)**:**

A PCR assay was performed to detect and differentiate the five families of acquired MBL encoding genes (VIM, IMP, SPM, GIM and SIM families) in a single reaction.

-Preparation of plasmid and genomic DNA: Plasmid DNA was isolated from the bacterial cells by alkaline lysis method (Strunburg and Mack, 2003), genomic DNA was purified by phenol extraction and ethanol precipitation method (Sambrook *et. al*.,2001) .

-Primer design: Conserved regions of all available blaIMP, blaVIM, blaSPM-1, blaGIM-1, blaSM-1 alleles were identified. Five primer pairs, specific for each family of acquired MBLs, were designed to amplify products of 188 bp (IMP), 390 bp (VIM), 271 bp (SPM-1), 477 bp (GIM-1), 570 bp (SIM-1):

IMP (188 bp) Forward 5´- GAATAGAGTGGCTTAAYTCTC-3´
Reverse 5´-CCAAACYACTASGTTATCT-3´
VIM (390 bp) Forward 5´-GATGGTGTTTGGTCGCATA-3´
Reverse 5´-CGAATGCGCAGCACCAG-39
SPM-1 (271 bp) Forward 5´-AAAATCTGGGTACGCAAACG-3´
Reverse 5´-ACATTATCCGCTGGAACAGG-3´
GIM-1 (477 bp) Forward 5´-TCGACACACCTTGGTCTGAA-3´
Reverse 5´-AACTTCCAACTTTGCCATGC-3´
SIM-1 (570 bp) Forward 5´-TACAAGGGATTCGGCATCG-3´
Reverse 5´-TAATGGCCTGTTCCCATGTG-3´

Amplification, The cycling conditions were: initial DNA release and denaturation at 94 °C for 5 min; followed by 40 cycles of 94 °C for 30 s, 52 °C for 40 s and 72 °C for 50 s; followed by a single, final, elongation step at 72 °C for 5 min. The amplified PCR product was analyzed with appropriate DNA size marker (100-1000 bp) on 2% agarose gel containing 1 ml ethidium bromide. The amplified product was examined for the presence of multiple bands under UV

3. Results:

The demographic and clinical criteria of the studied patients are shown in table (1), whereas the total number of the studied patients was 87 (57males and 30 females) with age ranged from 15 to70 years old. 55 (63.2%) of the studied patients had been admitted in the hospital ≥48 hours prior to ICU admission, 17 (19.5%) of the studied patients were surgical patients, while the remaining 70 (80.5%)

were medical patients, 65 (74.7%) had a previous exposure to antimicrobial drugs intake and 22 (25.2%) had been exposed to mechanical ventilation.

Table (2) shows the pattern of bacterial colonization in the studied patients, 46/87 (52.9%) of the studied patients were colonized by bacteria at one or more of the swabbed sites, Gram negative enterobactericeae were the commonest ESBL producing bacteria isolated from the colonized patients, as the highest rate of isolation was for *E.coli* 22/46 (47.8%), followed by *Klebsiella* pneumonia 16/46 (34.8%), Citrobacter spp. 3/46 (6.5%), and the lowest rate of isolation was for *Enterbacter spp.* 2/46 (4.3%).

Meanwhile, the rate of total MBL isolated cases were 37/46 (80.4%), *Pseudomonas aeruginosa* was the commonest detected organism and was in 22/46 (43.5%) of cases, followed by *Acinetobacter* 11/46 (23.9%), *E.coli* 3/46 (6.5%), *Klebsiella pneumonia* 2/46 (4.3%) and Enterbacter spp.1/46 (2.2%) as shown in table (3).

Regarding the genotypic detection of MBL-encoding genes in the 105 isolates (from nasal, oral or rectal swabs), blaIMP subtype was the commonest, and was detected in 48/105(45.7%) of overall isolates, the commonest organism detected was in *Pseudomonas earuginosa* (21), followed by in *Acinetobacter baumannii* (17), Klebsiella pneumonia (3*), Enterobacterspp* (2*), E. coli* (5). The blaVIM genotype was detected in 34/105 (32.4%), followed by blaSIM in 16/105 (15.2%), then blaSPM 4/105 (3.8%). Meanwhile, blaGIM was the lowest genotype detected in overall 105 isolates, it was detected only in 3/105 (2.9%) isolates, 2 of them was *Pseudomonas spp* and one isolate was *E coli* (Table 4).

Table (5) shows the risk factors for bacterial colonization on admission at ICU, the patients aged above 50 years have a higher rate of colonization (62.5%), than patients with age below 50 years (37.5%) (p=0.04). Regarding gender, males had a higher rate of colonization (61.4%) than females (36.7%), (p=0.02). An important risk factor is the hospital stay >48 hs, hospitalised patients had a significant higher rate of colonization (74%), than the non hospitalised (24%) (p=0.0001). Uses of antibiotics was observed as risk factors for colonization as patients who used antibiotic had a significant higher rate of colonization (60.7%) than the non user (20%) (p=0.004). Patients who undergone mechanical ventilation, had a significant higher rate of colonization (53.6%), than no mechanical ventilators (22%) (p=0.003). Smokers were significantly of higher bacterial colonization (80%) than non smokers (34.6%) (p=0.007).

Table (1) Demographic data of the studied patients upon admission to the ICU (No=87)

Variables	Number of patients	%
Age:		
≥50	48	55.2%
<50	39	44.8%
Gender		
Males	57	66%
females	30	34%
Diabetes mellitus	28	32.2%
Cardiac	16	18.4%
Cerebral stroke	11	12.8
Hepatic coma	21	24.1%
Renal failure	8	9.2%
Respiratory disease	25	28%
Surgical admission	17	19.5%
Previous hospitalization for ≥48hours	55	63.2%
Mechanical ventilation	22	25.2%
History of A.B intake	71	815%

Table (2) Prevalence of colonization by ESBL producing bacteria in ICU patients upon admission

Organisms	Patients colonized (N=46)	
	No	%
Prevalence of ESBL:		
E. coli	22	47.8%
Klebsiella pneumonia	16	34.8%
Citrobacter species	3	6.5%
Enterobacter species	2	4.3%
Total ESBL isolated	43	93.4%

Table (3) Prevalence of colonization by MBL producing bacteria in ICU patients upon admission

Organisms	Patients colonized (N=46)	
	No	%
Prevalence of MBL:		
Pseudomonas aeruginosa	20	43.5%
Acinetobacter baumannii	11	23.9%
E. coli	3	6.5%
Klebsiella pneumoniae	2	4.3%
Enterobacter species	1	2.2%
Total MBL isolated	37	80.4%

Table (4) PCR results of MBL phenotypes in bacterial isolates (N=105)

MBL subtype	Overall MBL types	*Pseudomonas aeruginosa*	*Acinetobacter baumannii*	*Klebsiella pneumonia*	*Enterobacter spp*	*E. coli*
blaIMP	48 (45.7%)	21	17	3	2	5
blaVIM	34 (32.4%)	16	9	5	1	3
blaSIM	16 (15.2%)	7	4	1	2	2
blaSPM	4 (3.8%)	3	1	0	0	0
blaGIM	3 (2.9%)	2	0	0	0	1
Total	105(100%)	49	31	8	5	11

Table (5) Study of risk factors for bacterial colonization on admission at ICU

Risk factors	Colonization on admission		*P* value
	Present (n=46) No (%)	**Absent (n=41)** No (%)	
Age (years):			
≥50 (n=48)	30 (62.5%)	18 (37.5%)	0.04*
<50 (n=39)	16 (41%)	23 (59%)	
Gender :			
Males (n=57)	35 (61.4%)	22 (38.6%)	0.02*
Females (n=30)	11 (36.7%)	19 (63.3%)	
Hospital stay>48 hrs.:			
Hospitalised (no=50)	37 (74%)	13 (26%)	0.0001**
Not hospitalised (no=37)	9 (24%)	28 (76%)	
Antibiotic intake ≥2groups :			
Yes (no=71)	43 (60.7%)	28 (39.4%)	0.004**
No (no=15)	3 (20%)	12 (80%)	
Mechanical ventilation (n=28)	15 (53.6%)	13 (46.4%)	0.003**
No mechanical vent. (no=59)	13 (22%)	46 (77%)	
Smoking:			
Smokers (no=35)	28 (80%)	7 (20%)	0.007**
Non-smokers (n=52)	18 (34.6%)	34 (65.4%)	

*Significant $p<0.05$, **highly significant $p<0.01$, <0.001.

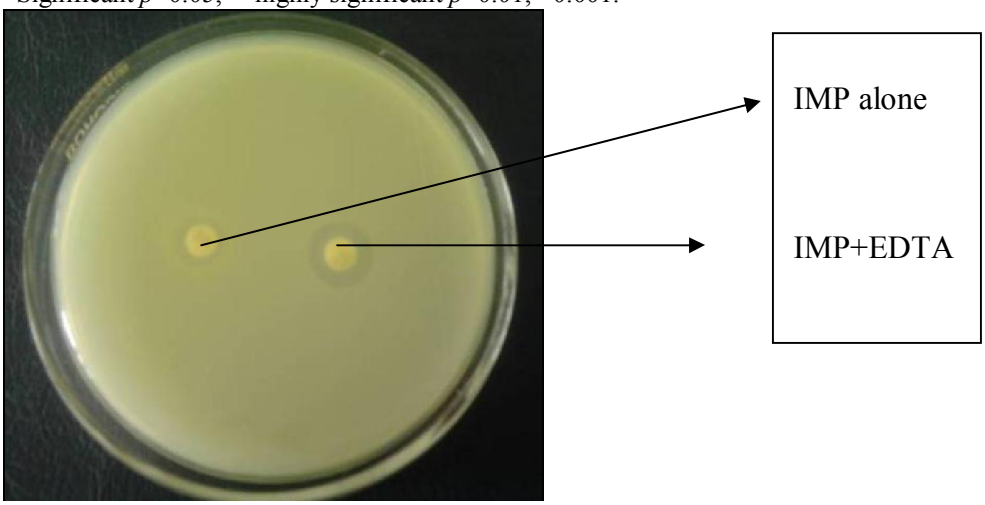

Figure (1) Combined disk test showing enhanced inhibition zone of ≥8 mm around IPM + EDTA disc indicating MBL producers.

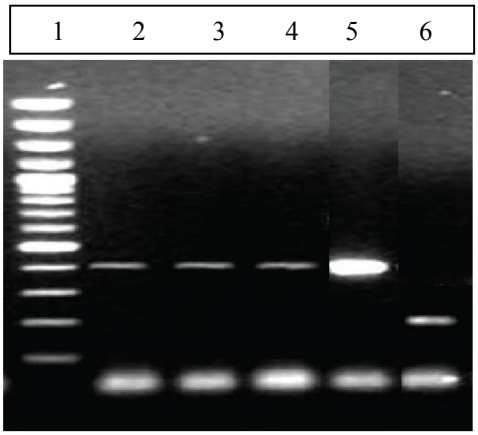

Figure (2) Shows PCR product of MBL phenotypes: lane 1 (100-1500 bp ladder), lanes 2, 3, 4 (390 bp positive strain for blaVIM), lane 5 (390 bp blaVIM positive control), lane 6 (positive strain for blaIMP

4. Discussion:

Extended spectrum beta lactamase (ESBL) – producing Gram negative bacteria are new emerging pathogens, Clinicians, microbiologists, infection control practitioners and hospital epidemiologists are concerned about ESBL producing microorganisms because of the increasing incidence of such infections, the limitation of effective antimicrobial drug therapy and adverse patients outcome (http/wwne.gov/eid/article/13/8/07-0071, article. htm≠r5|).

The socioeconomic impact of this colonization leading to nosocomial infections can cause economic burden on the personal and/or governmental scale burden. Therefore, the analysis of the incidence and pattern of bacterial colonization including multidrug

resistance Gram negative bacteria in patients on ICU admission is very important (Jarves, 1996).

Although MBL-producing strains represent a serious therapeutic challenge, to date, clinical experience regarding the most appropriate treatment and outcome of infections due to these strains is surprisingly rare. Therefore, detection of MBL producing gram negative bacilli especially *P. aeruginosa* is crucial for optimal treatment of patient's particularly critically ill and hospitalized patients and for better control the spread of resistance.

In the present study, bacterial colonization by Gram negative bacteria was detected in 46/87 (52.9%) of the studied patients, positive for ESBL production was detected in 43/46 (93.5%), the commonest of which was *E.coli* 22/46 (47.8%), followed by *Klebsiella pneumonia* 16/46 (34.8%), *Citrobacter spp*. 3/46 (6.5%), and the lowest rate of isolation was for *Enterbacter spp*. 2/46 (4.3%). These results were in agreement with the previous studies by Afazi et al. (2010), who reported that E.coli was the commonest isolates among ESBL producing Gram negative bacteria (57%) followed by *Klebsiella pneumonia* (59%),while it was (6%), and (8%) for *Enterobacter* and *Citrobacter spp* respectively. Another study by Ozgumus et al. (2008), E.coli was the commonest isolated ESBL producing pathogen followed by *Klebsiella spp* in ICU patients. In the same manner, Mona et al. (2009), reported that (60%) of isolates from ICU patients, were confirmed as ESBL producer G-ve bacilli, mostly *E.coli* (34%), followed by *Klebsiella spp*. (30.2%).

In the current study, the rate of total MBL isolated was 37/46 (80.4%), the non fermenter Gram negative bacterial; (*Pseudomonas aeruginosa* and *Acinetobacter baumamnii*) were the predominant MBL producing bacteria isolated from the studied patients as it was isolated at a rate of 31/46 (67.4%) of MBL isolates, Pseudomonas aeruginosa was detected in 22/46 (43.5%) isolates, followed by *Acinetobacter* 11/46 (23.9%) isolates, *E.coli* 3/46 (6.5%) isolates, *Klebsiella pneumonia* 2/46 (4.3%) isolates and *Enterbacter spp*.1/46 (2.2%).

Afazi *et al*. (2010) stated that the rate of detection of *Pseudomonas earuginosa* was 57% and the colonizing MBL producing G-ve bacilli for *E.coli* and *Klebsiella pneumonia* were 16% and 10% respectively. Over the last decade MBL producing isolates have emerged particularly in *Pseudomonas aeruginosa*. These isolates have been responsible for serious infections such as septicemia and pneumonia and have been associated with failure of therapy with carbapenems. MBL genes have spread from *P. aeruginosa* to *Enterobacteriaceae,* and a clinical scenario appears to be developing that could simulate the global spread of ESBL. Carbapenems are the drug of choice for treatment of infections by penicillin or cephalosporin resistant gram negative bacilli especially in ESBL producing gram negative infections (Pitout et al., 2007). It is well known that poor outcome occurs when patients with serious infections due to MBL producing organisms are treated with antibiotics to which the organism is completely resistant (Marra et al., 2006).

The occurrence of a MBL positive isolate poses not only a therapeutic problem but is also a serious concern for infection control management. As a result of being difficult to detect, such organisms poses significant risks particularly due to their role in unnoticed spread within institution and their ability to participate in horizontal MBL gene transfer, with other pathogens in the hospital (Agarwal *et al*. 2006).

Regarding the genotypic detection of MBL-encoding genes in the isolates, blaIMP subtype was the commonest, it was detected in 45.7% of overall isolates, the commonest organism detected was in Pseudomonas aeruginosa, while balGIM was the lowest genotype detected (2.9%). The blaVIM genotype was detected in 32.4%, followed by blaSIM in 15.2%, then blaSPM 3.8%. Meanwhile, blaGIM was the lowest genotype detected in overall 105 isolates; it was detected only in 2.9% isolates.

The presence of high prevalence of blaIMP may be due to the clonally spread of the plasmid containing this gene amongst the various members of the Gram negative bacilli in different hospitals including ours. This result agrees with the result of Afazi *et al*. (2010) which stated that the commonest genotype was blaMP (33.7%), followed by blaVIM (22.9%), while it was (20%) for blaGIM,and (0.2%) for blaSPM.

Most of Research conducted in this issue has focused on identifying risk factors for colonization with multidrug-resistant, Gram positive bacteria, in contrast, little research has been conducted to identify the risk factors for colonization with Gram negative multidrug-resistant bacteria in non outbreak setting (Harris *et al*., 2007). Understanding risk factors for colonization is important for several reasons. First, understanding the potential causal mechanisms of colonization can lead to successful infection control, involving antimicrobial stewardship and public health intervention aimed at controlling the emergence of ESBL –producing bacteria. Second, such knowledge can help identify which patients should receive empiric ESBL-targeted antimicrobial therapy (Harris *et al*., 2007).

The present study had focused on several risk factors for colonization. It was observed that patients aged above 50 years have a higher rate of colonization (63%), than patients with age below 50

years (41%) owing to their lower rates of immunity. Regarding gender, males had a higher rate of colonization (65%), than females (35%). An important risk factor, is the hospital stay >48 hours, as they had a significant higher rate of colonization (74%), than the non hospitalised (24%). Usage of antibiotics was observed as a risk factor for colonization. Patients who used antibiotics had a significant higher rate of colonization (60%) than non-users (20%). Severe nosocomial infection contributes to prolonged ICU stays, increased morbidity and mortality and of course increased resource utilization (Inan *et al*., 2005).

Another important risk factor is mechanical ventilation, patients who underwent mechanical ventilation, had a significant higher rate of colonization (68%), than others (22%), Smokers had significantly higher bacterial colonization (80%) than non smokers (34%). These findings corroborates the findings of some other related studies (Drakulovic *et al*., 2001; Harris *et. al*., 2007).It was reported that exposure to β-lactamases is a significant risk factor MBL producing Pseudomonas aeruginosa infections. Zavsaki et al. (2006)

Conclusion & Recommendation:

Colonization by ESBL and MBL producing Gram negative bacilli may occur in critically ill patients on admission to ICU particularly in the presence of risk factors. These patients may act as reservoir for occurrence and spread of nosocomial infections. There is a need for improved surveillance of antimicrobial resistance and strengthened quality control of antimicrobial drug. The routine clinical microbiology laboratories should employ simple methods to diagnose ESBL and MBL producing organisms. Rational antibiotic guidelines should be mandatory to minimize the problem along with infection control practices. Also incorporating microbial genetic typing infection control program is recommended.

Corresponding author
Tawfik Abd Motaleb
Departments of Microbiology & Immunology
National Liver Institute-Menoufyia University, Egypt.

References

1. (http/wwne.gov/eid/article/13/8/07-0071, article.htm≠r5|).
2. Afzai Azim, P. Bahaskar Roa, AK. Baroria, R.K.Singh,K.N. Prasad,Banani Podder. Epidemiology of bacterial colonization at intensive care unite admission with emphasis on Extended – Spectrum and Metallo beta lactamase producing Gram negative bacteria –An Indian experience .Journal of Medical Microbiology .August,2010 Vol.59 No.8,955-960 .……
3. Agarwai VA, Dongre SA, Powar RM (2006) .Antimicrobial resistance of Pseudomonas aeruginosa producing metallo-beta lactamase . Indian Journal Med. Res.124:588-590 .
4. Baird D. Staphylococcus: Cluster-forming gram-positive cocci. In : Collee JG, Fraser AG, Marmion BP, Simmons A, editors. Mackie and McCartney Practical Medical Microbiology. 14 th ed. New York: Churchill Livingstone;
5. 1996. p. 245-61.
6. Bhattacharya S. Extended spectrum β-lactamases from petridish to the patient. Indian J Med Microbial. 2006;24(1):20-24
7. Brawley, R. L., Weber, D. J., Samsa, G. P. & Rutala, W. A. (1989). Multiple nosocomial infections. An incidence study. Am J Epidemiol 130, 769–780.
8. Bryan-Brown, C. W. (1992). Pathway to the present: a personal view of critical care. In Critical Care, 2nd edn, pp. 5–6. Edited by J. M. Civetta, R. W. Taylor & R. R. Kirby. Philadelphia, PA: J. B. Lippincott.
9. Bush K, Jacoby GA, Medeiros AA. A functional classification scheme for β-
10. lactamases and its correlation with molecular structure. Antimicrob Agent
11. Chemother. 1995;39:1211-33
12. Chaudhary U, Aggarwal R. Extended spectrum β-lactamases and emerging
13. threat to clinical therapeutics. Indian J Med Micrbiol. 2004;22(2):75-80
14. Clinical and Lab Standards Institute (2006). Performance standards for Anti-microbial susceptibility testing. 16th informational supplements CLSI document. M2 Wayne Pa
15. Drakulovic, M.B., Bauer ,T. T., Torres, A., Gonzalez,J., Rodriguez, M.,J.& Angril, J.(2001). Initial bacterial colonization in patients admitted to a respiratory intensive care unit : bacteriological pattern and risk factors. Respiration 68, 58-66 .
16. Ellington, M. J., Kistler, J., Livermore, D. M. & Woodford, N. (2007). Multiplex PCR for rapid detection of genes encoding acquired metallo-b-lactamases. J Antimicrob Chemother 59, 321–322.
17. Empel J., A.Baraniak, E.literacka,A.Mrowka, J.Fiett, E.Sadowy, W. Hryniewicz, M. Giniadkowski, and the Beta-PL Study group. (2008) .Molecular survey of beta lactamases conferring resistance to newer beta lactamas in Enterobacteriaceae isolates from Polish hospitals. Antimicrob.Agents Chemother. 52: 2449-2454
18. Giakkoupi P., A.Xanthaki, M. Kanelopoulou, A.Vlahaki, V.Miriagou, S.Kontou, E.Papafraggas, H.Malamou-Lada, L.S.Tzouvelekis, N.J.Legakis and others. (2003). VIM-1 metallo beta lactamase- producing Klebsiella pneumonia strains in Greek hospitals. J. Clin. Microbiol. 41:3893-3896
19. Harris, A, D., McGregor, J. G., Johnson, J.A., Strauss, S.M., Moor, A. C., Standiford,H.C., Hebden. J.N.&Morris,J.G., jr (2007). Risk factors for colonization with extended-spectrum beta lactameses-producing bacteria and intensive care unit admission .Emerg.Infect.Dis. 13,1144-1149 .
20. Heyland, D. K., Cook, D. J., Griffith, L., Keenan, S. P. & Brun Buisson, C.
21. for the Canadian Critical Care Trials Group (1999). The attributable morbidity and mortality of ventilator-

associated pneumonia in the critically ill patient. Am J Respir Crit Care Med 159, 1249–1256.
22. Inan, D., Saba, R., Gunseren, F., Ongut, G., Turhan, O., Yalcin, A. N. Mamikoglu, L. (2005). Daily antibiotic cost of nosocomial infections in a Turkish university hospital. BMC Infect Dis 5, 5.
23. Jacoby GA. Extended-spectrum b-lactamases and other enzymes providing
24. resistance to oxyimino--lactams. Infect Dis Clin North Am. 1997;11(4):875-87.
25. Jarvis, W.R (1996). Selected aspects of socioeconomic impact of nosocomial infections :morbidity, mortality ,cost. And prevention .Infection Control Hosp Epidemiol 17,552-5 57.
26. Livermore D.M. 2008. Defining an extended-spectrum-$-lac tamase. Clin. Microbiol. Infect. 14: 3- 10
27. Luzzaro F., J.D. Docquier, C. Colinon, A. Endimiani, G. Lom- bardi, G. Amicosante, G.M. Rossolini and A. Toniolo. 2004. Emergence in *Klebsiella pneumoniae* and *Enterobacter cloacae* clinical isolates of the VIM-4 metallo-$-lactamase encoded by a conjugative plasmid. Antimicrob. Agents Chemother. 48: 648 650
28. Marra AR, Pereira CA, Gales AC, Menezes LC, Cal RG, desouza JM. Blood stream infections with metallo-ß-Lactamase- producing *Pseudomonas aeruginosa*: Epidemiology, Microbiology and clinical outcomes. Antimicrob. Agents Chemother., 2006, 50: 388-390.
29. Mary VJ, Kandathi AJ, Balaji V. Comparison of methods to detect carbapenamase and metallo β-lactamases production in clinical isolates.Indian J Med Res. 2005;121:780-83
30. Mathur P, Kapil A, Das B, Dhawan B. Prevalence of extended spectrum beta
31. lactamase producing gram negative bacteria in a tertiary care hospital.Indian J Med Res. 2002; 115:153-7
32. Mona A.Gharib, Nadia H. Ouda, Mohga S., Lamia Hamed, and Sameh Abd Rahman .Prevelance of Extended spectrum beta lactamase producers in an Egyptian critical care centre . Egyptian Journal of Medical Microbiology, October 2009.
33. NCCLS (2000). Performance Standards for Antimicrobial Disk Susceptibility Test, approved standard, 7th edn, vol. 20 no. 1, M2–A7.Wayne, PA: National Committee for Clinical Laboratory Standards.
34. Nordmann P, Poirel L. Emerging carbapenemases in gram negative aerobes. Clin. Microbiol. Infect., 2002, 8: 321-333.
35. Ozgmus o.p.,Tosun I., Aydin F.,and Osman A. K. . Horizontal dissemination of TEM –and SHV- type Beta-lactamase Genes carrying resistanance plasmids amongst clinical isolates of Enterobactericeae. Brazilian Journal of Microbiology (2008) 39:636-643 .
36. Paterson DL, Rossi F, Baquero F, Hsueh PR Woods GL, Satishchandran V, Snyder TA Harvey CM, TepplerH, DiNubile MJ, Chow JW (2005) *.In vitro* susceptabilites of aerobic and facultative anerobic Gram negative bacilli isolated from patients with intra abdominal infection worldwide : the 2003 study for monitoring antimicrobial resistance Trends (SMART) J. Antimicrob.. Chemother.55:965-973 .
37. Petropoulou D., Konstantina T, Vassiliki P, *et al.*(2006) : Evaluation of imipenam+EDTA disc method for detection metallo betalactamase producing *Klebsiella pneumonia* isolates from blood culture .Micro. Drug. RES. 12(1) 39-43 .
38. Pitout JD, Chow BL, Gregson DB, Laupland KB, Elsayed S, Chruch DL (2007). Molecular epidemiology of metallo-ß-lactamase producing *Pseudomonas aeruginosa* in the Calgary Health Region: Emergence of VIM-2 Producing Isolates. J. Clin. Microbiol., 45: 294-298.
39. Pournaras S., A. Ikonomidis, L.S. Tzouvelekis, D. Tokatlidou, N. Spanakis, A.N. Maniatis, N.J. Legakis and A. Tsakris. 2005. VIM-12 a novel plasmid mediated metallo-β-lactamase from Klebsiella pneumoniae that resembles a VIM-1/VIM-2 hybrid Antimicrob. Agents Chemother. 49: 5153 5156
40. Queenan A.M. and K. Bush. 2007. Carbapenemases: the versa tile β-lactamases. Clin. Microb. Rev. 20: 440 458
41. Rudnicka J., M. Wróblewska, H. Marchel and M. £uczak, 2005. Comparison of frequency and susceptibility to antimicro bials of bacterial strains of the family Enterobacteriaceae isolated from patients hospitalised in intensive care units (in Polish). Med. Dos w. Mikrobiol. 57: 185-191 .
42. Sambrook J,Fritsch EF, Maniatis T,molecular cloning ; A laboratory manual,2nnd ed.Cold spring Harbor ,NY:Cold Spring Laboratory Harbor; 2001 .
43. Shibata N , Doi Y, Yamane K , Yagi T, Kurokawa H, Shibayama K , Kato H, Kai K,Arakaway Y. PCR typing of genetic determinants for metallo- beta - lactamases and integrases carried by gram-negative bacteria isolated in Japan, with focus on the class 3 integron. *J. Clin. Microbiol*. (2003); 41: 5407-5413.
44. Sturenburg E. And Mack D. ESBLs; Implications for the clinical microbiology department , therapy and infection control. J. Infect. 2003;273-95 .
45. Walsh TR, Bolmstrom A, Qwarnstrom A, Gales A. Evaluation of a new Etest for detecting metallo-β-lactamases in routine clinical testing. *J Clin Microbiol* 2002; *40* : 2755-9.
46. Walsh TR, Toleman MA, Poirel L, Nordman P (2005). Metallo ß–lactamases: the quiet before storm? Clin. Microbiol. Rev., 18: 306-325.
47. Yong D, Lee K, Yum J H, Shin H B , Rossolini G M, Chong Y, Imipenem-EDTA disk method for identification of metallo-ß-lactamase-producing isolates of *Pseudomonas spp*. and Acinetobacter spp. Journal of Clinical Microbiology . 2002; 40: 3798- 801.
48. Zavascki AP, Barth AL, Gaspareto PB, Goncalvez AL,MORO AL, Fernandes JJF, Goldani LZ,(2006). Risk factors for nosocomial infections due to Pseudo monas aeruginosa producing metallo beta lactamase in low tertiary care teachin hospitals. J. Antimicrob.Chemother. , 58;882-885.