Clinical and Microbiological Profile of Nosocomial Infections in Adult Intensive Care Units at Assiut University Hospitals, Egypt

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Abstract: Infection caused by multi-drug resistant bacteria is a serious problem especially for intensive care unit patients (ICU) throughout the world. The aim of this study was to determine the rate of nosocomial infections (NI), risk factors, bacterial pathogens and their antimicrobial susceptibilities in the adult Intensive Care Units at Assiut University Hospitals to assist in planning hospital antibiotic policy. Nine hundred suspected cases of nosocomial infections were identified as per the CDC guidelines. Clinical specimens were collected according to the site of infection and traditional bacteriological identification was performed. Antibiotic susceptibility was determined by modified Kirby-Bauer disc diffusion method. The rate of NIs among adult ICU patients was 15%. The commonest type of NI was lower respiratory tract infection (59.9%). The most frequently isolated microorganisms were gram negative bacteria (54.2%) amongst which, *Klebsiella* spp. was the most common. Gram positive bacteria accounted for 45.8% with methicillin resistant *Staphylococcus aureus* (MRSA) being the predominant (23.6%). The highly significant risk factors for acquiring nosocomial infections were: burns (odds ratio [OR], 3.48 %, confidence interval [CI], 1.20 - 10.12), endotracheal tubes (OR, 9.85; 95% CI, 5.36 - 18.11), mechanical ventilation (OR, 2.96; 95% CI, 1.68 - 5.21), urinary catheters (OR, 2.77; 95% CI, 2.5-3.1), intravenous catheters (OR, 2..31; 95% CI, 2-2.7), and hospital stay for more than 2 weeks (OR, 1.41; 95% CI, 1.2-1.7). The majority of patients (72%) in the ICUs received one or more empirical antibiotics for prophylaxis. Various groups of antibiotics were commonly prescribed, with penicillins (32%) and cephalosporins (36.7%) being the most common. Most of the gram negative and positive bacteria showed high percentages of resistance to many groups of antibiotics. The best sensitivity was to imipenem, vancomycin and teicoplanin. We conclude that the prevalence of nosocomial infections in the adult ICUs at Assiut University Hospitals is considerable. Many risk factors for nosocomial infections were found. Empirical antibiotics were widely prescribed for prophylaxis with cephalosporins and penicillins being the commonest. Lower respiratory tract infection was the commonest nosocomial infection. Gram negative bacteria caused most of the nosocomial infections with *Klebsiella* spp. being the predominant. MRSA was the most commonest Gram positive bacteria isolated. All isolates showed very high resistance for most of the studied antibiotic groups. The best sensitivity was to imipenem, vancomycin and teicoplanine. These results may have important implications for formulating antibiotic policies in order to lower the frequency of antimicrobial resistant organisms in the ICUs at Assiut University Hospitals.

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1. Introduction

The benefit of intensive care units (ICUs) is undeniable, yet they are considered as sites where additional complications to medical management can arise (Ponce -de- Leon, 2001). Health-care-associated infection (HAI), in other words nosocomial infection (NI), affects as many as 50% or more of patients in intensive care units (ICUs) in developed countries (Vincent *et al.*, 2009; WHO, 2009) but remains underestimated in developing countries (Pittet *et al.*, 2005). The prevalence of HAI is higher in ICUs than in general hospital wards (Ruef, 2005) leading to an enormous impact on morbidity, hospital costs, and often, survival (Blot, 2008).

Along with the problem of nosocomial infection goes the burden of "multidrug" antimicrobial resistance (MDR). The ICU has even been described as a factory for creating, disseminating, and amplifying antimicrobial resistance (Carlet *et al.*, 2007). This burden of resistance, however, is probably more due to the higher rate of inappropriate empiric antimicrobial treatment associated with infections caused by MDR pathogens (Figueiredo Costa, 2008). The emergence of MDR is often dedicated to excessive use of broad-spectrum antimicrobial agents (more than 60% of all ICU patients receive antibiotics during their stay) (Borg, 2003).

Multi-drug antibiotic resistance (MDR) increases dramatically among Gram-positive and Gram-negative bacteria worldwide, while antimicrobial agents with new mechanisms of actions

are lacking. As a result, infections by MDR pathogens are rising and they are associated with significant morbidity, mortality and financial costs. This phenomenon particularly affects intensive care units where patients have multiple risk factors (long hospital stay, prior use of antibiotics, severity of illness, absence of normal anatomical barriers, high frequency of medical and nursing interventions) (Tacconelli, 2006).

Extended spectrum beta-lactamases (ESBL), enterobacteriaceae, carbapenem resistant *Pseudomonas aeruginosa*, vancomycin resistant enterococci (VRE) and methicillin resistant *Staphylococcus aureus* (MRSA) represent the most commonly reported MDR pathogens in the ICU (Rosenthal *et al.,* 2010, European Centre for Disease Prevention and Control, 2010). Certain HAIs are more common, that is, urinary tract infections (UTI), respiratory infections (RTI), surgical site infections (SSI), and blood stream infections (Jazayeri and Irajian, 2009).

The aim of this study was to determine the rate of nosocomial infections (NI), risk factors, bacterial pathogens and their antimicrobial susceptibilities in the adult medical and surgical Intensive Care Units at Assiut University Hospitals to assist in planning hospital antibiotic policy.

2. Material and Methods

Study population

This retrospective study was carried out in the surgical and medical ICUs in Assiut University hospitals over a one year period (from January to December 2010). Among a total of 5979 patients admitted, 900 patients developed signs suspecting nosocomial infections according to the criteria of Centers for Disease Control and Prevention (CDC) definitions (Garner *et al.* (1988).

Definition of nosocomial infections

An infection was defined as nosocomial when it originated in the hospital environment, i.e. it was not present upon admission and appeared 48 hrs or more following admission. CDC definitions of infections were followed (Garner *et al.,* 1988). A clinical suspicion of infection was based on the following infection parameters: temperature of >38 ˚C or < 35 ˚C, and leucocytosis or leucopenia. In addition, clinical findings such as a new or progressive infiltrate on chest X-ray, cloudy urine, purulent sputum or phlebitis in the absence of the above criteria were considered to be indicative of possible infection .

Surveillance data

Collected data included the patient's name, age, gender, ward, admission and infection onset dates and organism. The survey sought information on prior antibiotic intake (therapeutic or prophylactic), intrinsic and extrinsic risk factors and clinical features, including the presence of any suspicion symptoms of infection such as fever, sputum, coughing or diarrhea, and so on, and pertinent physical examination and laboratory data . Informed oral consent was obtained from all patients or their relatives prior to specimen collection. Ethical considerations including privacy of personal data were considered.

Samples

Samples were then taken from the suspected site of infection (urinary tract, respiratory tract, blood, wound drainage, etc.). They were subjected for full traditional bacteriological diagnosis including Gram's staining, culture and biochemical diagnosis. Antimicrobial susceptibility tests of isolates were performed by the modified Kirby Bauer disc diffusion test, and interpreted according to Clinical Laboratory Standards breakpoints (CLSI, 2008). The antibiotic discs (HiMedia, Mumbai, India) included penicillin, ampicillin, amoxicillin, carbinicillin, piperacillin / tozabactam , ampicillin / sulbactam, amoxicillin/ clavulanic acid, ticaracillin /clavulanic acid, cefaclor, cufuroxime, cefamandol, cefixime, cefpodoxime, cefotaxime , cefaclor, cephtazidime, cefoperazone, Cephtazidime / clavulanic acid , ceftriaxone, cefuroxime, cefipime, ciprofloxacin, norfloxacin, lomefloxacin , gatifloxacin, imipenem, amikacin, gentamycin, kanamycin, netilmicin, neomycin, streptomycin, tobramycin, spiramycin, tetracycline, oxytetracycline, chloramphenicol, trimethoprim, trimethoprim/ sulphamethoxazole, erythromycin, azithromycin, spiramycin, lincomycin, clindamycin, vancomycin and teicoplanin.

Statistical analysis

The data were evaluated using the SPSS statistical package (SPSS Inc, Chicago IL), version 14. The categorical variables were presented as numbers and percentages .

Logistic regression was used for determining the association of various risk factors and the development of Nls. The odds ratios and 95% confidence intervals (Cls) were determined against the reference categories. A *P* value of $\leq$0.05 was taken as significant

3. Results

During the study period, 15 % of patients admitted to different ICUs, developed different nosocomial infections (900/5979). We reported many risk factors for the development of nosocomial infections as burns, the presence of endotracheal tubes, ventilators, nasogastric feeding, central venous lines, intravenous lines and the stay in hospital for more than 2 weeks (Table 1).The majority of patients (72 %) received empirical antibiotics mostly for prophylaxis (98%). (Table 2). The most common

antibiotics prescribed were cephalosporins (36.7%) and penicillins (32%) (Table 3). The commonest nosocomial infection was lower respiratory tract infection followed by bacteraemia (Table 4). A total of 1113 aerobic bacteria were isolated from different specimens. Gram negative bacteria represented the majority of causes (54.2%), amongst which *Klebsiella* spp. was the most prominent (30%). On the other hand, gram positive organisms caused 45.8 % of the nosocomial infections, with MRSA being the commonest (23.6%) (Table 4). Resistance rates of some gram negative and gram positive isolates are shown in tables 5 , 6, 7 and 8.

Table (1) :Odds ratio, 95% confidence intervals, and *P* values for variables in logistic regression analysis of nosocomial infections.

Variables	Odds ratio	95% CIs	*P* value
Intrinsic risk factors			
Burns	3.48	1.20 - 10.12	0.04 *
Diabetes Mellitus	1.08	0.56 - 2.09	0.9
Obesity	1.74	0.41-7.41	0.7
Malnutrition (albumen < 3g/L)	1.05	0.32 - 3.44	0.8
Extrinsic risk factors			
Endotracheal tube	9.85	5.36 - 18.11	0.000 **
Ventilator	2.96	1.68 - 5.21	0.002 **
Urinary catheters	2.77	2.5-3.1	0.001**
Intravenous catheters	2.31	2-2.7	0.001**
Nasogastric feeding	1.69	1.04 - 2.74	0.04 *
Central venous line	2.30	1.55 - 3.08	0.05 *
Operation	1.76	0.93 - 3.35	0.1
Drainage tube	1.74	0.41-7.41	0.7
Dialysis	0.84	0.12-6.46	0.7
Length of Hopital stay			
< 1w	0.98	0.65-1.47	0.9
1-2 w	0.94	0.4-2.21	0.9
> 2 w	1.41	1.2-1.7	0.001**

*Significant; ** Highly significant

Table (2): Intake of empirical antibiotics in patients with nosocomial infections in the adult Intensive Care Units.

Intake of empirical antibiotics	No (%)
Patients receiving no empirical no antibiotics	252 (28)
Patients receiving empirical antibiotics	648 (72)
One	227
More than one	421
Indication	
Therapeutic	13 (2)
Prophylactic	635 (98)

Table (3):Commonly prescribed empirical antibiotics among the studied group.

Antibiotic	No (%) of patients
Penicillins	**732 (32)**
• Benzyl penicillin	147 (6.36)
• Extended spectrum:	
Ampicillin	1 (0.04)
Amoxacillin	1 (0.04)
Piperacillin and tazobactam	1 (0.04)
• Penicillin + B lactam inhibitor	
Amoxacillin + clavulanic acid	312 (13.5)
Ampicillin Sulbactam	43 (1.86)
• Amoxycillin and flucloxacillin	227 (9.8)
Cephalosporins	**841(36.7)**
•First generation	
Cephradine	1 (0.04)
Cephalexin	2 (0.09)
Cephazoline	8 (0.35)
• Second generation	
Cefrozil	3 (0.13)
• Third generation	
Cefotaxime	315 (13.6)
Cephtazidime	244 (10.6)
Cefoperazone	106 (4.6)
• Fourth generation	
Cefepime	162 (7.00)
Quinolones	**253 (11.1)**
Ciprofloxacin	148 (6.40)
Levofloxacin	107 (4.63)
Gatifloxacin	4 (0.17)
Aminoglycosides	**223 (9.7)**
Amikacin	131 (5.66)
Gentamycin	92 (3.98)
Carbapenerns	**62 (2.7)**
Meropenem	60 (2.59)
Imipenem	2 (0.09)
Glycopeptides	**40 (1.7)**
Vancomycin	39 (1.69)
Teicoplanin	1 (0.04)
Lincosamides	**96 (4.2)**
Clindamycin	93 (4.02)
Lincomycin	3 (0.13)
Macrolides	**41 (1.8)**
Clarithromycin	36 (1.56)
Azithromycin	5 (0.22)
Others	
Sulfathiazole	**1 (0.04)**

Table (4): List of bacteria isolated from nosocomial infections according to the site of infection

Bacteria	Blood	Endo-tracheal swab	Sputum	Urine	Wound swab	Bed sore swab	No (%)
Gram negative							
Klebsiella	52	130	103	35	20	0	**603 (54.2)**
E coli	10	28	20	20	7	3	340 (30)
Pseudomonas	8	38	17	9	8	5	88 (7.9)
Acinetobacter	7	20	13	10	0	0	85 (7.6)
Proteus	0	8	4	18	6	4	50 (4.5)
Gram positive							40 (3.6)
MRSA	60	125	45	10	15	10	**510 (45.8)**
CoNS	55	15	14	10	2	3	263 (23.6)
VSE	10	25	25	5	8	5	99 (8.9)
MSSA	8	17	12	5	5	3	62 (5.6)
VRE	8	10	7	5	4	2	50 (4.5)
Total	**218 (19.3)**	**416 (36.8)**	**260 (23)**	**127 (11.2)**	**75 (6.6)**	**35 (3.1)**	36 (3.2) **1113 (100)**

MRSA: methicillin resistant *Staphylococcus aureus*
CoNS: Coagulase negative *Staphylococci*
VSE: Vancomycin sensitive enterococci.
MSSA: Methicillin sensitive *Staphylococcus aureus*
VRE: Vancomycin resistant enterococci

Table (5): Resistance rates of isolated Gram negative bacteria to penicillins and cephalosporins

Antibiotics	% of Resistant Bacteria				
	Klebsiella spp.	***E coli***	***Pseudomonas*** spp.	***Proteus*** spp.	***Acinetobacter*** spp.
•Penicillins					
Ampicillin	95	90	97	99	88
Amoxacillin	93.7	90	96	98	90
Piperacillin	88	86	90	90	69
Carbinicillin	96.8	90	98	99	70
Piperacillin /tazobactam	83	85	95	97	67
Amoxacillin/ clavulanic acid	91	90	94	95	68
Ticaracillin/ clavulanic acid	90.4	90	95	96	65
•Cephalosporins					
2nd generation					
Cefaclor	93	90	94	96	71
Cefuroxime	92	90	96.5	95	70
Cefamandol	93	92	98	96.5	75
3rd generation					
Cefixime	91	90	89	90	72
Cefpodoxime	88	92	92	70	75
Cefotaxime	90	91.5	90	91	68
Cephtazidime	93	90	89	90	65
Cefoperazone	93.6	93	88	92	70
Cephtazidime/ clavulanic acid	92	91	90	91	62
Ceftriaxone	91	85	89.5	86.7	66
Cefuroxime	92	90	91	90	68
4th generation					
Cefipime	88	89	90	89.5	76

Table (6): Resistance rates of isolated Gram negative bacteria to other groups of tested antimicrobials.

Antibiotics	% of Resistant Gram Negative Bacteria				
	***Klebsiella* spp.**	***E coli***	***Pseudomonas* spp.**	***Proteus* spp.**	***Acinetobacter* spp.**
Quinolones					
Ciprofloxacin	81.9	70	92	94	72
Norfloxacin	74.5	68	83	84	68
lomefloxacin	75	70	80	79	62
Gatifloxacin	72	77	78	33.3	60
Carbapenems					
Imipenem	19	14.7	28	27	29
Aminoglycosids					
Amikacin	68	70	57.9	70.5	64.5
Gentamycin	70	60	89	66.7	61.5
Kanamycin	80	85	90	88	63
Netilmicin	85	80	92	50	64
Neomycin	86	85	85	88	65
Streptomycin	87	90	82	80	66
Tobramycin	78.7	65	86	79	67
Spiramycin	75	70	79	76	67
Tetracyclines					
Tetracycline	40	39	45	50	23
Oxytetracycline	39	38	44	52	22
Chloramphenicol	79	70	78	83	56
Trimethoprim					
Trimethoprim/ sulphamethoxazole	77 75	75 76	80 79	82 80	67 65

Table (7): Resistance rates of isolated Gram positive bacteria to penicillins, cephalosporins and carbapenems.

Antibiotics	% of Resistant Gram Positive Bacteria				
	MRSA	**CoNS**	**MSSA**	**VRE**	**VSE**
Penicillins					
Natural penicillin	100	99	95	100	98
Oxacillin	100	88	0	70	80
Ampicillin	87	86	85	72	60
Amoxacillin	79	78	73	70	67
Carbinicillin	88	86	78	70	65
Amoxacillin/ ciavulanic acid	80	75	65	35	40
Ticaracillin/ clavulanic acid	79	72	70	40	59
Cephalosporins					
1ST generation					
Cefazoline	85	82	79	86	85
2nd generation					
Cefaclor	89	79	75	85.7	86
Cefuroxime	89	79	75	85	83
3rd generation					
Cefixime	92.6	85.7	79	85.9	82
Cefotaxime	90	85	83	89	82
Cephtazidime	92	89	82	85	80
Ceftriaxone	85	73	79	85	82
Cefuroxime	89	79	80	83	85
4th generation					
Cefipime	98	95	93	96	92
Carbapenerns					
Imipenem	24	23	29	30	25

MRSA: Methicillin resistant *Staph. aureus*.; VRE: Vancomycin resistant enterococci, CoNS: Coagulase negative *Staphylococci*
MSSA: Methicillin sensitive *Staph. aureus* ; VSE: Vancomycin sensitive enterococci.

Table (8): Resistance rates of isolated Gram positive bacteria to other studied antimicrobials.

Antibiotics	% of Resistant Gram Positive Bacteria				
	MRSA	CoNS	MSSA	VRE	VSE
Aminoglycosides					
Amikacin	70	58.6	55	68	60
Gentamycin	80	83	70	70	59
Kanamycin	89	85	78	68	58
Streptomycin	86	85	83	72	57
Tobramycin	85	75	70	70	55
Tetracyclines					
Tetracycline	78	75	65	80	75
Oxytetracycline	77	76	65	82	72
Chloramphenicol	70	65	57	48	40
Trimethoprim	68	65	59	59	57
Trimethoprim/ Sulphamethoxazole	64	63	56	54	53
Macrolides					
Erythromycin	80	82	85	89.9	80
Azithromycin	90	92	89	90	82
Spiramycin	92	89	90	86	83
Lincosamides					
Lincomycin	80	79	77	76	70
Clindamycin	83	75.9	80	79	72
Glycopeptides					
Vancomycin	39	35	29	35	0
Teicoplanin	20	7	5	10	0
Quinolones					
Ciprofloxacin	78	75	72	75	64
Norfloxacin	65	70	68	69	61
Lomefloxacin	66	69	70	69	60
Gatifloxacin	67	65	65	62	55
Bacitracin	69	70	67	70	63

MRSA: Methicillin resistant *Staph. aureus* ; VRE: Vancomycin resistant enterococci
CoNS: Coagulase negative *Staphylococci* ; MSSA: Methicillin sensitive *Staph. aureus*
VSE: Vancomycin sensitive enterococci.

4. Discussion

Intensive care units (ICUs) have the highest prevalence of nosocomial infections (Nls) in the hospital setting. The high prevalence of Nls in the ICUs is also associated with high antibiotic consumption (Emmerson, 2000).

A recent systematic review and meta-analysis of studies investigating the burden of HAIs in developing countries pointed out that some regions, in particular the Eastern Mediterranean region were poorly represented in the global mapping of HAIs due to paucity of data describing their burden (Allegranzi *et al.*, 2011).

In the present study, we reported that the frequency of hospital acquired infections in the adult medical and surgical ICUs in Assiut University Hospitals was 15%. Assiut University Hospitals are teaching hospitals. It has been reported that the university/teaching hospitals that usually function as referral hospitals and accept patients requiring more complex care generally report higher infection rates (Nejad *et al.*, 2011). This is lower than the percentage reported in a recent Turkish study, where 20.1% of the patients developed a total of 40 intensive care unit-acquired infections (Ozer *et al.*, 2011). In Africa, the overall prevalence of HAI ranged from 2.5% to 14.8%, up to twice as high as the average European prevalence (7.1%) reported by the European Centre for Disease Prevention and Control (European Centre for Disease Prevention and Control, 2008).

Regarding the important risk factors, multivariate analysis demonstrated in this study that burns, the presence of endotracheal tubes, ventilators, urinary catheters, intravenous catheters, central venous lines and nasogastric tubes were important risk factors for NIs. In a previous study in these ICUs, previous administration of antibiotics; diabetes, central intravenous lines, leucocytopenia,

and surgery were independently associated with blood stream infections (Ahmed *et al.*, 2009).

The commonest site of NI varies in different studies; but generally lower respiratory tract infections, bacteraemia and urinary tract infections are the commonest triad in many studies with variable percentages. In our study we found that most of the ICU-acquired infections were lower respiratory tract infections occurring in 60% followed by blood stream infections (19.3%), urinary tract infections (11.2%) and lastly, wound and bed sore infections (9.7%). Studies reported respiratory tract infections to be the commonest but with lower percentages (Azzam and Dramaix, 2001). On the other hand, urinary tract infection was the commonest in other studies (Zoldann *et al.*, 2005). Bloodstream infections were reported to be the predominant in other studies (Erbay *et al.*, 2005). In the present study we reported blood stream infections in 19.3%. This is higher than the previous percentage reported in the same study area by Ahmed *et al.* (2009) where the percentage was only 7.6%. This may be explained by the heavy use of broad spectrum empiric antimicrobials during the study period that may have led to increase MDR bacteria with consequent increase in the frequency of NIs. Our rate is is also higher than the percentage reported by Pourakbari *et al.* (2012) in Iran who found the frequency of blood stream infections during a five year period to be 10.23%.

We found a shift to Gram positive organisms being responsible for 64.7% of the nosocomial blood stream infections, while Gram negative bacteria caused 35.3% only. MRSA was the commonest cause of Gram positive bacteria (27.5%) followed by CoNS (25.2%). The commonest Gram negative bacteria was *Klebsiella* sp (23.9%). Our findings were similar to a previous study done in our intensive care units by Ahmed *et al.*(2009) who reported that Gram positive bacteria were responsible for 69% of isolates, while Gram negative isolates accounted for 29% of them. They also reported these 3 organisms to be the most common causes, but with variable percentages. They found coagulase negative *Staphylococci* in 30.3% followed by *S. aureus* in 29.2% and lastly *Klebsiella pneumoniae* in 10.3%.

Regarding antibiotic consumption in the ICUs, 72% of patients had one or more antibiotics empirically prescribed mostly for prophylaxis in 98%. All classes of antibiotics were prescribed with penicillins and cephalosporins being the most common. This high consumption rate was previously reported in Egypt by El-Teheawy *et al.* (1988) who found that >80% of admitted patients in their study had antibiotics prescribed, and in many cases without documented proof of infection. Among these patients, >30% received repeated courses. The problem of antibiotic resistance is worsened, when the consumption of antibiotics is increased (Oteo *et al.*, 2009), or when used inappropriately or prescribed by the physicians without the availability of proper sensitivity report. Antibiotic resistance is one of the most pressing health problems. It can cause significant danger and sufferings for people who have common infections that once were easily treatable with simple antibiotics. The outcomes of antibiotic resistance are long lasting illnesses leading to high level of morbidity and mortality (Ahmed *et al.*, 2011)

In this study, Gram negative bacilli were the commonest causes of nosocomial infections (54.2%), amongst which *Klebsiella* sp. were the most predominant (30%). Gram positive organisms caused 45.8% of all NIs, with MRSA representing 23.6% followed by CoNS in 8.9%. Our results are comparable to many studies that reported Gram negative isolates as the predominant causative agents. Ashour and El-Sharif **(**2009) reported that the most frequently isolated Gram-negative bacteria from all clinical specimens were *Klebsiella pneumonia* followed by *Escherichia coli* in Egypt. In addition, Azzam and Dramaix (2001) reported that Gram negative bacteria represented 78.6% of the-causative agents. In a Tunisian study, Gram negative rods constituted 80.8%, among which, *Klebsiella pneumonia* was also the predominant (23.1%) (Kallel et al 2005). In the current study, *Klebsiella* spp. was mostly isolated from sputum and samples from the endotracheal tubes. This finding is in accordance with the result of a previous Egyptian study (Ashour and El-Sharif, 2009).

On the contrary some studies reported Gram positive microorganisms to be the major causative agents (Diekema and Pfaller, 2003). Over the past two decades, there has been a shift in the spectrum of nosocomial pathogens from Gram negative to Gram positive bacteria. Increasing antibacterial resistance is an important factor associated with the emergence of Gram positive cocci (Diekema and Pfaller, 2003). Jones et al (2004) assimilated *in vitro* susceptibility data from over 220000 isolates from ICUs in five countries (France, Germany, Italy, Canada, and the United States) over the period 2000 to 2002. The most frequent gram-negative species isolated from infections in the ICU was *E coli* (7.7%-15.5%), and *P aeruginosa* (10.8%-22.3%) being most common in three (USA, Canada, France) of the five countries following only *S aureus*.

For coagulase negative *Staphylococci* isolated in our study, 88% were resistant to oxacillin . This is in accordance with the study of Pourakbari *et al.* (2012) in Iran who reported it in 89% of the isolates. Also, MRSA isolates were generally more resistant to various groups of the studied antibiotics

in comparison to MSSA. This finding was also documented by Pourakbari *et al.* (2012).

In accordance with Ashour and El-Sharif , (2009)**,** the newest fluoroquinolones (as gatifloxacin) had enhanced activity against gram-positive and negative bacteria compared with second-generation quinolones (ciprofloxacin).

Infection caused by multi-drug resistant bacteria is a serious problem for especially intensive care unit patients (ICU) throughout the world (Jazayeri and Irajian, 2009)..

The phenomenon of multi drug resistant pathogens had emerged in Egypt and worldwide in recent years due to excessive antibiotic misuse (El Kholy *et al.*, 2003). Multidrug-resistant organisms are resistant to one or more classes of antimicrobial agents, such as β-lactams, including penicillins, cephalosporins, and monobactams, carbapenems, fluoroquinolones, and aminoglycosides. The severity and extent of disease caused by these pathogens varies by the population(s) affected and by the institution(s) in which they are found (Chopra *et al.*, 2008).

European surveillance has documented that MRSA, VRE and multidrug -resistant Gram negative bacteria are increasing rapidly in importance (Biedenbach *et al.*, 2004). The stiking finding in this study, is the high percentage of antimicrobial resistance among the isolates. Many multidrug resistant bacteria were isolated including MRSA, VRE and many multiresistant Gram negative rods.

In the present study, Gram negative bacteria showed very high resistance (50-100%) to many groups of antimicrobials, as penicillins, cephalosporins, quinolones, aminoglycosides. Intermediate levels of resistance were to tetracyclines (23-50%), choramphenicol (56-83%) and trimethoprim (65-82%). The least resistance was reported to imipenem (14.7-29%). Many studies also reported multidrug-resistance in many Gram negative isolates. Ashour and El-Sharif (2009)**,** reported high resistance to many groups of antibiotics in Egypt. Erbay *et al.* (2005) reported high resistance rates to first and third generation cephalosporins which were commonly used. Wattal *et al.* (2005) found a correlation between resistance to third generation cephalosporins and increased cephalosporin use. A high resistance rate was also reported by EI- Kholy *et al.* (2003) who performed a similar study in five hospitals in Cairo, but with lower resistance rates.

Karlowsky et al., (2006) reported that *E. coli* isolates showed high resistance to third generation cephalosporins and quinolones. Ahmed *et al.* (2011) reported also high level of quinolone resistance in *E. coli* isolates. Other regions of the world as Spain had much lower rates of *E coli* resistance to ciprofloxacin (22%) (Jones *et al.* , 2010). In the recent study of Pourakbari *et al.* (2012), *E. coli* susceptibility against gentamicin was 77%.

Ceftazidime and cefotaxime resistance are markers for the presence of extended spectrum β lactarnases (ESBL) (EI- Kholy *et al.*, 2003). Regarding ceftazidime and cefotaxime resistance, we found that around 90% of *Klebsiella* , *E coli,* and *Pseudomonas* isolates were resistant suggesting a high prevalence of extended spectrum β lactamase producing strains. Our results were similar to Pourakbari *et al.*, (2012) regarding the resistance of *Klebsiella* to ceftazidime but for *E coli* isolates, they reported that nearly 63% were susceptible. Our isolates were not tested further to confirm ESBL production. In the Egyptian study of Ashour and El-Sharif **(**2009) , all gram-negative species examined were highly resistant to third-generation cephalosporins. Previous reports suggested that ESβL-producing strains were endemic in Egypt (El Kholy *et al.*, 2003). Our results are much higher than the results of a recent Egyptian study where the resistance of *Klebsiella pneumoniae* to ceftazidime was reported in 76.2% (El-Kholy *et al.*, 2012). In a previous study of EI- Kholy *et al.* (2003), the resistance to ceftazidime and cefotaxime was 38% and 60% respectively for both antibiotics. Other studies in developing countries reported the rate of ceftazidime resistance to be 68% (Rosenthal *et al.*, 2008). On the other hand very low rates of ceftazidime resistance was reported in the United States (6.2%).(Edwards *et al.*, 2009). The excessive consumption of empirical cephalosporins especially 3rd generation reported in this study may have led to this very high levels of resistance.

Of the important multidrug resistant organisms are *Pseudomonas aeroginossa* resistant to imipenems. In the present study we reported a much lower rate of resistance (28%) compared to 56% in the recent Egyptian study of El-Kholy *et al.* (2012). Other studies reported various rates of resistance as 36.6 % (Rosenthal *et al.*, 2008) and 19.1% (Edwards *et al.* ,2009).

In the present study, imipenem resistance by *Klebsiella* and *E coli* spp. were 19% and 14.7% respectively . These were higher than those reported in another Egyptian study ,where the resistance of these two organisms was reported to be 13.9% and 8% respectively. On the other hand resistance of *Pseudomonas* and *Acinetobacter* sp was 28 % and 29% respectively in our study compared to 40% and 40.9% in the Egyptian study of Ashour and El-Sharif (2009).

The majority of the gram-positive isolates in the present study were highly resistant to penicillins , cephalosporins chloramphenicol , tetracyclines, aminoglycosides, macrolides and lincosamides. Most of these antibiotics were commonly prescribed empirically in the ICUs. They showed intermediate resistance to quinolones (55-79%), bacitracin (63-70%), trimethoprim (53-68%), and chloramphenicol (40-70%). The least resistance was to vancomycin (29-39%) and to teicoplanin (5-20%).

On comparing the pattern of resistance of some gram positive bacteria to the results of other studies, we found that we have very high levels of resistance. Ahmed *et al.* (2011), reported the resistance of gram positive bacteria to macrolides to be 64.3 % and 66.4%..compared to 80-92%in our study. Methicillin resistance in *S. aureus* strains has become widespread in hospitals and ICUs (Diekema *et al.*, 2004). In this study, we reported a very high prevalence of MRSA (23.6%) and CoNS (8.9%). This is much higher than other studies. The Egyptian study of El Kholy *et al.* (2003) reported that 71% of the *Staphylococcus aureus* isolates and 77% of coagulase negative staphylococcal isolates were oxacillin resistant in contrast to our very high percentage where 84% of the total *Staphylococcus aureus* isolates and 88% of coagulase negative *Staphylococci* were methicillin resistant. Different studies showed a great variation in the prevalence of MRSA. A European multicenter study showed that MRSA widely varied among 26 contributing countries. The prevalence was reported as <1 % in northern Europe but >40% in southern and western Europe (Tiemersma *et al.* , 2004). National Nosocomial Infection Surveillance (NNIS) System data demonstrate a steady increase in the incidence of nosocomial infections caused by MRSA among ICU patients over time. MRSA accounts for >60% of *S. aureus* isolates in US hospital ICUs (National Nosocomial Infections Surveillance System, 2004)

The percentage of enterococcal isolates exhibiting vancomycin resistance increased from 0.3% to 7.9% between 1989 and 1993 (CDC, 1993). The prevalence of VRE varies significantly in different countries as it may be influenced by the amount of antimicrobial agents used. The percentage of VRE, in our study (3.2%), was comparable to the result of El Kholy *et al.* (2003) who reported that <5% of the enterococcal isolates were vancomycin resistant. On the other hand, EI- Bialy and Elsharkawy, found VRE to account for 1.1% of the total nosocomial bacteria isolated at Zagazig University hospitals, Egypt .The higher percentage in our study may be explained by the difference in the study area, where our study was conducted in the ICUs, where a high percentage of resistant microorganisms are found, while the other study was performed in different hospital departments. On the contrary, the frequency of VRE was higher in other studies, it was 27% in the ICUs in USA in 2002 (NNIS System,2003).

The high rates of antimicrobial resistance identified in the present study might be attributed to the lack of antibiotic use policies and guidelines in the majority of hospitals in Egypt. More focused studies and efforts are required to establish and regulate the use of antibiotics in Egyptian ICUs (El-Kholy *et al.*, 2012). The major contributing factors to increasing level of resistance to commonly used antibiotics may be free availability of antibiotics over the counter, prescription of antibiotics without susceptibility report, de-escalation according to clinical course, etc. Further research at molecular level is required on those resistant genes carried by these pathogens and the sequences may be compared with similar genes reported from other parts of the World (Ahmed *et al.*, 2011).

Apart from general infection control measures that are considered valuable and irreplaceable, strict antibiotic hospital policies are of urgent need. Several methods have been proposed for the restriction of broad spectrum antibiotics use, including antibiotic cycling, broad empiric antibiotic treatments, prompt de-escalation accordingly to cultures' results and shorter courses of antimicrobial treatment.(Hayash and Paterson 2011; Arnold *et al.*, 2011). Clinicians treating critically ill patients should consider antimicrobial resistance as an important part of their routine treatment plans. Careful, focused attention to this problem at the local ICU level, using a multidisciplinary intervention, will have the greatest likelihood of limiting the development and dissemination of antibiotic-resistant infections.

Epidemiological information will help to implement better infection control policies in ICUs and help collaboration between different ICUs as more knowledge is gained. Therefore, developing nationwide antibiotic policy and guidelines is essential nowadays due to increasing resistance patterns. Also, developing a local antibiogram database will improve the knowledge of antimicrobial resistance patterns to help improve treatment strategies based on unit-specific data. Policies on the control of antibiotic usage have to be enforced and implemented to avoid the evolution of newer generations of pathogens with higher resistance, not only to the older generation drugs, but also to the relatively new ones.

Our study has some limitations. First, molecular typing was not performed on ICU-acquired MDR organisms. Thus the role of patient to patient transmission in the acquisition of such

resistant strains could not be determined. Second, the adequacy of the antimicrobial dosage and the duration of treament was not investigated. Although these limitations, yet our results highlighted the problem of MDR bacteria among ICU patients in Assiut University Hospitals which should be inflicted on the policy of antimicrobial prescription.

We conclude that the prevalence of nosocomial infections in the adult ICUs at Assiut University Hospitals is considerable. Many risk factors for nosocomial infections were found. Empirical antibiotics were widely prescribed for prophylaxis with cephalosporins and penicillins being the commonest. Lower respiratory tract infection was the commonest nosocomial infection. Gram negative bacteria caused most of the nosocomial infections with *Klebsiella* spp. being the predominant. MRSA was the most commonest Gram positive bacteria isolated. All isolates showed very high resistance for most of the studied antibiotic groups. The best sensitivity was to imipenem, vancomycin and teicoplanine. These results may have important implications for formulating antibiotic policies in order to lower the frequency of antimicrobial resistant organisms in the ICUs at Assiut University Hospitals.

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