Nosocomial Device Related Infections inside Intensive Care Units and Inhibitory Effect of Reserpine on Ciprofloxacin Resistance among Clinical Isolates of *Acinetobacter Baumannii*

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Abstract: The goal of this research is to investigate the prevalence of nosocomial device related infections (DRIs) and to study the resistance mechanisms of nosocomial microorganisms to antibiotics, as well as to evaluate the effect of reserpine as efflux pump inhibitor (EPI) among ciprofloxacin resistant *A. Baumannii*that was meropenem resistant. Patients are admitted to intensive care units (ICUs) due to some medical problems which require medical intervention using medical devices which contribute to the incidence of nosocomial infection. One hundred and ninety eight infected cases hospitalized in some Kingdom of Saudi Arabia hospitals from November 2011 to January 2013. A total of 255 isolates were recovered from198 different cases from many sources as sputum, endotracheal tube (ETT), central venous catheter (CVC),central venous pressure (CVP), nasogastric tube (NGT), cerebrospinal fluid (CSF), eye, nose, urine, pus, bedsore, blood, surgical drain and surgical wound. Microbiological identification revealed that (31.20%) of isolates were Gram positive and (68.80%) were Gram negative. In this study 141 nosocomially infected cases that were device and undevice related examined for occurrence of device related infections (DRIs), these cases revealed 157 bacterial isolates. The rate of DRI was (47.77%) and the rate of undevice related infection was (52.23%). Susceptibility to ciprofloxacin and meropenem for *A. Baumannii* isolates was done by Kirby Bauer disc diffusion method. Also minimum inhibitory concentration (MIC) for ciprofloxacin was determined in presence and absence of 25mg/L reserpine by microtitre broth dilution method. Antimicrobial resistance is a serious problem inside ICUs, among the mechanisms of resistance to antimicrobial agents is the efflux pump system. The growing number and rapid increase in antibiotic resistant Acinetobacter isolates to β- lactam antibiotics and carbapenems has prompted us to investigate the resistance mechanisms among *A. Baumannii* isolates. It was found that 0.00% of meropenem resistant *A. Baumannii* (MRAB) isolates were carbapenemase producer by Modified Hodge test (MHT), 100% were Metallo β -lactamase (MBL) producers by EDTA disc synergy test (EDST) and 52% were AmpC by β-lactamase AmpC producers. Detection of potential resistance to ciprofloxacin among MRAB isolates was done by efflux pump test using reserpine as EPI, this test revealed that 76% of MRAB isolates had efflux pump which confer complete or partial resistance to ciprofloxacin. This study demonstrated that EDTA disc synergy test seems to be a better method for detection Carbapenemases than MHT as well as AmpC β-lactamase was a contributory factor for carbapenem resistance among isolates of MRAB, also reserpine was inhibitor for efflux pump which was responsible for ciprofloxacin resistance. Presence reserpine with ciprofloxacin decrease the MIC four folds and one fold in 56% and 20% of MRAB isolate respectively, on the other hand the MIC of (24%) of MRAB isolates were not affected by the presence of reserpine.

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Key words: Nosocomial infections**,** Intensive care units, Reserpine, A. Baumannii.

1. Introduction

Nosocomial infections are today by far the commonest complications affecting hospitalized patients; they are infections which are acquired in hospital by a patient who was admitted to hospital for a reason other than that infection and this infection occurred after more than 48 hours (WHO, 2002; Zsolt Filetot, 2003; Silvano Esposito and Sebastiano Leone, 2006). Intensive care unit is a unit in the hospital where seriously ill patients are cared for by specially trained staff. Patients are admitted to the ICU for a variety of reasons; some patients need close monitoring immediately after a major surgical operation, others may have problems with their lungs that require ventilator support with breathing or may have heart and blood vessel problems (for example, very low or very high blood pressure, a heart attack, or an unstable heart rhythm) needing observation, also patients in the ICU may have an imbalance in the level of chemicals, salts, or minerals in their bloodstream that require close

monitoring as these levels are corrected (Çelik *et al*, 2005). All the previous medical problems require medical intervention using medical devices which cause breaches of the mucosal defences of the innate immune system providing ready access for pathogens in an already immunocompromised patient. The medical intervention to correct the previous mentioned problems results in occurrence of infection inside ICUs. So, an ICU acquired infection is defined as an infection that develops at least 48 hours after admission into ICU (Ylipalosaari, 2007 and Stephenson, 2008).Device related infections (DRI) can be provided by endotracheal intubation, ventilation, urinary catheters, using of peripheral venous catheter, arterial catheters as well as central venous catheter(Stephenson, 2008).

Active efflux is now recognized as an important component of bacterial resistance to most of classes of antibiotics. This mechanism is mediated by efflux pumps which membrane-associated active transporters are promoting the extrusion of toxic compounds including antibiotics from the cells (Zechini and Versace. 2009). Reduced uptake across the outer membrane (which is considered as the significant permeability barrier for both hydrophilic and hydrophobic compounds) of Gram-negative bacteria, constitutes such a mechanism (Lomovskaya *et al,* 2008). Efflux pumps may be specific for one substrate such as tetracycline or may be non-selective i.e. transport a range of structurally dissimilar compounds (including antibiotics of multiple classes) and confer a multiple drug resistance (MDR) phenotype for antimicrobials from at least three different classes. Antimicrobial resistance in an efflux mutant is due to one of two mechanisms either increased expression of the efflux pump protein or the protein contains an amino acid substitution(s) that makes the protein more efficient at export. In either case, the intracellular concentration of the substrate antimicrobial is lowered and the organism becomes less susceptible to that agent so, the minimal inhibitory concentration (MIC) of the antibiotics substrates for the strains that are over expressing an efflux pump are usually two-to eightfold higher than those for a susceptible strain of that species (Piddock, 2006; Zechini and Versace, 2009).It is interesting to note that the newer molecules developed from the main antibiotic classes are less susceptible to efflux than older ones, as demonstrated for the third and fourth generation quinolones versus first and second generation quinolones, for ketolides versus macrolides, or for glycylcyclines versus tetracyclines (Bambeke *et al*, 2006).

Bacterial antibiotic efflux pumps belong to five superfamilies that are classified in two mechanistically distinct types, the first type is primary transporters that couple drug extrusion from the cell with ATP hydrolysis while, the second type is secondary transporters which energized by trans-membrane electrochemical gradients of either protons (proton motive force) or sodium ions (Zechini and Versace, 2009). The inhibition of the proton motive force (PMF) dependent pump may involve a direct interaction with the pump and a reduction in the trans-membrane potential. These inhibitors include Carbonylcyanide m-chlorophenyl hydrazone (CCCP), dinitrophenol (DNP) and valinomycin. Other groups of EPIs that have been investigated have their origin in other therapeutic areas include the antihypertensive reserpine and verapamil. Reserpine enhances the activity of fluoroquinolones on MDR Gram-positive (Piddock, 2006; Zechini and Versace, 2009). The present study aimed that invasive medical intervention is an important contributory factor for the incidence of nosocomial infection in ICUS, and to study the resistance mechanisms, as well as inhibitory effect of reserpine on ciprofloxacin resistant among clinical isolates of *A. baumannii*.

2. Material and Methods

Cases and isolates:

In the present study a total of255 isolates were recovered from198 different cases hospitalized in the following ICUs:medical ICUs, cardiac care units, paediatric ICUs, neonatal ICUsand surgical ICUs; (52) in some Kingdom of Saudi Arabia hospitals from November 2011 to January 2013. These isolates were recovered from different sites as sputum, endotracheal tube (ETT), central venous catheter (CVC), central venous pressure (CVP), nasogastric tube (NGT), cerebrospinal fluid (CSF), nasal, urine, pus, bedsore, blood, surgical drain and surgical wound.

Isolation and identification of microorganisms:

Gram positive and Gram negative isolates were identified microscopically and biochemically according to Koneman *et al*. (1997) and Hawkey (2006[a&b]) as described in appendix A and B.On the other hand *A.baumannii*isolates were confirmed and identified to species level by API10S.

Inhibitory effect of reserpine on ciprofloxacin resistance

It was studied on 25 isolates of meropenem resistant *A. baumannii* (MRAB) that were subjected to ciprofloxacin susceptibility test by Kirby Bauer disc diffusion method using Ciprofloxacin (CIP 5µg) disc from HIMEDIA. Laboratories, PVT. Limited, India, also minimum inhibitory concentration for ciprofloxacin was done for these isolates in absence and presence of reserpine by microtitre broth dilution method.

Kirby Bauer disc diffusion method:

The inoculum was prepared from isolation culture plate by touching with a loop the tops of each of 3–5 colonies of similar appearance of the tested organism. This growth was transferred to a tube of saline after the inoculum has to be made from a pure culture a loopful

of the confluent growth was suspended in another tube of saline, the prepared bacterial suspension in the second saline tube was compared with the turbidity standard and the density of the test suspension was adjusted to the turbidity standard (0.5 -1.0 McFarland) by adding more bacteria or more sterile saline. Cotton swab was dipped into the adjusted bacterial suspension of tested organism. The swab was streaked three times all over the surface of the Mueller-Hinton agar (MHA) which purchased from Difco Laboratories, U.S.A. by rotating the plate through an angle of 60° after each application. Finally, the swab was passed around the edge of the agar surface. The inoculum was left to dry for a few minutes at room temperature with the lid closed. The antimicrobial discs were placed on the inoculated plates using a sterile forceps. The results were interpreted according to the critical diameters as shown below according National Committee of Clinical Laboratory Standards (NCCLS) 2002 (Vandepitte *et al,* 2003).

Determination of MIC by microtitre broth dilution:

MIC of ciprofloxacin was done (with and without reserpine). A 50 µl of double strength nutrient broth were dispensed into all wells of a microtitre plate using multipipettor. Each isolate needs 3 row in the microtitreplate; one row for determination of MIC of ciprofloxacin alone and two rows for determination of MIC of ciprofloxacin with reserpine. The test was done as following: 50 µl from ciprofloxacin stock solution were pipetted into the 3rd well of row A. The ciprofloxacin was mixed into the 3rd well in row A by sucking up and down 6-8 times using the multipipettor, then 50 µl from 3rdwell in row A were withdrawn and added to4th well, this makes the concentration of ciprofloxacin in 4thwell is one fold diluted than the 3rd well, then 50 µl from 4thwell were withdrawn and added to 5th well and mixed well, then 50 µl from 5th were withdrawn and added to 6th well, the procedure was repeated till well No.12 in row A, after adding the ciprofloxacin to all wells 50 µl from bacterial inoculum were added to these wells and mixed well. The same procedure was done for determination of MIC of ciprofloxacin with reserpine but the last 50 µl that withdrawn from well No.12 in row B were added to well No. 1 in row C in which the concentration of ciprofloxacin in this well was 0.48µg/ml, then 50 µl were withdrawn from well No. 1in row C and added to well No.2 in row C, where the final concentration of ciprofloxacin in this well was 0.24 µg/ml, the last 50 µl that withdrawn from well No. 2 in row C were added to well No.3 in row C in which the concentration of ciprofloxacin in this well was 0.12 µg/ml, the last 50 µl that were withdrawn from this well were discarded out, then 50 µl from inoculum and 50 µl from reserpine were added to these wells. Regarding to negative control the first well in row A and row B were filled with ciprofloxacin and broth only. Regarding to positive control 50 µl of the bacterial inoculum added to the broth in the second well in row A and row B. To exclude any antibacterial activity for reserpine solution, 50 µl of 750µg/ml were added to broth and inoculums in 3rdwellof row C. The plates were incubated at 37^{o}C for 18 hours and read by eye (Amsterdam, 1996 and Andrews, 2001).

Phenotypic detection of resistance mechanism of *A. baumannii* isolates to cephalosporins, meropenem and ciprofloxacin by: Modified Hodge test (MHT) to evaluate Carbapenemase, EDTA disc synergy test (EDST) to determine Metallo β –lactamase, Amp C disc test to Amp C and Efflux pump to determine Efflux pump inhibitor.

3. Results

This study revealed that 78/255 (30.58%) of isolates were Gram positive and 177/255 (69.42%) were Gram negative. Gram positive isolates were *S. aureus*, coagulase negative staphylococci (CONS)and *Enterococcus spp.* with the following frequencies 12.94% (33/255), 16.86 % (43/255) and 0.78% (2/255) respectively, where Gram negative isolates were *E.coli, K.pneumoniae, Enterobacterspp, A.baumannii, Ps. aeruginosa, Proteus mirabilis and Proteus vulgaris* with following frequencies 15.69% (40/255), 14.12%(36/255),3.92% (10/255), 12.16% (31/255), 12.55% (32/255), 5.10% (13/255) and 5.88% (15/255) respectively as described in Fig. (1).

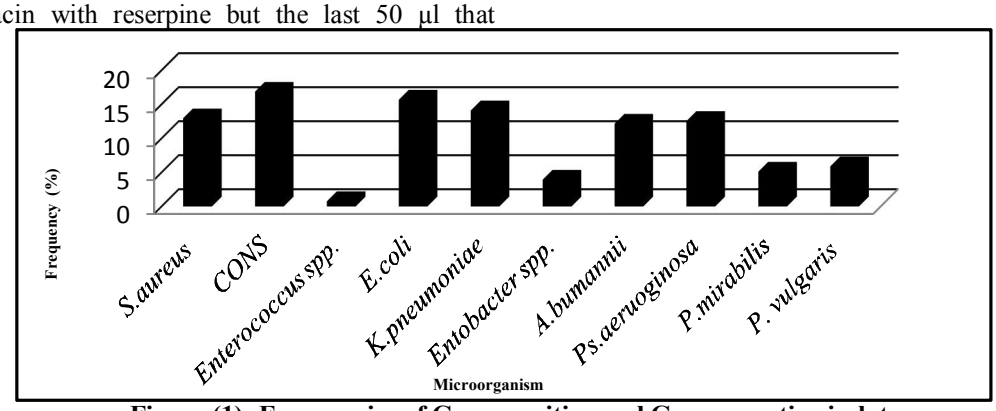

Figure (1): Frequencies of Gram positive and Gram negative isolates

The frequencies of Gram positive isolates inside MICU, SICU, NICU, PICU and CCU were 9.41% (24/255), 8.63% (22/255), 7.45% (19/255), 2.74 % (7/255) and 2.35% (6/255) respectively, on other hand the frequencies of Gram negative isolates inside MICU, SICU, NICU, PICU and CCU were 32.16% (82/255), 27.47% (70/255), 4.70% (12/255), 0.78% (2/255) and 4.31% (11/255) respectively as described in Fig. (2).

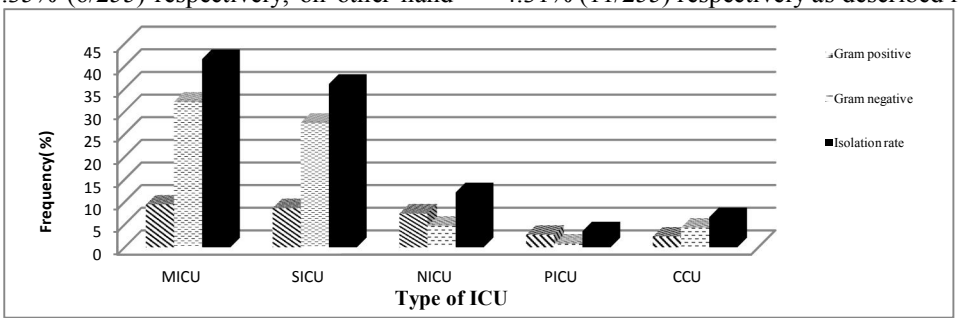

Figure (2): Frequencies of Gram positive and Gram negative isolates inside different ICUs

Seventy five infections (47.77%) out of the 157 infections were device related, while the remaining 82 infections were undevicerelated (52.23%). The frequencies of Gram positive and Gram negative isolates in DRIs were 15.28% (24/157) and 32.49% (51/157) respectively, on the other hand the percentages of Gram positive and Gram negative isolates in undevice related infection were 15.92% (25/157) and 36.31% (57/157) respectively (Figure. 3).

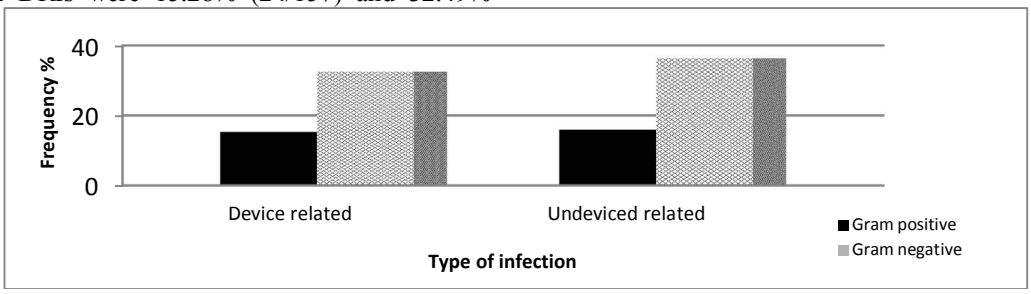

Figure (3): Frequencies of device and undevice related infections among Gram positive and Gram negative isolates.

By examination of urinary tract infection it was found that 47.10 % (16/34) were due insertion of urinary catheter for a long time, while 52.90% (18/34) were undevice related. Investigation of respiratory tract infection revealed that 31.25% (15/48) were due endotracheal intubation and 68.75% (33/48) were undevice related. Regarding to surgical wound infection 19.35% (6/31) were related to insertion of postoperative drains, while 80.65% (25/31) were undevice. Blood stream infection was the most type of nosocomial infection that was device related in which 69.77% (30/43) of infections were attributed to insertion of venous catheters. This study revealed that the one case of meningitis was undevice related (Figure. 4).

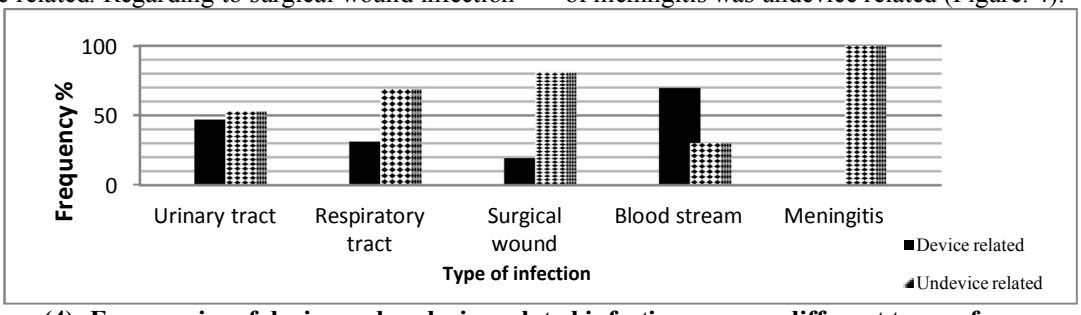

Figure (4): Frequencies of device and undevice related infections among different types of nosocomial infections.

On the other hand to investigate *A.baumanni i*isolates were confirmed and identified to species level by API10S as described in Tab.(1) to further investigation the efflux pump inhibition among *A. baumannii* using efflux pump inhibitor.

Table (1): Results of biochemical identification of *A. baumannii* using API 10 S.

Reaction / Isolate No.	ONPG*	GLU*	ARA*	LDC*	ODC*	CIT*	H_2S*	URE*	TDA*	IND*	OX*	NO_2*
7	-	+	+	-	-	+	-	-	-	-	-	-
89	-	+	+	-	-	+	-	-	-	-	-	-
112	-	+	+	-	-	+	-	-	-	-	-	-
117	-	+	+	-	-	+	-	-	-	-	-	-
125	-	+	+	-	-	+	-	-	-	-	-	-
143	-	+	+	-	-	+	-	-	-	-	-	-
145	-	+	+	-	-	+	-	-	-	-	-	-
147	-	+	+	-	-	+	-	-	-	-	-	-
154	-	+	+	-	-	+	-	-	-	-	-	-
159	-	+	+	-	-	+	-	-	-	-	-	-
160	-	+	+	-	-	+	-	-	-	-	-	-
166	-	+	+	-	-	+	-	-	-	-	-	-
178	-	+	+	-	-	+	-	-	-	-	-	-
181	-	+	+	-	-	+	-	-	-	-	-	-
189	-	+	+	-	-	+	-	-	-	-	-	-
191	-	+	+	-	-	+	-	-	-	-	-	-
192	-	+	+	-	-	+	-	-	-	-	-	-
194	-	+	+	-	-	+	-	-	-	-	-	-
196	-	+	+	-	-	+	-	-	-	-	-	-
199	-	+	+	-	-	+	-	-	-	-	-	-
206	-	+	+	-	-	+	-	-	-	-	-	-
207	-	+	+	-	-	+	-	-	-	-	-	-
210	-	+	+	-	-	+	-	-	-	-	-	-
219	-	+	+	-	-	+	-	-	-	-	-	-
223	-	+	+	-	-	+	-	-	-	-	-	-
227	-	+	+	-	-	+	-	-	-	-	-	-
236	-	+	+	-	-	+	-	-	-	-	-	-
240	-	+	+	-	-	+	-	-	-	-	-	-
248	-	+	+	-	-	+	-	-	-	-	-	-
251	-	+	+	-	-	+	-	-	-	-	-	-
254	-	+	+	-	-	+	-	-	-	-	-	-

* **ONPG;**O-nitro-phenyl-β-D-galactopyranoside**, GLU;** Oxidation/fermentation of glucose, **ARA;** Oxidation/fermentation of arabinose, **LDC**; Lysine decarboxylase, **ODC**; Ornithine decarboxylase, **CIT;** Citrate, **H_2S**;Hydrogen sulfide, **URE;** Urease, **TDP**; Tryptophan deaminase, **IN;** Indole**, OX;** Oxidase and **NO_2;** NO_2 production.

Identification of antimicrobial resistance:

The frequency of potential ESβL production among *E.coli*, *K.pneumoniae*, *Enterobacter*spp, *A. baumannii, Ps. Aeruginosa*and *Proteus* spp. was 85% (34/40), 83.33% (30/36), 60% (6/10), 100% (31/31), 71.87% (23/32) and 71.42% (20/32) respectively as described in Tab.(2) and Fig.(5).

Table (2): Frequencies of different resistance mechanisms among MRAB

Test	No. of isolates	Positive		Negative	
		No.	%*	No.	%*
Modified Hodge	25	0	0.0	25	100.0
EDTA disc synergy	25	25	100.0	0	0.00
AmpC disc	25	13	52.0	12	48.0
Efflux pump	25	19	76.0	6	24.0

*Percentage was correlated to the number of MRAB

The growing number and rapid increase in antibiotic resistant *Acinetobacter* isolates to β- lactam antibiotics and carbapenems has prompted us to investigate the resistance mechanisms among *A. Baumannii* isolates. It was found that 0.00% of MRAB isolates were carbapenemase producer by MHT, 100% were MBL producers by EDST and 52% were AmpC producers. Detection of potential resistance to ciprofloxacin among MRAB isolates was done by efflux pump test using reserpine as EPI, this test revealed that 76% of MRAB isolates had efflux pump which confer complete or partial resistance to ciprofloxacin. This study demonstrated that EDTA disc synergy test seems to be a better method for detection carbapenemases than MHT as well as AmpC β-lactamase was a contributory factor for carbapenem resistance among isolates of MRAB, also reserpine was inhibitor for efflux pump which was responsible for ciprofloxacin resistance.

Phenotypic resistance mechanisms among *A. baumannii* isolates:

This investigations that done on *A.baumannii* to detect some potential resistance mechanisms that play a role in its resistance to β-lactam, carbapenems and quinolones, revealed that 0% (0/25) were negative for carbapenemase production by modified Hodge test,

while 100% (25/25) were metallo β- lactamase producers by EDS with ceftazidime and meropenem and 52% (13/25) were positive AmpC producers. Efflux pump test that was done to detect the role of potential efflux in resistance to ciprofloxacin among MRAB showed that 76% (19/25) of these isolates were affected by the action of EPI as described in Tab.(2) and Fig. (6-10).

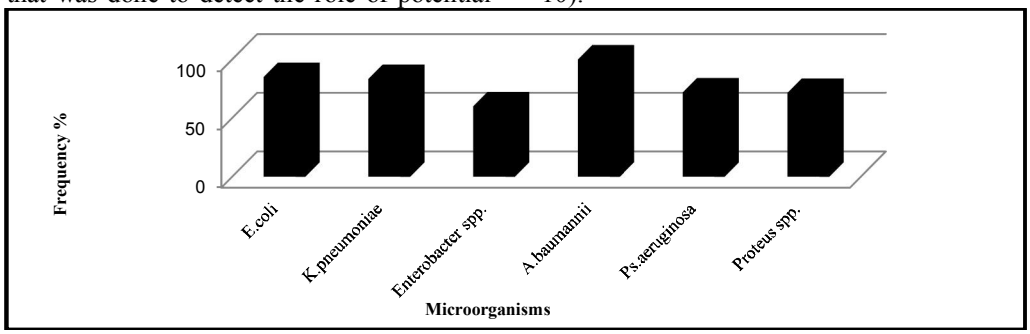

Figure (5): Frequencies of potential ESβL producers among Gram each negative

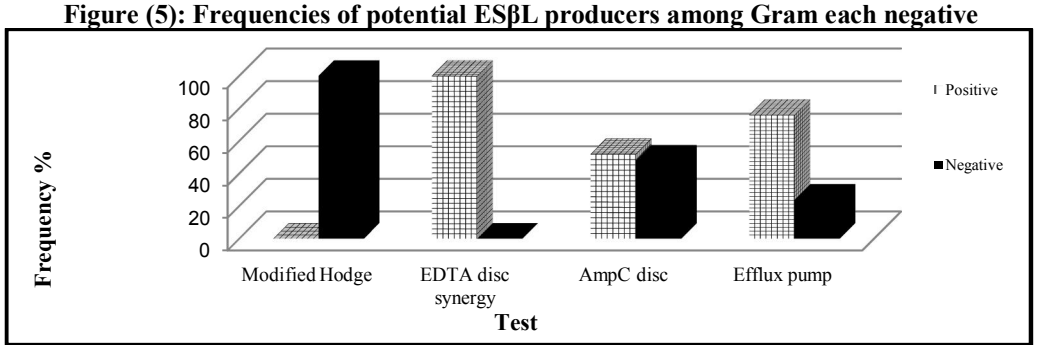

Figure (6): Frequencies of different resistance mechanisms among MRAB

Table (3): Minimum inhibitory concentration values of efflux pump test using efflux pump inhibitor (reserpine) among MRAB

Isolate No.	Antimicrobial agent	Antimicrobial agent + efflux pump inhibitor
	Ciprofloxacin	Ciprofloxacin + reserpine 25mg / L
	MIC (µg /mL)	MIC (µg /mL)
A.baumannii 1	7.81	0.48
A.baumannii 2	3.9	0.24
A.baumannii 3	3.9	0.24
A.baumannii 4	7.81	3.9
A.baumannii 5	3.9	0.24
A.baumannii6	3.9	0.24
A.baumannii 7	7.81	0.48
A.baumannii 8	3.9	0.24
A.baumannii9	3.9	0.24
A.baumannii 10	3.9	3.9
A.baumannii 11	3.9	3.9
A.baumannii 12	7.81	3.9
A.baumannii 13	3.9	0.24
A.baumannii 14	7.81	3.9
A.baumannii 15	3.9	0.24
A.baumannii 16	3.9	3.9
A.baumannii 17	7.81	3.9
A.baumannii 18	3.9	3.9
A.baumannii 19	3.9	3.9
A.baumannii 20	7.81	3.9
A.baumannii 21	3.9	0.24
A.baumannii 22	3.9	0.24
A.baumannii 23	3.9	0.24
A.baumannii 24	3.9	0.24
A.baumannii 25	3.9	3.9

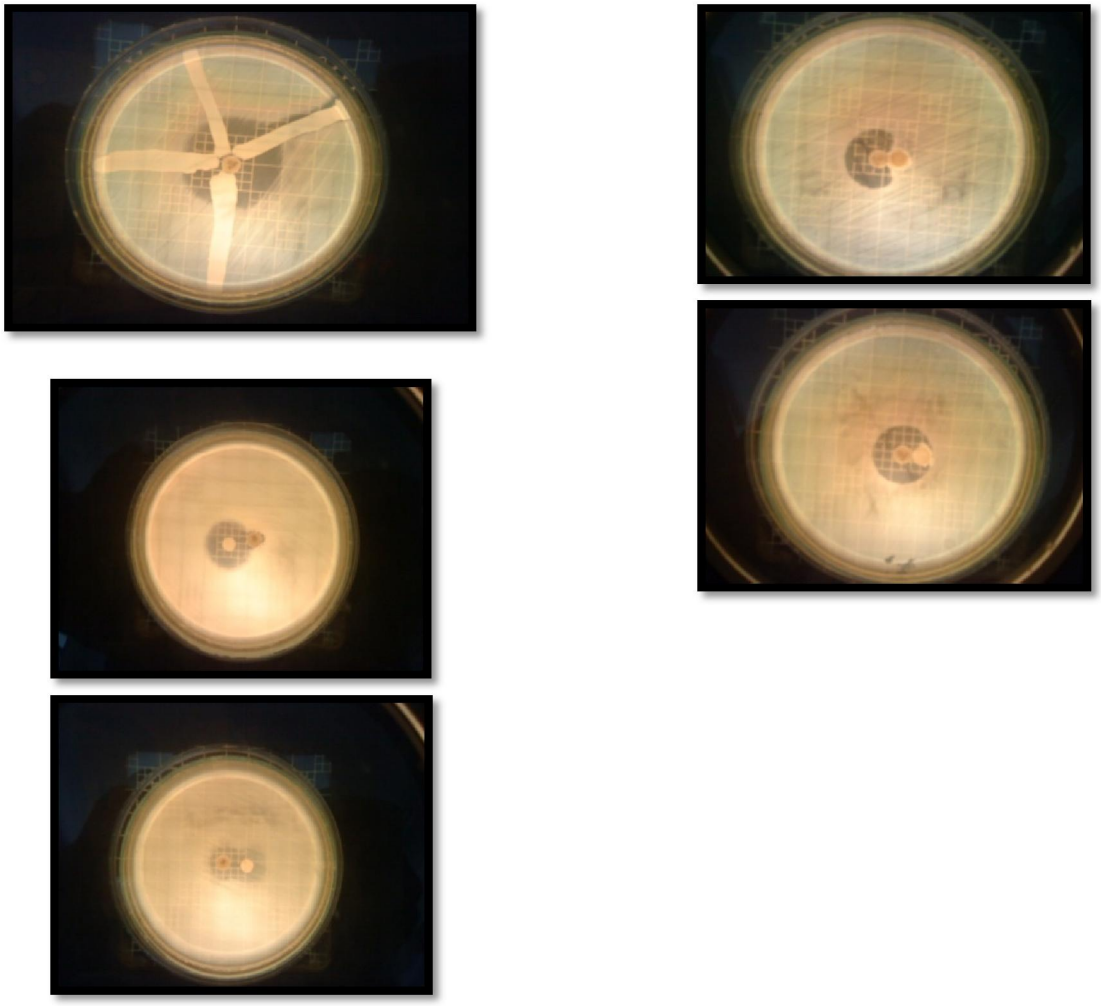

Figure (8): Positive EDTA disk synergy test

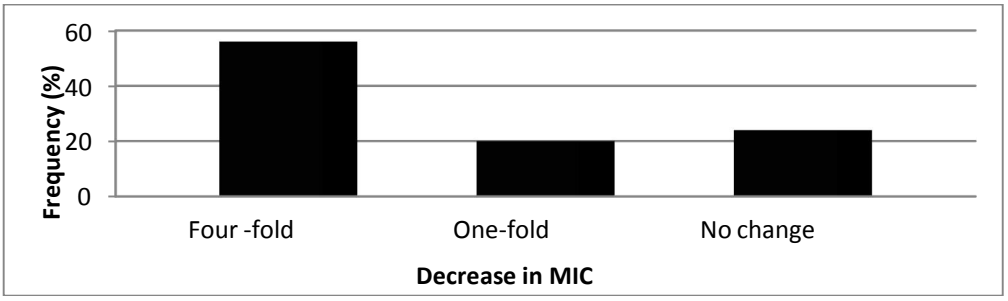

Figure (11): Variation of MIC values among MRAB due to efflux pump inhibitor.

4. Discussion

Nosocomial infections are one of the leading causes of death. Also nosocomial infections lead to disability and emotional stress of the patient and may lead to disabling conditions that reduce the quality of life. The economic costs are considerable impacts of nosocomial infections due to increased length of hospital stay for infected patients, the increased use of drugs, the need for isolation and the use of additional laboratory and other diagnostic studies (WHO, 2002).

The intensive care unit (ICU) is an integral part of hospital care and provides a higher level of monitoring and treatment for patients than would be found on general wards. The role of the ICU is particularly valuable for patients with serious but potentially reversible, life threatening conditions and is provided regardless of age (Stephenson, 2008). The main risk factors for ICU acquired infection can be divided into three key groups which are related to patient characteristics and underlying diseases, acute disease

process, and to the use of invasive diagnostic or therapeutic procedures. Underlying diseases can impair host defence mechanisms, predisposing to the development of ICU infection. Patients on immunosuppressive medication, older patients and patients with malnutrition are at an increased risk. These endogenous risk factors are hard to influence by preventive methods (Ylipalosaari, 2007). There are four main types of ICU acquired infection: urinary tract infection (UTI), blood stream infection (BSI), respiratory tract infection (RTI) and surgical wound infection. Urinary tract infectionsare the most common nosocomial infections which account for about 40% of all hospital acquired infections and constitute a major source for nosocomial septicemia and related mortality in acute care hospitals **(**Taher and Golestanpour, 2009).

In the present study a total of 255 isolates were recovered from198 different cases hospitalized in the following ICUs:medical ICUs, cardiac care units, paediatric ICUs, neonatal ICUs and surgical ICUs; (52), these isolates were recovered from different sites assputum, ETT, CVC, CVP, NGT, CSF, eye, nose, urine, blood, pus, surgical drain, wound and bedsore. This study found that out of 198 cases 141 cases were infected (71.21%), whereas 57 cases were colonized (28.79%). Single bacterial isolates were recovered from 142 cases (71.72%), while 56 cases were found to be carrying more than one bacterial isolates (28.28%). This study revealed that 78/255 (30.58%) of isolates were Gram positive and 177/255 (69.42%) were Gram negative. Gram positiveisolates were *S. aureus, coagulase negative staphylococci (CONS) and Enterococcus spp.* with the following frequencies 12.94% (33/255), 16.86 % (43/255) and 0.78% (2/255) respectively, where Gram negative isolates were *E. coli, K. pneumoniae, Enterobacterspp, A. baumannii, P. aeruginosa and Proteus mirabilis and Proteus vulgaris* with following frequencies 15.69% (40/255), 14.12%(36/255), 3.92%(10/255), 12.16%(31/255), 12.55% (32/255), 5.10% (13/255) and 5.88% (15/255). Our study included many types of nosocomial infections, the main type of these infection was respiratory tract infection 30.58% (48/157) followed by BSI 27.38% (43/157),UTI 21.66% (34/157), surgical wound infection 19.75% (31/157) and CNS infection 0.63%(1/57). It was found that 47.77% (75/157) of ICU acquired infections were device related and 53.23% (82/157) were undeviced related.

The present study was agree with different studies all over the world which reported that the nosocomial BSI creates a serious health problem in hospitals all over the world. In addition, patients admitted to ICUs have a higher risk of nosocomial BSI than those admitted to other types of units(Ahmed *et al*, 2008). While in healthy subjects the lower respiratory tract is usually sterile, the incidence of bacterial colonization can be as high as 80% in subjects who are intubated and mechanically ventilated (Drakulovic *et al*, 2001). Intensive care unit acquired infection of the respiratory tract is a common complication among patients who receive medical care in this setting. Colonization of the respiratory tract by Gram-negative and Gram-positive bacteria may precede infection of the lower respiratory tract, including pneumonia that is associated with considerable morbidity and mortality (Falagas *et al*, 2006). Surgical wound infections are increasing in frequency. They have a negative effect on length of stay among ICU patients and patients on other wards. The reported incidence of surgical wound infections ranges from 15% to 38% (Çelik, 2007).Infection inside ICU can occur due to invasive diagnostic and assisted procedures which cause breaches of the mucosal defences of the innate immune system provided by endotracheal intubation, ventilation, urinary catheters; use of peripheral venous and arterial catheters, as well as central venous catheters can all provide ready access for pathogens in an already immunocompromised patient (Stephenson, 2008).

The rates of nosocomial infections inside ICUs are approximately 3 times higher than elsewhere in hospitals. The important risk factors for acquisition of infection are invasive procedures, which include operative surgery, intravascular and urinary catheterization and mechanical ventilation of the respiratory tract (Sallam *et al*, 2005). The risk of UTI increases if the patient has an indwelling urinary catheter for a longer duration in which the most nosocomial UTIs develop after urinary tract manipulation (Damani, 2004).Over the last 50 years, the use of invasive ventilator support has undoubtedly represented an advance in the treatment of respiratory insufficiency. Although it saves many lives, the application of positive airway pressure via a prosthesis placed in the airways can produce higher frequency of respiratory infections due to the impairment of local defence mechanisms caused by the presence of the tube (De Carvalho, 2006). Regarding to blood stream infection, this type of nosocomial infection occur two to seven times more often in ICU patients than in ward patients. Patients admitted to SICUs carry an even higher risk of nosocomial BSI than those admitted to other types of critical care units(El-Hawary and Abdel Haleim, 2004).

In this study, the growing number and rapid increase in antibiotic resistant *Acinetobacter* isolates to β- lactam antibiotics and carbapenems has prompted us to investigate the resistance mechanisms among *A. Baumannii* isolates. It was found that 0.00% of MRAB isolates were carbapenemase producer by MHT, 100% were MBL producers by EDST and 52% were AmpC producers. This study demonstrated that EDTA disc synergy test seems to be a better method for detection

Carbapenemases than MHT as well as AmpC β-lactamase was a contributory factor for carbapenem resistance among isolates of MRAB, also reserpine was inhibitor for efflux pump which was responsible for ciprofloxacin resistance

Detection of potential resistance to ciprofloxacin among MRAB isolates was done by efflux pump test using reserpine as EPI, this test revealed that 76% of MRAB isolates had efflux pump which confer complete or partial resistance to ciprofloxacin.There is increasing evidence that the efflux pump mechanism plays an important role in high level resistance to antibiotics. Reserpine is a well-established EPI for non-fermenting Gram negative bacteria(Wei-Feng *et al*, 2005).This study revealed that efflux pump system plays a role with/without other mechanisms in ciprofloxacin resistance among MRAB in which the MIC value of ciprofloxacin for MRAB isolates were determined in presence and absence of reserpine, when reserpine was added the MIC values decrease in 76% (19/25) of isolates, on the other hand 24% (6/25) of isolates did not exhibit any change in MIC values after addition of reserpine which indicating that efflux pump system plays a role with/without other mechanisms in resistance to ciprofloxacin. Vila *et al.* (2007) reported that 33% (14/42) of clinical isolates of *A. baumannii* showed at least 4 folds decrease in MIC of ciprofloxacin after addition of reserpine, these findings agreed with the findings of the present study in which the MIC value of ciprofloxacin decreased 4 folds in 56% (14/25) of isolates, on the other hand our findings showed that 20% (5/25) of MRAB isolates showed one fold decrease in MIC value of ciprofloxacin after addition of reserpine. These findings are relatively compared with the findings of Vila *et al*. (2002)in which the MIC of ciprofloxacin was decreased one fold after addition of reserpine in 45.2% (19/42).

The present study revealed the real effect of reserpine (four folds decrease in MIC value) on the MIC value of ciprofloxacin was reported in 66.66% (12/18) of isolates that showed MIC value < 4µg/mL. These findings are consistent with the results of Vila *et al*. (2002) in which the real effect of reserpine was reported in 88.9% (8/9) of isolates that showed low MIC value < 4µg/mL. The present study suggests that the potential resistance mechanisms to ciprofloxacin for both MRAB isolates that not affected by reserpine or those that exhibited one fold decrease in MIC after addition of reserpine may be attributed to other mechanisms rather than efflux pump system such as target modification (mutation in gyrA and parC) and / or decreased uptake due to decreased expression of the outer membrane proteins (Vila *et al*, 1997;Vila *et al*, 2002; SU *et al*, 2005 and Vila *et al*,2007). All strains of *A.baumannii* that included in this study were able to grow in the presence of 256µg/mL reserpine. Therefore, the observed effect of 25µg/mL reserpine on MIC of ciprofloxacin was due to its effect not due to the antibacterial effect of reserpine (Vila *et al*, 2002).

Conclusion

From the present study we might conclude that: nosocomial infections are one of the most frequent medical complications affecting patients admitted to the ICUs.The important risk factors for acquisition of infection are invasive procedures such as surgery, intravascular, urinary catheterization and mechanical ventilation of the respiratory tract. Meropenem was the most effective antibacterial agent against Gram negative isolates and vancomycin was the effective antibacterial agent against Gram positive isolates. As well asthe indiscriminate use of antibiotic inside ICUs lead to emergence of high resistant strains.Multidrug resistant *A.Baumannii*became a problematic organism inside ICUs since *A.baumannii* became resistant to the majority of commercially available antimicrobials (aminoglycosides, cephalosporins, quinolones and imipenem). Efflux pump system plays a role in ciprofloxacin resistance among some *Acinetobacter* isolates. *Presence reserpine with ciprofloxacin decrease the MIC four folds and one fold in 56% and 20% of meropenem resistant A. baumannii (MRAB) isolate respectively*.

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