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KAHRAMANMARAŞ SÜTÇÜ İMAM UNIVERSITY

GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

ISOLATION AND IDENTIFICATIONOF MULTI DRUG RESISTANT *ACINETOBACTER BAUMANNII* IN THE CLINICAL SAMPLES

OMER ALI NAMIQ

MASTER THESIS

DEPARTMENT OF BIOENGINEERING AND SCIENCES

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M.Sc. thesis entitled “ISOLATION AND IDENTIFICATION OF MULTI DRUG RESISTANT *Acenetobacter Baumanii* IN THE CLINICAL SAMPLES” by Omer Ali NAMIQ, who is a student atthe Department of Bioengineering and Sciences, Graduate School of Natural and Applied Sciences, Kahramanmaraş Sütçü İmam University, was certified by all themajority jury members, whose signatures are given below at the date of 28.10.2015.

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ÇOKLU İLAÇLARA DIRENÇLI *ACINETOBACTER BAUMANNII'*LERINKLINIK ÖRNEKLERDEN İZOLASYON VE İDENTIFIKASYONU

(Yüksek Lisans)

Omer Ali NAMIQ

ÖZET

Bu çalışmanın amacı, Haziran-2014-2015 tarihleri arasında Kuzey Irak'ın Süleymaniye kentinde bulunan Central Health Laboratory ve Burn and Plastic Surgery Hospital/Emergency isimli hastanelerinde; çeşitliklinik örneklerinden *Acinetobacterbaumannii* bakterilerini izole ve identifiye ederek, suşların antibiyotik duyarlılıklarını belirlemekti.Suşların izolasyon ve identifikasyonlarında, klasik yöntemler ve VITEK 2 (bioMérieux, Fransa) otomatize sistemi kullanılmıştır. *A.baumannii*suşunun klinik örneklere göre dağılımı;400 yanık yarası ,170 yara eksudatı, 130 idrar ve 30 kulak örneği şeklindeidi. Toplam 730 klinik örneğin 80'inden *A. baumannii* izole edildi.İzole edilen suşların %100.0'ı ampicillin/sulbactam, pipracillin ve colistine duyarlı iken,%97.5'iceftazidime,% 96.25'ici profloxacin vegentamicin, % 93.75cefepime, % 91.25 imipenem ve meropenem, % 87.5tetracycline, % 78.75 levofloxacin, % 75cefoperazone/sulbactam, % 66.25 netlimicin,% 43.75 amikacin, % 11.25 tigecyclineve % 9 trimethoprim/sulfamethoxazole duyarlılık oranında bulundu.

Çalışmamızda, hastanelermizden izole edilen *A. baumannii* suşlarına in-vitro etkinliği en duyarlı antibiyotik kolistindir.İzolatların, beta lactam grubu antibiyotiklere, karbapenemlere ve diğer antibiyotiklere direnç oranlarının yüksek olduğu belirlenmiştir.

Anahtar kelimeler: *Acinetobacterbaumannii*, Antimikrobiyal direnç, Nozokomiyal Patojenler

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ISOLATION AND IDENTIFICATION OF MULTI DRUG RESISTANT *ACINETO-BACTER BAUMANNII* IN THE CLINICAL SAMPLES

(M.Sc. THESIS)

Omer Ali NAMIQ

ABSTRACT

The purpose of this study was to isolation and identification of *Acinetobacterbaumannii* from different clinical samplesfrom Central Health Laboratory and Burn and Plastic Surgery Hospital/Emergency in Sulaimani city, in North Iraq, between June 2014 and June 2015. The strains were isolated and identified by the conventional methods and VITEK 2® (bioMérieux, France) automated system and multi-drug resistance of the bacteria to determine with antimicrobial susceptibility tests.The strains were isolated from the following clinical samples (730): 400 burn, 170wound exudates, 130 urine and 30 ear samples. 80 *A. baumannii* isolated from total 730 clinical samples.*A. baumannii* strains tested were susceptible to ampicillin/sulbactam (100%), pipracillin (100%),colistin (100%) levofloxacin(78.75%), ceftazidime(97.5 %), ciprofloxacin (96.25%), cefepime(93.75%), gentamicin (96.25%), imipenem(91.25%), meropenem(91.25%), tetracycline(87.5%), cefoperazone/sulbactam(75%), netlimicin(66.25%), amikacin(43.75%),tigecycline(11.25%) andtrimethoprim/sulfamethoxazole (9%) in respective percentages.

It was concluded that colistin was the most effective antibiotic for *A. baumannii* isolated in our Hospitals. High rates of resistance to beta-lactam antibiotics including carbapenems, and other antibiotics were remarkable.

Key words: *Acinetobacterbaumannii,* Antimicrobial resistance, Nosocomial pathogens

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LIST OF ABBREVIATIONS

A.baumannii:	Acinetobacter baumannii
MDR-AB	Multi-drug resistance Acinetobacter baumannii
UTI:	Urinary Tract Infection
DNA:	Deoxyribonucleic acid
RNA:	Ribonucleic acid
CR-AB	Carbapenem resistance Acenetobacter baumannii
PDR-AB:	Pan-drug resistance A. baumannii
ICU	Itensive care unite
USA	United state of emerica
MDROs:	Multi-drug resistance organisme
MIC:	Minimum Inhibitory Concentration
TSI:	Triple suger iron
DPPH:	1,1-diphenyl-2 picrylhydrazyl
HPLC:	High Performance Liquid Chromatography
LC:	Liquid Chromatography
MS:	Mass Spectrometry
CE:	Conventional Extraction
MAE:	Microwave Assisted Extraction
ASE:	Accelerated Solvent Extraction
ATCC:	American Type Culture Collection
G–ve:	Gram-negative bacteria
G+ve:	Gram-posative bacteria
ESBLs:	Extended-spectrum beta-lactamases
FRAP:	Ferric reducing antioxidant power
MHA:	Mueller-Hinton agar
SDA:	Sabouraud dextrose agar
DCM:	Dichloromethane
IDSA	Infecious Society of America
VPA	Ventilator associated pneumonia
CLIS	Clinical laboratory Standard Institue
NNIS	National Nosocomial Infection Survellance
OMPs	Outer membrane protiens

1.INTRODUCTION

Acinetobacter. baumannii, a Gram-negative, coccobacilli that are oxidase negative, obligate aerobic and nonmotile bacterial species, has emerged as a nosocomial pathogen. The distribution of *A. baumannii* is widely spread in nature and it can be generally isolated from soil, water, sewage, and also in healthcare settings (Baumann et al., 1968).

Originally, the genus was placed under the family *Neisseriaceae*, but now it has been moved to family *Moraxellaceae*(Washington Winn et al 2006 and Young et al., 2007).

Currently, at least 30 Deoxyribonucleic acid(DNA) homology groups are described within the genus *Acinetobacter*, The important species belonging to this genus are *A. baumannii, A. lwoffii, A. haemolyticus, A. calcoaceticus and A. junii* (Murray Patrick et al., 2003).

The typical Gram stain morphology of *Acinetobacter* species is helpful in theinitial identification. They appear as Gram-negative coccobacillary cells oftenappearing as diplococci. It is important to remember the fact that *Acinetobacter*species may initially appear as Gram-positive cocci in direct smears from clinicalspecimens and in smears prepared from positive blood culture bottle. Most of the strains grow well on MacConkey agar and produce pale or slightly pinkish colonies.Colonies on blood agar appear small to medium sized, smooth, translucent to opaque with entire edges (woodford et al., 2006).

*A. baumannii*is the species most commonly isolated from theclinical specimens 54. It is sacchrolytic and acidifies most of the OF carbohydrates.Rapid identification is made by the ability of *A. baumannii* in fermenting 1% and10% lactose.

Multi-drug resistant *A. baumannii*(MDR-AB) have emerged as a substantial public health problem worldwide. Its clinical significance over the last 15 years has been propelled by its remarkable (Metan et al., 2013).

The ability of this species to acquire resistance to multiple drugs and its high capacity to survive for extended periods in the environment and tolerate wide range of pH, salinity, both wet and dry conditions, and unique ability to survive on almost all nutrient sources utilizes a wide variety of carbon and other energy sources and grows well on routine laboratory media. (Bergogne-BereZin and Towner, 1996)

These abilities have allowed this pathogen to be ubiquitous in the hospital environment as well as the community and have made it one of the frontline pathogens threatening the current antibiotic era (Falagas and Karveli, 2007 b).

*Acinetobacter*s can survive more than four months in the environment,on clothing and bedclothes, bed rails, ventilators and other surfaces in the environment, including sinks and doorknobs, which is longer than 7 days survival period for *S. aureus* (Deitz et al., 1988), *A. baumannii* colonizes healthy humans transiently at a low density on the warm and moist skin of axilla, groin, between toes, throat, nares and intestinal tract but it generally does not cause infection (Young et al., 2007).

Because of this and aforementioned features, it has successfully involved in several outbreaks across the globe. making nosocomial transmission extremely difficult to control. on most of the environmental surfaces has led to an increased concern regarding hospital acquired infections.

In early 1980s, *A. baumannii* nosocomial outbreaks were described in South Europe, particularly in France, Germany, England, Netherlands, and Spain (Villegas and Hartstein, 2003; Fournier and Richet, 2006). The spread to Northern European countries like, Belgium and Germany has been linked with international transfer of colonized patients and airline travel (Peleg et al., 2006a).

International *A. baumannii* clones known as European clones I, II and III have been reported in several European countries and also the United States (van Dessel et al., 2004; Nemec et al., 2004 and Wroblewska et al., 2007).

A data collected by National Nosocomial Surveillance Infection System from several hospitals in New York, United State of America (USA) from 1984 to 2003 on the prevalence of multidrug resistant *Acinetobacter* strains showed that the commonest manifestation was inIntensive Care Unite(ICU) acquired pneumonia due to *A. baumannii*, which was found to be 4% in 1986 and 7% in 2003 (Gaynes and Edward, 2005). This substantial rise in USA was observed to have been contributed by the injured military personnel returning from war in Iraq and Afghanistan (Davis et al., 2005). Similar surveillance from 1997 to 2001 in South American countries like, Argentina, Colombia, Chile, and Brazil showed increase prevalence of MDR-AB (Tognim et al., 2004).

The Infectious Disease Society of America (IDSA) identified *A. baumannii* among the most common seven pathogens threatening the health-care delivery system (Talbot et

aal., 2006). In other statistical studies of European hospitals, *A. baumannii*was among 2% - 10% of all Gram-negative bacterial infections in ICU (Euzeby, 2006).

Acinetobacter species are organisms of low virulence, but it is capable of causing infections, *A. baumannii* is primarily a health care associated pathogen commonly isolated from the hospital environment and hospitalized patients. *Acinetobacter* species are have great potential for rapid spread in hospital settings, and nowadays, they are considered as one of the most important causes of nosocomial opportunistic infections in immunocompromised patients, particularly in the ICU have been identified, including advanced age, severity of illness, immunosuppression, surgery, burns, prolonged mechanical ventilation, prior treatment with broad-spectrum antimicrobials, prior colonization with *Acinetobacter-spp*., and prolonged hospital or ICU stays (Jain and Danziger, 2004; Rungruanghiranya et al., 2005).

Digestive tract is an important reservoir for MDR-AB in hospital environments. It is increasingly reported as the cause of outbreaks and nosocomial infections such as bloodstream infections, ventilator-associated pneumonia (VAP), urinary tract infections (UTI) and wound infections, septicemia and skin infections (Bergogne-Berezin and Towner,1996; Villers et al., 1998: Falagas et al., 2006; Sharma and Johnson, 2008)

Other rare infections include meningitis, endocarditis, osteomyelitis, peritonitis in dialysis patients, and arthritis. Rare cases of community acquired pneumonia caused by *A. baumannii* have also been reported (Leung et al., 2006 and Baran et al., 2008).

In the past few years, *A. baumannii* has emerged as acommon pathogen in burn units, often withincreasing antimicrobialresistance. Burn infections are very sensitive due to loss of skin which is a barrier to prevent the penetration of microorganisms. On the other hand, burn units are suitable environment for the growth of bacteria such as*Acinetobacter* sp. and others, often with increasing antimicrobial resistance.

Once MDR strains become established in hospital environments they can persist for months. Therefore, the growth of MDR organisms such as *Acinetobacter*, resistant to quinolones, cephalosporins, and carbapenems should be considered as key risks of burn wound infections. Microbial colonization and antibiotic sensitivity trends in burn over time necessitate periodic monitoring of these changes in each burn center separately. Aggressive infection control measures should be applied to restrict the emergence and spread of MDR pathogens. (Rafla et al., 2011 and Bayram et al., 2013).

Chim et al (2007). found *Acinetobacterspp*. to be highlyprevalent in Singapore, mainly due to constant introduction of*Acinetobacterspp*. carried onhuman skin (endemic to tropical climate) with every admitted patient. The impact of the affected patients had clinical evidence of infection requiring a treatment with a carbapenem antibiotic and acquisition of *A. baumannii*, associatedin the past few years.

In the 1970s, *Acinetobacter* infections were easily treated with ampicillin, second-generation cephalosporins, minocycline, colistin or gentamicin. Since then, the occurrence of antibiotic resistance has hindered the therapeutic management of *Acinetobacter* infections, particularly *A. baumannii*. The incidence of (MDR-AB) and carbapenem resistant *A. baumannii* (CR-AB) increased between 1980 and 1990.4 - 6 In 1994, Go and coworkers reported a nosocomial outbreak of infections due to *A. baumannii* in a New York hospital. Isolates of *A. baumannii* in this outbreak were resistant to all routinely tested antibiotics, including all cephalosporins and carbapenems. Other studies showed *A. baumannii* was susceptible to only polymyxin B and sulbactam. *A. baumannii* which was resistant to all clinically useful antibiotics was referred to as (PDR-AB).

In the literature, various terms have been used to describe the resistance rate of *A. baumannii* to antibiotics like MDR-AB is used to describe the isolates which are resistant to at least three classes of antibiotics including Penicillins, cephalosporins, fluroquinolones and aminoglycosides. While the term Pandrug- Resistant (PDR)" meaning "all," pandrug resistance is often defined as resistance to all antimicrobials that undergo first-line susceptibility testing that have therapeutic potential against *A. baumannii*.which is used to describe the *A. baumannii* isolates which are Non-susceptibile to all agents in all antimicrobial categories were tested exception of polymyxin (E) and Tigecycline (Cisneros et al., 2002).

The widespread of MDR-AB nosocomial infections in recent years was contributed, at least in part, to the ability to endure for prolonged periods throughout the wide range of environment and the tendency to acquire diverse mechanisms of resistance to antimicrobials (Bergogne-Berezin and Towner, 1996; Maragakis and Perl, 2008).

Acinetobacterspp. possesses a remarkable ability to accumulate resistance mechanisms, in particular to β-lactams (penicillin, cephalosporin and carbapenem), tetracycline, aminoglycoside and fluoroquinolones. The emergence of carbapenem resistance in thiscontext is the most crucial issue because carbapenem are the antibiotic molecules that are used

to treat patients in ICU when all other antibiotics have failed to work (Poirel Nordmann, 2008). *Acinetobacter* infections have become more difficult to treat owing to the emergence of isolates resistant to all commonly prescribed antimicrobial drugs, particularly PDR-AB (Bergogne-Berezin and Towner, 1996 and Poirel Nordmann, 2008).

The phenomena of emergence of MDR in this bacterium have also been traced to mutation in the drug target sites, acquisition of drug resistance genes, or emergence of new acquired mechanisms. The evolution of antibiotic resistance in *Acinetobacter*s can be divided into two major eras: 1) before 1975, that marked the discovery of penicillin and awareness of this pathogen; and 2) after 1975, when there were substantial advances in invasive procedures in ICUs and discovery of powerful antibiotics. During this second period, new pattern of emergence has emerged and have been disseminated to many health care facilities.

Resistance of bacteria against antibiotics is a major problem in ICU. Multidrug-resistant organisms (MDROs) cause longer length of stay, increased mortality and treatment costs, on the other hand, the use of broad-spectrum antibiotics can cause the colonization of the pathogens and serious infections (Manchanda et al., 2010 and Kart et al., 2011)

The potential role played by the capacity of *A. baumannii* to survive in the hospital environment in the spread of epidemic strains is reflected by the success of infection control measures, including environmental decontamination with hypochlorite solutions (Fournier et al., 2006).

More than its virulence characteristics, the main danger associated with *A. baumannii* resides in its capability to acquire antimicrobial-resistance genes extremely rapidly, leading to multidrug resistance (Fournier & Richet, 2006). This extremely rapid development of antimicrobial resistance is likely to result from the ability of *A. baumannii* to respond rapidly to challenges issued by antimicrobials, coupled with the widespread use of antimicrobials in the hospital environment. In particular, the influence of wide use of extended-spectrum cephalosporin and quinolone has been demonstrated (Fournier & Richet, 2006 and Bassetti et al., 2006).

The mortality rate associated with bacteremia is approximately 52% andthat associated with pneumonia ranges from 23% to 73% (Jain and Danziger, 2004).This organismwas responsible for 6.9% of pneumonia, 2.4% of bloodstreaminfections, 2.1% of surgical site infections and1.6% of UTI inICU across USA in 2003 (NNIS, 2004).

From the SENTRY antimicrobialsurveillance program between 1997 and 1999, the prevalence of *Acinetobacterspp.*recovered from respiratory tract infections ranged from 2.0% in Canada, 2.5% in theUSA, and up to 9.7% in Latin America which was 2-fold more frequently foundfrom wounds (Gales et al., 2001).

Also the study in 100 patients whowere admitted to Siriraj Hospital, Thailand in 2014. It was shown that the lowerrespiratory tract was the most common site of MDR-AB nosocomial infection(74.8%) followed by urinary tract (11%), surgical site infection (4.5%) and systemicinfection (4.5%) (Surasarang et al., 2007).

A. baumannii can rapidly modify transmembrane proteins and efflux pumps to prevent current antibiotics from penetrating its inner membrane and executing their mechanism of action. Furthermore, the enhanced ability of *A. baumannii* to obtain DNA from the external environment has allowed the species to obtain novel drug and heavy metal ion resistance genes. With resistance documented to all known classes of antibiotics, as well as cellular mechanisms that prevent desiccation and the action of antimicrobials, the world is in great need of new antimicrobials that can eliminate this dangerous pathogen (Manchanda et al., 2011).

The most common resistance mechanism to carbapenem in *Acinetobacter* is Oxacillin production from Class D (Turton et al., 2006; Woodford et al., 2006; Lu et al., 2008).

BlaOXA-51 genes inherently exist in 97% of *A. baumannii*isolates. The trace of this gene is a sensitive and accurate method for detection of bacteria compared to the commonly used biochemical tests (Turton et al., 2006).

Typically, most laboratories use Disc Diffusion method, which is a qualitative one for assessment of antibiotic susceptibility. In this method based on the size of zone of inhibition, susceptibility or resistance is reported and no number is reported in this technique. This method is not adequate for the report of the cases of resistance associated with the increase in Minimum Inhibitory Concentration (MIC) or intermediate resistance (I), or for those antibiotics with insufficient disk diffusion due to the high molecular weight Non-fermentative Gram-negative bacteria such as *A. baumannii*need longer time to reach the cell mass as required for assessing the susceptibility because of the slower growth comparing with the family of *Enterobacteriaceae*. This feature makes the results of Disk Diffusion, as a qualitative susceptibility testing, to be misinterpreted (Giske1et al., 2007).

In our study undertaken over a period of 12 months (June 2014 - June 2015) we report the prevalence of *A. baumannii* isolates and their antibiograms. Antibiotic sensitivity testing was performed through VITEK 2 AST-N262 (bioMerieux Inc, Mercy L'etoil, Fransa) automated system for ampicillin/sulbactam, piperacillin, piprofloxacin, gentamicin, trimethoprim/sulfamethoxazole, ceftazidime, cefepime, imipenem, meropenem, tetracycline, levofloxacin, cefoperazone/sulbactam,, netilmicin, amikacin, colistin, tigecycline. Outcomes were interpreted according to CLSI (Clinical and Laboratory Standards Institute) standards. The most active agents in vitro against the MDR-AB are polymyxin E (Colistin) and tigecycline.

From the increasing occurrence of MDR *A. baumannii* over recent years accompany with the deficiency of new antimicrobials discovery caused limited therapeutic options globally.

Consequently, colistin that was abandoned in most parts of the world in the early 1980 because of the reported high incidence of nephrotoxicity and neurotoxicity has been revived (Falagas and Kasiakou, 2005; Li, Nation, et al., 2006).

Carbapenem resistance amongst *Acinetobacterspp*. has been increasing in the last decade, and outbreaks due to CR-AB have been identified worldwide (Partridge et al,. 2008; Lu et al., 2006).

During the past decade, the terms PDR and MDR have been commonly applied to *A. baumannii* strains to designate, respectively, resistance to all, to all but one or two, and to three or more classes of potentially effective antimicrobial agents, have caused *A. baumannii*become the most important pathogen after *P. aeruginosa* among non-fermentative Gram-negative bacteria (Albrecht et al., 2006).

1.1. Aim of the Study

Isolaton and identification of *A. baumannii* from different clinical speciemens through Vitek 2 system compact system

The antimicrobial susceptibility of clinical isolated *A. baumnnii* from different clinical spiciement with the majore focus on carbapenems.

To investigate the incidence of MDR-AB and PDR-AB of clinical isolated obtained from Central Health Laboratory and Burn and Plastic Surgery Hospital/Emergency in Sulaimani city during the period from Jun, 2014 to Jun, 2015. from sulaimania city

Also to investigate antimicrobial activity of tegecycline and colistin against MDR-AB and PDR-AB

2. LITERATURE REVIEW

2.1. The history of the genus *Acinetobacter*.

The history of the genus *Acinetobacter* dates back to theearly 20th century, in 1911, when Beijerinck, a Dutch microbiologist,described an organism named Micrococcus calcoaceticusthat was isolated from a soil (Henriksen, 1973).*Acinetobacterspp* were first thought to be non-virulent saprophytes.

Since then, it has had several names, becoming known as *Acinetobacter* in the 1954s (Akinetos Greek adjective, unable to move, Baherion, Greek noun, rod) was proposed by Brisou and Prévot to separate the non-motile micro-organisms from the motile ones within the genus Achromobacter, The *Acinetobacter* group was inappropriately defined and reclassified to different families and genera (Gordon and Wareham 2010). *Acinetobacter*, as a separategenus, was proposed in 1954, but since then it has gone through several comprehensive revisions. For a long time, the genus belonged to the family *Neisseriaceae*, but currently it is designated, together with *Moraxella* and *Psychrobacter*, to the family *Moraxellaceae*(reviewed by Towner *et al*., 1991; Berezin and Towner, 1996; Gordon and Wareham, 2010).

Baumann *et al*. in (1968), first published a complete survey and concluded that the different species listed belonged to a single genus, this was when the name the name *Acinetobacter* was first proposed. Further sub-classification into different species based on phenotypic characteristics was not possible.

All these discoveries resulted in the official acknowledgment of the genus *Acinetobacter* by the Subcommittee on the Taxonomy of *Moraxella* and Allied Bacteria in 1971 (Peleg *et al*., 2008).

Go *et al*. (1994) in USA confirmed that The first reported outbreak of CR-AB occurred in 1994. CR-AB isolates were isolated from a leukaemia patient in the oncology ward of a Taiwanese hospital in May 1998 (Hsueh *et al*., 2002). These isolates were observed to be resistant to almost all antibiotics e.g. cephalosporins, aztreonam, aminoglycosides and ciprofloxacin and were therefore named PDR-AB (Hsueh *et al*., 2002). The rise in the MDR-AB strains has been due to the extensive use of antimicrobial chemotherapy against bacterial infections (Hsueh *et al*., 2002).

2.3. Classification/Taxonomy of *A. baumannii*

The genus *Acinetobacter* has been revised extensively since 1986. It comprises of 33 different species of which 22 are named. (Bouvet *et al*., 1986,Dijkshoorn *et al*., 2007, Nemec *et al*., 2009) and further 28 groups have been identified. *Acinetobacter* are grouped into three main complexes: i) *Acinetobacter calcoaceticus-baumannii* complex, which is glucose oxidizing and non-haemolytic; ii) *Acinetobacter lwoffii*, which are glucose negative and non-haemolytic and iii) *Acinetobacter haemolyticus*, which is haemolytic (Ehlers *et al*., 2012).

Table 1. Nomenclature of *A. baumannii* (Euzeby, 2008)

Domain	**Bacteria**
Phylum	*Proteobacteria*
Class	*Gammaproteobacteria*
Order	*Pseudomonadales*
Family	*Moraxellaceae*
Genus	*Acinetobacter*
Species	*A. baumannii, A. Baylyi, A. Beijerinckii, A. bouvetii, A. calcoaceticus, A. Gerneri, A. grimontii, A. gyllenbergii, A. haemolyticus, A. johnsonnii, A. Junnii, A. Lwoffii, A. Parvus, A. radioresistens, A. schindleri, A. soli, A. Tandoii, A. tjernbergiae, A. Towneri, A. Ursingii, A.venetianus* and 14 species are still unnamed

2.4. Properties of *A. baumannii*

2.4.1. Physical characteristics

Acinetobacter species are able to grow at various temperatures and pH conditions(Kurcik-Trajkovska, 2009), These bacteria can grow in simple mineral medium containing ammonium or nitrate salts and a single carbon and energy source such as acetate, lactate or pyruvate. Most *Acinetobacter* strains grow between 20°C and 37°C with most strains having an optimum temperature of growth is in the range of 30^0C - 35^0C. The capacity to grow at 44^0C serves as a distinguishing characteristic between *A. Baumannii* and other genospecies(Bergogne-Berezin; Towner,1996; Forbes *et al*.,2007)Theversatile organ-

ism exploits a variety of both carbon and energy sources. These properties explain the ability of *Acinetobacter* species to persist in either moist or dry conditions in the hospital environment, thereby contributing to transmission (Smith *et al*., 2007).

*Acinetobacter*species of human origin grow well on solid media that are routinely used in clinicalmicrobiology laboratories such as sheep blood agar or tryptic soy agar at 37°C incubation temperature.

2.4.2. Growth and Cultural Characteristics

In a study carried out by Bergogne-Bérézin,(2009),*Acinetobacter* grows rapidly on 5% sheep blood , Eosin-methylene blue (EMB) agar and MacConkey agars. Characteristic of colonies(after 18–24 h incubation at 37°C) *A. baumannii* on, 5% sheep blood agar are producing smooth, opaque colonies, in which some isolates are nonhaemolytic. on (EMB) colonies are bluish to bluish gray, Colonies on MacConkey agar are light lavender colour indicating but do not non- lactose fermenting colonies lactose.

2.4.3. Biochemical characteristics

The present study are shown in table (1.2). *A. baumannii* is a Gram-negativenon lactosefermenting bacteria, catalase positive and oxidase negative and urease positive, aerobic, often capsulate and non-motile . It shows no reaction with indole and methyl red. In the Triple Sugar Iron (TSI) agar, it shows alkaline slant and neutral butt and it does not produce gas (H_2S).(Holt *et al*.,1994; Macfaddin *et al*., 2004).

2.4.4. Physiology and morphology

The isolates grown on blood agar medium for 24 to 48 h at 37°C showed non-haemolytic colonies that were about 2 to 3 mm in diameter.

Members of the genus *Acinetobacter* are non-motile coccobacilli that are frequently confused with *Neisseriae* in Gram stained samples. They are generally encapsulated, oxidase negative, catalase positive, obligate aerobic and they do not ferment carbohydrates. *Acinetobacterspp*. are short, plump, Gram negative (but sometimes difficult to destain) rods, typically 1.0 to 1.5 μm by 1.5 to 2.5 μm in size during the logarithmic phase of growth but often becoming more coccoid in the stationary phase (Bergogne-Berezin and Towner, 1996; Constantiniu *et al*., 2004; Perez *et al*., 2007; Peleg *et al*., 2008;).

Table1. 2. The biochemical characteristics of *A. baumannii*.

Tests	***A. baumannii***
Catalase	+
Oxidase	-
Simmons citrate	+
Hemolysin production	_ (γ hemolysis)
produce acid from carbohydrates Glucose Xylose Mannitol Sucrose Galactose Manose Rhamnose Lactose Maltose	+ - -
Kliglar iron agar (KIA)	Alkaline slant / No change bottom, No gas , No H2S
Urease production	-
Motility	-
Growth at 44ºC	+
Growth at 37ºC	+

2.5. Epidemiology

Members of the genus *Acinetobacter* are widely distributed in nature and can be isolated from soil and fresh-water samples, as well as from humans and animals. Human carriage of *Acinetobacter* has been demonstrated in normal healthy individuals. Certain *Acinetobacter spp*., chiefly *A. johnsonii, A. lwoffii* and *A. radioresistens*, are part of the bacterial flora of the skin, where they are found predominantly in moist skin areas.

Peleg., *et al* in (2008) indicated that *A. baumannii* is the main species associated with outbreaks of nosocomial infections. It is a common misconception that *A. baumannii* is a ubiquitous organism that can be readily found in soil and water, and that it is a

frequent skin and oropharyngeal commensal of humans. While these statements certainly apply to members of the genus *Acinetobacter* when considered as a whole, *A. baumannii* (and its close relatives of clinical importance) are not ubiquitous organisms.

Acinetobacter is well-recognised for its ability to cause nosocomial outbreaks, and particular strains are able to cause epidemics in multiple hospitals within a city, in various regions in a country and can even spread worldwide (van Dessel, Dijkshoorn *et al*. 2004; Coelho, Turton *et al*. 2006).

Wisplinghoff *et al*. in (2004) reported that During an outbreak, *A. baumannii* can be isolated from numerous sources in the hospital environment , and this wide dissemination in the hospital environment results in frequent carriage of *Acinetobacter* by hospital staff and patients. Airborne transmission and patient-to-patient transmission have also been demonstrated. However, although the hands of hospital personnel, coupled with contamination of environmental surfaces and medical equipment, may play a role in the spread of *A. baumannii* during an outbreak, it seems likely that the infected patient forms the primary reservoir of infection, with such patients often shedding extremely large numbers of *A. baumannii* cells into their surrounding environment.

2.6. *Acinetobacter* Infection

A. baumannii is a ubiquitous pathogen capable of causing both community and health care associated infections(HAIs)

A. baumannii is responsible for only 2%–10% of Gram-negative bacilli recovered from patients in intensive care units in Europeand the United States (Fridkin and Gaynes, 1999; Hanberger *et al*,.1999; Richet, and Fournier, 2006)

2.6.1. Hospital Acquired Infection

Acinetobacter species most commonly cause nosocomial infections, includingbloodstream infections, ventilator-associated pneumonia, skin and soft-tissue infections, wound infections, surgical site infections, major trauma, burns, premature birth, previous hospitalization, stay in an ICU, catheter-related bacteremia, respiratory and and urinary tract infections, secondary meningitis(Graser at el., 1993; Ayats *et al*., 1997; Garcia-Garmendia *et al*., 2001 Wisplinghoff, 2004).

The assessment of the outcome of *Acinetobacter* infection is difficult and reported mortality rates range from 5% in general wards to 54% in the ICU (Poutanen, Louie *et al*.

1997; Siau, Yuen *et al*. 1999). Seifert *et al*. showed the crude mortality rate of *A. baumannii* bacteraemia to be as high as 44%. However, it is difficult to determine morbidity and mortality directly attributable to *Acinetobacter* as opposed to comorbidity, which is very common in these patients. Death attributable to *A. baumannii* bacteraemia, at 19%, was assessed to be much lower than the crude mortality rate (Seifert, Strate *et al*. 1995). Several predisposing factors to infections with *Acinetobacter* have been identified. These include immunosuppression, unscheduled hospital admission, respiratory failure at admission, previous antimicrobial therapy, previous sepsis in ICU and invasive procedures; all of which have been recognised as risk factors for *Acinetobacter* infection (Garcia-Garmendia, Ortiz-Leyba *et al*,. 2001). *Acinetobacter* can be cultured from different environmental sites within hospitals and it is thought that cross contamination between sites is a major mode of transmission in hospital outbreaks (van den Broek, Arends *et al*. 2006). Carriage of *Acinetobacter* on the hands of hospital staff and on medical instrumentation can contribute to the spread of the organism. The ability of certain *Acinetobacter* species to survive on dry surfaces for extended periods of time may also increase transmissibility. It has been suggested that desiccation tolerance, along with multidrug resistance demonstrated by some strains, may explain why *Acinetobacter* is able to establish itself in the hospital environment and cause recurring nosocomial outbreaks (Jawad, Seifert *et al*. 1998).

2.6.2. Community Acquired Infection

Acinetobacter spp. have been reported occasionally as causative agents of community-acquired infections such as wound infection, urinary tract infection, otitis media, eye infections, meningitis and endocarditis. In addition, *Acinetobacter spp*. other than *A. baumannii* and its close relatives are normal commensals, often colonizing the skin and mucous membranes of humans, and their isolation may therefore have been misinterpreted as being indicative of agents causing infection. Nevertheless, *A. baumannii* is recognized as a rare but important cause of severe community-acquired pneumonia in tropical areas of Asia and Australia (Bassetti and Repetto, 2008). Chen, Hsueh *et al* in (2001) conducted astudies Those type of infection is usually associated with underlying conditions such as alcoholism, smoking, chronic obstructive pulmonary disease and diabetes mellitus and is a particular problem in tropical climates such as Southeast Asia and Australia, where skin carriage is more common due to environmental conditions (Anstey, Currie *et al*. 1992; Chu, Leung *et al*. 1999; Chen *et al*., 2001; Falagas *et al*.,2007). In these areas *A. baumannii*

can be a cause of severe community-acquired pneumonia, especially in young alcoholic patients.

2.7. Clinical manifestations of *A. baumannii* infections

2.7.1. Respiratory Infections

Acinetobacter most commonly infects the respiratory tract, causing tracheobronchitis and/or pneumonia. ventilator-acquired pneumonia (VAP), is the most commonly identified clinical manifestation of *A. baumannii* (Dijkshoorn *et al*. 2007; Peleg *et al*. 2008; Munoz-Price and Weinstein, 2008).

the data from the National Nosocomial Infections Surveillance (NNIS) System in USA showed that the proportion of *Acinetobacter* species associated with intensive care unite VAP rates due to *A. baumannii* reached 7% in 2003 (Gaynes and Edwards,2005; Falagas & Kopterides, 2006). the mechanical ventilation has been reported the mortality rates of 30 to 75% due to *A. baumannii* in ventilator dependent paitients (Bergogne-berezin and Towner, 1996).

2.7.2. Wound and burn infections.

Joly-Guillou, 2008. Reported that *A. baummanii* is also a common cause of hospital infection in burn patients, but the infection caused is often less severe when compared to other organisms.

Epidemic of this organism in burn units have often been linked to multidrug resistant strains and lasting epidemic is usually connected to the contamination of the hospital environment. This bacterium gained a bad reputation among soldiers injured during the Iraqi war and it was named “Iraqibacter” due to clones spreading from Iraq to American military hospitals in Germany and US (Petersen, *et al*. 2007; Peleg, *et al*. 2008).

2.7.3. Blood stream infections and sepsis

Peleg *et al*. (2008) in the United States investigated that Bacteremia caused by *Acinetobacter* had the third highest crude mortality and it was the 10th most common aetiologic agent, responsible for 1.3% of all nosocomial bloodstream infections.

sources of bacteremia are infected intravascular and respiratory tract catheters, canules and tubes. , *A. baumannii* was more likely to be isolated from patiens in ICUs than patiens in non-ICU ward (1.6% versus 0.9% of blood stream infections, respectively, in

those locations). The crude mortality rate of*A. baumannii* blood stream infection was 34.0% in total, 43.4% which is the third rank in the ICU, and 16.3% in the non-ICU ward (Wisplinghoff *et al*., 2004; peleg *et al*., 2008).

2.7.4. Meningitis

Acinetobacter was a rare cause of Nosocomial meningitis(Fernandez-Viladrich.,1999; Filka 1999; Wroblewska., 2004).

Garcia- Garmendia *et al*. in(2001) observed that post-neurosurgical *A. baumannii* meningitis is becoming an increasingly important entity with mortality rates as high as 64% been reported in patients with meningitis due to *A. baumannii* and rare cases of community-acquired primary meningitis have also occurred. The main cause of these cases is thought to be a prolonged connection between the brain ventricles and the external environment, a ventriculostomy, or a cerebropspinal fluid fistula(Siegman-Igra *et al*,. 1993; Chang *et al*., 2000; Joly-Guillou, 2008).

2.7.5. Urinary tract infections

Nosocomial UTI was rarely occurred by *A. baumannii*, being responsible for 1.6% of ICU-acquired UTIs in 2003 (Gaynes and Edwards, 2005).It occurs most commonly in elderly debilitated patients, in patients confined to ICUs, and in patients with permanent indwelling urinary catheters, The use of endotracheal tubes, intravascular, ventricular, or urinary catheters often leads to opportunistic bacteria colonizing the site (Fournier and Richet 2006; Loehfelm *et al*., 2008).

2.7.6. Skin and soft tissue infections

Acinetobacter infections involving skin and soft tissue have become increasingly reported and highlight the importance of environmental contamination in causing infections. This may be because strains of *Acinetobacter* species are the only group of Gram-negative bacteria that may be present naturally on the human skin (Seifert *et al*., 1997).

It was reported by Gaynes and Edwards (2005) that *Acinetobacter* was responsible for 2.1% of ICU-acquired skin/soft tissue infection.

Most reports are from wounded military personnel from Iraq-Kuait region or Afghanistan, as It is a very difficult pathogen to eradicate from a burns unit (Hujer *et al*.,

2006; Trottier *et al*., 2007; Adams-Haduch *et al*., 2008; Davis *et al*., 2005; 2006; Sebeny *et al*., 2008).

In astudy presented by Johnson *et al*. (2007), Such infections are not associated with high mortality rates but may be a source of bacteraemia and sepsis if inappropriate therapy is given. It was the most commonly isolatedpathogen from patients with open tibial fractures, but due to its low pathogenicity atthis site, it was completely eradicated.

2.8. Pathogenesis-Virulence factors

*A. baumannii*have very few virulence factors or little known about its true pathogenic potential (Cisneros & Rodriguez-Bano, 2002),however some strains have virulence factors associated with invasiveness, transmissibilityor the enhanced ability to colonise immunocompromised patients (Dijkshoorn *et al*., 1996).

Susceptible patients include those who have recently undergonemajor surgery, malignancy, burns or immunosuppression, use of antimicrobial agents, presence of invasive devices such as endotracheal and gastric tubes, and type of respiratory equipment and particularly the elderly, although outbreaks of *Acinetobacter* infection have also been associated with bacteremia, sepsis in neonatalintensive care units and pediatric oncology units. (Buxton., *et al*., 1978: Castle.,*et al* 1978; Peacock., 1988; Bergogne-Berezin and Joly-Guillou, 1991;; Lortholary *et al*., 1995;; Struelens *et al*., 1993)

Several specific potential virulence mechanisms have been identified like outer membrane proteins (OMPs), Biofilm formation, Quorum sensing (Tomaras *et al*., 2003; Choi*et al*.,2005; Marti *et al*.,2006; Braun, 2008; Choi *et al*., 2008; Kim *et al*., 2009;Gaddy *et al*., 2009).

2.8. Multi-drug resistance (MDR) and Pan-drug resistant (PDR) *A. baumannii*

2.8.1. Multi-drug resistance (MDR) *A. baumannii.*

MDR clinically, is the ability of disease causing microorganism to withstand a wide variety of antimicrobial compounds (Mooij, 2009). Hence, a strain is considered a MDR if an isolate is resistant to representatives of three or more classes of antibiotics. However, transference of resistance determinants by mobile genetic elements including plasmids, transposons, and gene cassettes in integrons between and across different bacterial species are important factors that can contribute to the increase in multi-resistant strains (Livermore, 2007).

MDR-AB has been reported worldwide and is now recognized as one of the most difficult health care associated infections to control and treat. Burn ward and ICU patients and those with central intravenous catheters are the main targets of this organism (Simmonds *et al*., 2009). Several outbreaks in ICUs, burns units and NICUs have been reported previously (Schloesser *et al*., 1990 and Chan *et al*., 2007)

Definitions of MDR-AB the reviewed studies are different. Appleman *et al*. (2000) in USA reported that MDR-AB isolates were resistant to imipenem, ceftazidime, efotaxime, gentamicin, tobramycin, piperacillin–tazobactam, ticarcillin–clavulanate, ciprofloxacin, ofloxacin and trimethoprim–sulfamethoxazole. Corbella *et al*. (2000) in Spain Described MDR-AB as carbapenem-resistant isolates also Alarcon *et al*. (2001) in Spain Described MDR-AB strains resistant to all tested antibiotics, including imipenem, doxycycline and colistin, and intermediate only to tobramycin.. Giamarellos-Bourboulis *et al*. (2001) in Greece Described MDR *A. baumannii* isolates resistant to ampicillin–sulbactam, cefotaxime, ceftriaxone, ceftazidime, cefepime, amikacin and ciprofloxacin. Levin *et al*. (2001) in Brazil Described MDR-AB isolates resistant to all commercially available antimicrobials except colistin. Podnos *et al*. (2001) in USA Described MDR-AB isolates sensitive only to polymyxin B and resistant to amikacin, imipenem and sulbactam. Cawley *et al*. (2002) in USA Described MDR-AB isolates resistant to piperacillin–tazobactam, tobramycin, levofloxacin, ceftazidime, aztreonam, imipenem–silastin and amikacin. Oh *et al*. (2002) in Korea Defined MDR-AB as resistance to eight or more of the following antibiotics: ampicillin, carbenicillin, piperacillin, ticarcillin, cefotaxime, cefoxitine, ceftazidime, aztreonam, imipenem, amikacin, gentamicin, kanamycin, tobramycin, ciprofloxacin, norfloxacin, sulfamethoxazole and trimethoprim. Simor *et al*. (2002) in Canada Defined MDR-AB as resistance to penicillins, cephalosporins, piperacillin–tazobactam, trimethoprim–sulfamethoxazole, ciprofloxacin, gentamicin and tobramycin. All isolates were susceptible to imipenem and some to amikacin. Garnacho-Montero *et al*. (2003) in Spain Described MDR-AB isolates that were resistant to imipenem and sensitive only to colistin in one treatment arm, and at least carbapenem sensitive in the other treatment arm. Gorman *et al*. (2003) in Canada Described MDR-AB an isolate sensitive only to imipenem and intermediate to amikacin.

2.8.2. Pan-drug resistant -*A. Baumannii*(PDR-AB) in the reviewed studies

Kuo *et al*. (2003) in Taiwan Described PDR-AB was defined as resistance to all antibiotics commercially available (ceftazidime, ciprofloxacin, cefepime, aztreonam, ciprofloxacin, piperacillin–tazobactam, ticarcillin–clavulanate, ofloxacin, amikacin, imipenem and meropenem). Not tested for colistin or ampicillin–sulbactam. Wang *et al*. (2003) in Taiwan was defined PDR-AB as resistance to all currently available antimicrobials (including carbapenems), except colistin (polymyxin B). Kuo *et al*. (2004) in Taiwan was defined PDR-AB as resistance to all antibiotics routinely tested (i.e. ampicillin–sulbactam, ceftazidime, piperacillin–tazobactam, cefepime, aztreonam, ciprofloxacin, trovafloxacin, moxifloxacin, garenoxacin, amikacin, imipenem and meropenem).

A review of resistance distributions of antibiotics used in our study over a period of five years showed increasing rates of MDR and PDR strains. Particularly, the 4.7% PDR strain rate in 2007 was found to be 20.9% in 2011. These resistance rates are considered indicators of a gradual increase in difficulties treating *Acinetobacter* infections (Çelik*et al*., 2014).

Definitions of MDR species vary, referring to a wide array of genotypes and phenotypes (Falagas *et al*., 2006). MDR clinically, is the ability of disease causingmicroorganism to withstand a wide variety of antimicrobial compo-unds (Mooij,2009). MDR isolates may be due to empirical usage of broad spectr-um antibiotics and non adherence to a hospital antibiotic policy.

Two of the most common definitions of multidrug resistance are carbapenem resistance or resistance to ≥3 classes of antimicrobials (Falagas *et al*., 2006; Fournier *et al*., 2006).

Antimicrobial category	Antimicrobial agent
Aminoglycosides	Amikacin Gentamicin and Netilmicin
Fluoroquinolones	Ciprofloxacin and Levofloxacin
Tetracycline	Tetracycline
Folate pathway inhibitors	Trimethoprim-sulphamethoxazole
Extended-spectrum cephalosporins	Ceftazidime and Cefepime
Beta-lactamase inhibitors + penicillins,Penicillins	Ampicillin-sulbactam, Cefoperazone- sulbactam and Piperacillin
Carbapenems	Imipenem and Meropenem
Polymyxins	Colistin
Glycylcyclines	Tigecycline

By virtue of the previous studies, *A. baumannii*isolates which showed resistance to three or more antibiotics, including fluoroquinolones (Levofloxacin, ciprofloxacin), broad-spectrum cephalosporins (ceftazidime and cefepime), aminoglycosides (amikacin and tobramycin), comp-ound β-lactam / β-lactamase inhibitor (ampicillin / sulbactam, Cefoperazone- sulbactam, Pipera-cillin) and carbapenems (imipenem and meropenem), were considered as strains of MDR.

PDR-AB was defined as the isolates that were resistant to all tested antibiotics except colistin and tigecycline(Chaiwarith *et al*., 2005).

A. baumannii isolates were also susceptible to colistin which is rarelyused in clinical practice. Colistin and tegecyclin were not available in those two hospitals during the our study period. because we worked on the last two definitions

2.9. Mechanisms of Antibiotic Resistance

Perez *et al*. in (2007) mentioned that *A. baumannii* is attracting much attention owing to the increase inantimicrobialresistance and occurrence of strains that are resistant to virtually allavailable drugs.

It also has a remarkable capacity to acquire mechanisms that confer resistance to broadspectrum-lactams, aminoglycosides, fluoroquinolones and tetracyclines. *A. bau-*

*manniie*xhibits a remarkable ability to rapidly develop antibiotic resistance that led to multidrugresistance within a few decades(Bergogne-Berezin *et al*., 1996).

some strains of *A. baumannii* have become resistant to almost all currently available antibacterial agents, mostly through the acquisition of plasmids,transposons, or integrons carrying clusters of genes encodingresistance to several antibiotic families (Segal *et al*., 2003; Poirel *et al*., 2003; Joshi *et al*., 2003; Looveren *et al*., 2004; Smith *et al*., 2007).

However, because of the scarcity of large-scale surveillancestudies from the 1970s to the 1990s and the difficulties in comparing local reports, suchtrends are difficult to quantify on a global level. Resistance rates can vary according tothe country and the individual hospital, and depend on biological, epidemiological ormethodical factors (reviewed by Wisplinghoff *et al*., 2007). resistance topolymyxins and tigecycline have also been described, which indicates that *A. baumannii* can cause infections that are fully refractory to the currently availableantimicrobial drugs (reviewed by Li *et al*., 2006; Peleg *et al*., 2007).The resistance of *A. baumannii* to antimicrobial agents is mediated by all of the majorresistance mechanisms that are known to occur in bacteria, including modification oftarget sites, enzymatic inactivation, active efflux and decreased influx of drugs (Poirel *et al*., 2003). Beta-lactamases are the most diverse group of enzymes that are associatedwith resistance, and more than 50 different enzymes, or their allelic forms, have beenidentified so far in *A. baumannii* (Dijkshoorn *et al*., 2007) Another study reported that resistance to tetracyclines has been associated with tet (A) and tet (B) genes that encodetetracycline specificefflux pumps (Huys *et al*., 2005). ISAba1 was also thought to have a key rolein some carbapenem-resistant strains by enhancing the expression of the intrinsic OXA-51-like carbapenemases (Turton *et al*., 2006). Another chromosomal system that istypically found in *A. baumannii* is the AdeABC efflux system (Magnet *et al*., 2001).Reduced susceptibility to carbapenems has also been associated with the modification ofpenicillin-binding proteins and porins or with regulation of the AdeABCefflux system, which might result in high-level carbapenem resistance in *A. Baumannii* (Bou *et al*., 20001). Another study showed that production of ß-lactamases andreduced expression of PBP 2 (penicillin-binding protein biotype 2) are the mostfrequently observed mechanisms of resistance to carbapenems (.Fernandez-Cuenca *et al*., 2003)

2.9.1. Non-enzymatic mechanisms

2.9.1.1. Efflux

Efflux pumps have a potent ability to actively export beta-lactams, quinolones, and sometimes even aminoglycosides from cell cytoplasm.*Acinetobacter* species possess efflux pumps that are capable of actively removing a broad range of antimicrobial agents from the bacterial cell. Increased expression of chromosomal efflux pumps and acquisition of additional efflux systems can then lead to MDR (Peleg *et al*. 2008; Giamarellou *et al*. 2008 and Courvalin *et al*. 2011).

Three components make up an efflux pump: AdeB forming the transmembrane component, AdeA forming the inner membrane fusion protein and AdeC forming the outer-membrane protein (reviewed by Peleg *et al*. 2008). It is also widely distributed in *A. baumannii* isolates and is chromosomally encoded (Dijkshoorn *et al*. 2007; Peleg *et al*. 2008). Interestingly, AdeABC acts against virtually all classes of antibiotics including carbapenems (Giamarellou *et al*. 2008; Nikaido 1998).

2.9.1.1.1. RND pumps

The resistance nodulation cell division (RND) family are the most common efflux systems in MDR *Acinetobacter* and a number of pumps of this type have been identified in species belonging to the Acb complex. RND Pumps are three component pumps with broad substrate specificity consisting of a common tripartite structure with periplasmic, inner and outer membrane components. Two systems have been characterised in *A. baumannii*, AdeABC and AdeIJK (Marchand *et al*., 2004).

AdeABC is the most well characterised RND system in *Acinetobacter*. Found in both *A. baumannii* and other clinically relevant species (Magnet, Courvalin *et al*. 2001; Roca, Espinal *et al*. 2011), it is chromosomally encoded but has only been identified in clinical isolates and only confers MDR when overexpressed (Magnet, Courvalin *et al*. 2001). studies reveal that substrates for this pump include aminoglycosides, β-lactams, fluoroquinolones, tetracyclines, tigecycline, macrolides, chloramphenicol and trimethoprim (Magnet, Courvalin *et al*. 2001; Marchand, Damier-Piolle *et al*. 2004). Studies showed that a correlation between carbapenem resistance and overexpression of AdeABC (Héritier, Poirel *et al*. 2005; Huang, Sun *et al*. 2008). Also, a synergic effect in a recombinant strain, which harboured both the OXA-58 oxacillinase and overexpressed the AdeABC efflux pump, resulted in a higher level of carbapenem resistance (Seward *et al*., 1998).

AdeIJK the second **RND** pump, is a very common efflux system in *A. baumannii*. It was found to have a similar substrate profile to that of AdeABC but responsible for the low-level intrinsic resistance phenotype as no adjacent regulatory genes were identified (Damier-Piolle *et al*., 2008).It exports β-lactams, fluoroquinolones, tetracyclines, tigecycline, lincosamides, rifampicin, chloramphenicol, co-trimoxazole, novobiocin and fusidic acid, but not aminoglycosides (Magnet *et al*. 2008 and Guigon *et al*. 2010)

AdeFGH the third **RND** type efflux pump responsible for high-level antibiotic resistance to various groups of antibiotics fluoroquinolones, chloramphenicol, trimethoprim, clindamycin, tetracyclines, tigecycline and sulfamethoxazole(Coryne *et al*., 2010 and Rosenfeld *et al*. 2010).

2.9.1.1.2. Non-RND pumps

Chromosomally encoded non-RND efflux systems: For the MFS efflux pump, chloramphenicol resistance *Acinetobacter* (CrA), and Tet (tetracycline efflux system) were the major members identified in the *Acinetobacter* the substrate profile includes aminoglycoside, fluoroquinolones, trimethoprim (Roca, Marti *et al*. 2009; Rajamohan *et al*., 2010; Roca *et al*., 2012; Vila *et al*., 2007 and Su, Chen *et al*. 2005) and AbeS is a small multidrug resistance (SMR) efflux pump involved in chloramphenicol, fluoroquinolone, erythromycin (Srinivasan, Rajamohan *et al*. 2009). For the Tet efflux system, TetA and TetB are the predominant efflux pumpsfound in *A. baumannii* and TetA responsible for tetracycline resistance while TetB is responsible for tetracycline and minocycline resistance, and TetB was more commonly encountered in *Acinetobacter* in Marti's study (Marti, Fernandez-Cuenca *et al*. 2006).

2.9.1.2. The role of penicillin-binding proteins (PBPs) and outer-membrane proteins (OMP)

The role of PBPs in conferring antibiotic resistance in *A. baumannii* has been poorly investigated, but the reduced expression of PBPs has been reported to contribute to carbapenem resistance (Bou *et al*., 2000; Giamarellou *et al*., 2008).

2.9.1.3. The role of target site modifications(Resistance to quinolones)

Alteration of the target or cellular functions due to mutations in *A. baumannii* seem to confer resistance to quinolones and aminoglycosides. finally, selective pressure exerted by the use of broad-spectrum antimicrobials and transmission of strains among

patients may be the causes of the emergence of resistance (Mussi *et al*., 2005& Li *et al*., 2005).

2.9.2. Enzymatic mechanisms of resistance

2.9.2.1. β-lactam resistance

In astudy carried out by Opal and Pop-Vicas, (2010), β-lactamases are enzymes, which inactivate the β-lactams by splitting the amide bond of the β-lactam ring. These enzymes evolved allowing organisms to resist β-lactam compounds produced by various microorganisms in their natural habitat (mostly soil) and encountered by other, competing members sharing the same niche Eventually, these genes found their ways to organisms. the β-lactamases can be divided into four groups, namely classes A, B, C, and D

2.9.2.1.1. Class A β-lactamases

Class A β-lactamases are extended-spectrum β-lactamases (ESBLs)such as the TEM, SHV and CTX-M enzymes. They are sensitive to β-lactamase inhibitors. ESBL, demonstrate high-level resistance to penicillins and broad spectrum of cephalosporins including 3rd and 4th generation drugs(ceftazidime; cefepime) (Poirel *et al*, 2012).

2.9.2.1.2. Class B β-lactamases

Class B β-lactamases often referred to metallo-β-lactamases (MBLs) require zinc as a divalent cation for their activity.MBLs are class B beta-lactamases that are able to hydrolyze carbapenems as well as every other β-lactams antibiotic with the exception of aztreonam. They differ from class A and D β-lactamasesby having a metal ion in the active site, usually zinc, which participates in catalysis (Walsh 2005; Pfeifer *et al*. 2010).

2.9.2.1.3. Class C β-lactamases

Class C β-lactamasesare often referred to as AmpC-type enzymes, are widespread in Gramnegative bacteria, and are naturally occurring classC β-lactamases in *A. baumannii*, with a serine at its active site, similar to class A β-lactamases (Jacoby, 2009). It is a chromosomally encoded cephalosporinase, also known as *Acinetobacter*-derived cephalospoinase ADC (reviewed by Peleg *et al*., 2008).

ISAba1 is commonly associated with the overexpression of blaADC, providing a strong promoter resulting in high-level ceftazidime resistance (Corvec *et al*., 2003; Héritier *et al*., 2006). ADC enzymes are also penicillinase, , conferring resistance to amino-

pencillins, to first,second, and some representatives also to third generation cephalosporins (Opal and Pop-Vicas, 2010). which are able to hydrolyse narrow and broad spectrum cephalosporins, but not carbapenems or cefepime (Giamarellou *et al*. 2008; Perez *et al*. 2007). Observed resistance to cephalosporins can therefore be mediated by bott class A and class C β-lactamases.

2.9.2.1.4. Class D β-lactamases

Class-D β-lactamases are also known as oxacillinases (OXA-type β-lactamases),some OXAs (i.e., OXA ESBLs) are also able to hydroloyze extended-spectrum cephalosporins (Adams *et al*., 2008; Marcusson *et al*.,2009). OXA-type β-lactamases have carbapenem-hydrolysing activity (CHDL), which is alarming in the clinical setting (Walther-Rasmussen & Høiby, 2006). The designation "OXA" refers to its preferred substrate oxacillin and can hydrolyze amoxicillin, methicillin, cephaloridine, cephalothin, and to some extent carbapenemase (Brown and Amyes, 2006; Poirel and Nordmann, 2006; Walther-Rasmussen, and Hoiby. 2006). OXA-23, plasmid-encoded enzyme, were first reported in 1993 in a CR-AB strain identified in Scotland, initially named ARI-1 (*Acinetobacter* resistant to imipenem) (Paton *et al*.,1993).

The remainder of the carbapenem-hydrolyzing oxacillinases are OXA-23, OXA-40, and OXA-58 are commonly identified acquired CHDL associated with carbapenem resistance in *A. baumannii*, whereas OXA-51-like enzymes are naturally occurring (reviewed by Peleg *et al*., 2008). In an outbreak of CR-AB clones were identified both harbouring blaOXA-23 as the resistance determinant (Chakravarti *et al*., 2000Coelho *et al*., 2006).

2.9.2.2. Aminoglycoside resistance

Nemec *et al*. in (2004) mentioned that Aminoglycoside resistance in *A. baumannii* is mediated by the AdeABC efflux pump as well as aminoglycoside modifying enzymes(AMEs): phosphotransferases, acetyltransferases and nucleotidultransferases, It degrades aminoglycoside by modifying the hydroxyl group or amino group of the antibiotic and decreases the affinity for target site binding. In *A. baumannii*, the Genes encoding AMEs can be found in chromosomes, in plasmid, transposons, and some have been identified on class-1 integrons.

Recently, a new type of AME, encoded by aac(6_)-Iad, has been discovered and found to play a central role in amikacin resistance among *Acinetobacterspp*. in Japan (Carlos, and ARUJ, 2007)

2.9.2.3. Integrons and their association with resistance genes

(Poirel *et al*. in (2012) reported that the Integrons are capable of capturing, mobilizing and expressing resistance genes organised in gene cassettes. Several antibiotic resistance genes can be captured in the same gene cassette, thereby expressing resistance to several different antibiotic classes simultaneously (Weldhagen, 2004). Class 1 integrons areparticularly associated with class A β-lactamases (ESBLs), with VEB-1 being the first-enzyme found to be encoded on a gene cassette in an integron (Naas *et al*., 2006; Poirel *et al*., 2012). Various resistance genes are found associated with class 1 integrons with the mostcommonly encountered being the aminoglycoside and sulfonamides resistance genes.

2.10. Treatment of *Acinetobacter* Infections

2.10.1. Sulbactam

The β-lactamase inhibitor sulbactam, It is one of the member in β-lactamase inhibitor which has thegreatest intrinsic inhibitory effect against *Acinetobacter* (Falagas*et al*., 2006). It acts by binding to the PBP2 protein and two forms of sulbactam combination, namely ampicillin-sulbactam (SAM) and cefoperazone-sulbactam (SCP), are available in clinical use for treatment of *Acinetobacter* infection(Levin 2002). Preparations containing ampicillin/sulbactam have beenshown to be effective in the treatment of bloodstream, respiratory tract and urinarytract infections (Levin., *et al* 2003: Smolyakov *et al*., 2003). Ina randomised controlled trial of high-dose ampicillin/sulbactam versus colistin for the treatment of ventilator-associated pneumonia (VAP), comparable safety and efficacywere reported (Betrosian *et al*., 2008). Combinations of carbapenems and sulbactam have also beenshown to be effective even in carbapenem-resistant isolates (Lee et la., 2007 and Ko *et al*., 2004). If isolates aresusceptible, sulbactam-containing compounds may be a safe and effectivetherapeutic option, although as with the other classes the development and spreadof resistance is likely to limit future use (Higgins *et al*., 2004). sulbactam is widely considered as a treatment option, either alone or in combination, for mild to severe infections in sulbactam susceptible isolates.

2.10.2. Polymyxins

The majority of MDR strains remain susceptible to the polymyxins, resulting in increased reliance on these compounds despite previous concerns regarding toxicity (Falagas *et al*., 2005). Two forms of this class of antibiotics were in clinical usage, namely colistin (polymyxin E) and polymyxin B (Yam *et al*., 2006; Yahav *et al*., 2012). Colistin (polymyxin E) has proven clinical efficacy in the treatment of blood stream, wound and urinary tract infections (Gounden *et al*., 2009) and in modern use has not been associated with significant neurotoxicity, although nephrotoxicity remains a concern (Linden *et al*., 2006). Despite the relatively poor penetration of intravenous colistin into the lung, it has been shown to be comparable with imipenem in the treatment of VAP (Garnacho-Montero *et al*., 2003). Favourable outcomes have also been reported with the use of nebulised colistin (Kwa *et al*., 2005: Michalopoulos *et al*., 2008).

Studies have suggested that intravenous colistinand polymyxins B have satisfactory therapeutic value for the treatment of seriousinfection such as bacteremia, pneumonia, skin and soft tissue infections, and urinary tract infections (Falagas *et al*., 2006). Colistin resistance, particularly heteroresistance, has been reported (Ko *et al*., 2007: Hawley *et al*., 2008) but can be difficult to detect using routine susceptibility testing methodologies (Lo-Ten-Foe *et al*., 2007), meaning current rates of resistance may be under estimated. There is also confusion surrounding the dosing of colistin, a problem that is exacerbated by limited pharmacokinetic information and the fact that current commercial preparations contain different amounts of the active drug (Markou *et al*., 2008).

Most of the microbiological studies have shown a synergistic effect between colistin and rifampicin and carbapenems in vitro whereas the results from and human studies have also shown good activity (Cai *et al*., 2012).

2.10.3. Tigecycline

Tigecycline is a new glycylcycline agent which has shown bacteriostatic activity against MDR- *Acinetobacter* species (Pachon-Ibanez *et al*., 2004).

The use of polymyxins and tigecycline has emerged in recent years to overcome carbapenem and multi-drug resistance pan-drug resistance, and have proven success in treating severe *A. baumannii* infections (Dijkshoorn *et al*., 2007; Gordon & Wareham, 2010). A high level of resistance has now been documented by this drug, which has shown overexpression of efflux pumps in the strain. (Ruzin *et al*., 2007).

In recent studies, *Acinetobacter* isolates with a decreased susceptibility to tigecycline, due to overexpression of a multidrug efflux pumps, was documented (Peleg *et al*., 2007 and Maragakis and Perl., *et al* 2008:). tigecycline is an effective alternative for salvage therapy when it is properly administered by experts. The use of tigecycline for bloodstream infections is debatable as it is most effective in treating skin and soft-tissue infections, and its enhanced tissue penetration leads to serum concentrations below the pharmacodynamic breakpoint, resulting in recurrent bacteraemia and even aid in the emergence of resistance (Gordon & Wareham, 2009).

2.10.4. Aminoglycosides

Among aminoglycosides, amikacin and tobramycin are agents that retain activity against many *A. baumannii* isolates. There is a concern regarding its toxicity profile and as with other drugs, their resistance were growing with increased toxicity.

A study conducted by Gounden. *et al*. in(2009) which compared the activity and toxicity of tobramycin against colistin demonstrated no statistical significance between these two in mortality, also in a study done by Hallal *et al*. the efficacy and safety of inhaled and intravenous tobramycin were compared, in which inhaled tobramycin proved to be little better than the intravenous one.

2.10.5. Combination therapy

Given the lack of new treatments, there has been considerable interest in the use of dual or even triple antimicrobial combinations. Although significant synergy can be observed in vitro when colistin is combined with rifampicin, minocycline, ceftazidime or imipenem, and when sulbactam is combined with meropenem (Ko *et al*.,2004). evidence for any clinical benefit is lacking (Petrosillo *et al*., 2008).

In some instances less favourable outcomes have been reported with the use of combinations involving imipenem and amikacin or rifampicin (Bernabeu-Wittel., 2005 and Saballs., 2006). Studies with tigecycline-containing combinations found synergy with colistin, levofloxacin, amikacin and imipenem but antagonism when tigecycline was combined with piperacillin/tazobactam. Again, whether these interactions are relevant in vivo is undefined, but given that patients with MDR-AB often receive multiple antimicrobial agents these findings should be taken into consideration when formulating treatment regi-

mens to ensure that individuals are not given combinations that are potentially antagonistic.

In a study carried out by Housman *et al*. (2013), compared monotherapy and combination therapy with ampicillin/sulbactam, doripenem and tigecycline against MDR-AB using an in vitro pharmacodynamic model. Although specific combination regimens displayed an additive activity at aggressive doses against these MDR-AB, none of the regimens was able to maintain reductions in colony forming units against the more resistant isolates.

Recent data has suggested that glycopeptides, in particular, vancomycin, may have a unique activity against laboratory-adapted and clinical strains of *A. baumannii*, alone and in combination with colistin. In vivo, the authors studied the effect of combinations of vancomycin, colistin, and doripenem on clinical strains of CR-AB and found promising results. Their findings suggested that regimens containing vancomycin may confer a therapeutic benefit against infections caused by CR-AB (O'Hara *et al* 2013).

2.12. Vitek 2

Automation in clinical microbiology started much later than other clinical laboratories. Automation in clinical lab began in clinical chemistry with introducing chemical analyser machines based on "continuous flow analysis (CFA)". It was invented by Leonard Skeggs in 1957, and commercialized by Technicon® Corporation in 1960 decade (Coakly, William, 1981 and Galen Wood, 1990). Soon after, flow cytometry instruments were developed cell counter machines in haematology. Coulters counter machine based on flow cytometry introduced by Beckman Coulter Inc. and Becton Dickinson in 1970 decade after technical development (Wallace Coulter 1953 and Wolfgang Göhde). Clinical serology and enzymes studies were joined to the automated departments. But it took more time to introduce first auto analyser in microbiology lab.

Application of automated systems in clinical microbiology is different than other clinical laboratories. The main difference is the sterile working condition in microbiology and impure clinical bacterial samples which makes one day extra operation for isolation of pure isolated samples. Automated analyse machines still are not popular in daily operation in microbiology laboratories. Where other clinical laboratories are completely replaced automated systems instead of manual methods.

Sterile working condition nowadays has been replaced by higher concentrations of bacteria in feeding samples. But pure colonies preparation which is needed for identification process in microbiology still is a problem that needs conventional overnight culture plates. Thus diagnosis process couldn't be start directly after sampling. (David and Pincus, 2005)

The diagnosis process in the clinical laboratories starts with a chemical or substrate which exists in sufficient amount before sampling. But in clinical microbiology isolation and reproduction of recordable amount of the pure bacteria are needed before analyse process. Considering these culture and subculture process diagnosis process takes about 2 days. Till now technologic development wouldn't be able to make this preparation interval shorter. But after preparation of pure bacterial colonies auto analyser machine could be used to reach a shorter diagnosis time.

The BioMerieux® introduced Vitek2, the new generation of Vitek® microbiology analyzers and its associated ID-GN card in 1997. The Vitek2 system is developed on fluorescence-based technology and designed for the identification of wide range of micro organisms including Gram-negative &Gram positive bacteria, Neisseriacea and yeasts in clinical or industrial samples. There are different marked cards containing 64 chambers for identification tests or antibiotic susceptibility testing (AST).In clinical microbiology Vitek®2 used as an auto analyzer system for the identification (ID) and antibiotic susceptibility testing (AST) of the bacteria in clinical samples.

2.12.1. Reagent Cards

There are 64 micro spaces in this series of the reagent cards that each micro chamber contains a special substrate for individual tests. The chambers are limited between two clear walls on both sides of the card. This structure provides possibility of optical observation of contents inside chamber. Observation takes place once every 15 minutes for each chamber, by an electronic sensor. The walls are permeable to oxygen and needed oxygen level inside of micro chambers, could be provided throw walls where the sealing is protected also.

Different tests exists in different cards as expected; for instance acidification, alkalinization, enzyme hydrolysis, and growth in the presence of inhibitory substances (antibiotics) known as antibiotic susceptibility test (AST). For GN cards (Fig. 09), 47 biochemical tests, some assimilation tests and one negative control well (well No 1) are located in

these micro spaces. The list of tests in GN cards used in this study is given in Appendix 2. The GN card is based on established biochemical methods and developed substrates measuring carbon source utilization, enzymatic activities, and resistance.to variety diseses (Brumley,2012).

3. MATERIALS AND METHODS

3.1. Materials

3.1.1. The instruments

The apparatuses used for preparing the appropriate experiments in the present study are listed in table (3.1).

Table 3.1.The aparatuses used in the present study.

Name	**Company**	**Country**
Autoclave	Memmert	Germany
Incubator	Melag	Germany
Bunzen Burner	Shenma	China
Inoculating Loop	HiMedia	India
Water Distillator	Schott	Germany
Oven	Memmert	Germany
Light Microscope	Olympus SX31	Japan
Magnetic stirrer hot	Labtech	India
Micropipette	Sigma	Germany
pH meter	HANNA	Germany
Sensitive balance	Stuart scientific Co. Ltd	UK
Water bath	Memmert	Germany
Microbiology safetcabinat	DlabTech	Korea
Electronic balance	PSAW	India
Refrigerator	BEKO	Turkey
Vitek 2 Compact system	Biomeriux	French
Micropipette different volumes	Eppendrof	Germany
UVlamp trolley air purification	Dhgate	China
Table lamp	Turmed	Turkey

3.1.2. The equipments

The equipments used for preparing the appropriate experiments in the present study are listed in table (3.2).

Table 3.2. The equipments used used in the present study.

Name	**Company**	**Country**
Beaker different size	Bro3.3	Germany
Conical flask different size	Bro3.3	Germany
Graduated cylinder	Bro3.3	Germany
Disposable swab	Citotest	China
Microscope slide	Beromed GmbH	Germany
Cover slide	Beromed GmbH	Germany
Petri dishes	Plasti Lab	Lebanon
Forceps	Vantage	Pakistan
Aluminum foil	Sanita	Lebanon
Wooden stick	Citotest	China
Cotton	NCPI	Iraq
Micropipette tips	Plasti Lab	Lebanon

3.1.3. Chemical agents

The chemical agents and stains used for preparing the appropriate experiments in the present study are listed in table (3.3).

Table 3.3. Chemical agents and stains used in the present study.

Culture Media	**Company**	**Country**
Gram stain	Syrbio	S.A.R.
Hydrogen Peroxide(Catalase test) Solution	BDH	England
Tetra methyl-p-phenylene diamine dihydrochloride (Ox-idase reagent)	HiMedia	India
Normal saline (phosphate buffer saline pH 7.4powder)	Pioneer	Iraq
Ethanol 95%	BDH	England
Methyl red	Fluka	Switzerland

3.1.4. Culture Media

The culture media used for identification of *A. baumannii* in the present study are shown in table (3.4).

Table 3.4. Culture media used in the present study.

Culture Media	Company	Country
MacConkey agar	HiMedia	India
Blood agar base	HiMedia	India
Eosin methylene blue (EMB) agar	HiMedia	India
Brain Heart Infusion Agar Medium	HiMedia	India

3.1.4.1. MacConkey agar

MacConkey agar is a differential and selective medium used for the isolation of Gram-negative organisms and also used to separate between lactose-fermenter and non lactose-fermenter bacteria. Lactose-fermenting bacteria appear as red to pink colonies. Lactose non fermenting bacteria appear as colorless or transparent colonies (Atlas, 2010).

This medium was prepared by suspending 51.5g of dehydrated medium in 1000 ml distilled water and gently heated it while stirring until boiling to dissolve the medium completely then it is sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Avoid overheating. Cooled it to 45°C -50°C and mixed well before pouring into sterile Petri plates. The surface of the medium should be dry when inoculated. This medium was storied at 4^{o}C.

3.1.4.2. Blood agar

It is a differential and enriched media supported with blood, promote the growth of most Gram-positive and Gram-negative organisms and are also use for the isolation, cultivation, and detection of hemolytic activity of staphylococci, streptococci, and other fastidious microorganisms (Atlas, 2010).

This medium was prepared by suspending 40.0 g in 1000 ml distilled water and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes, then Cooling to 50°C and aseptically added 5% sterile defibrinated blood. It is mixing thoroughly and pour into sterile Petri dishes, this medium was storied at 4^{o}C.

3.1.4.3. Nutrient agar

Nutrient media are basic culture media support the growth of microorganisms and are also use for subculturing pathogens from differential or selective media prior to performing biochemical testing. It is one of the several non-selective media useful in routine cultivation of microorganisms. This relatively simple media has been retained and is still used extensively in the microbiological examination of a variety of materials and is also advised by normal methods (Cheesbrough, 2006).

It was prepared by suspending 28g in 1000 ml distilled water then mixed thoroughly, gently heated to boiling to dissolve the medium completely. Dispense it as desired and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cooled it to 45°C–50°C and mixed well before pouring into sterile Petri dishes. This medium was storied at 4^{o}C.

3.1.4.4. Eosin methylene blue (EMB) agar

EMB agar is a selective medium for Gram-negative bacteria. EMB agar also differentiates lactose-fermenting bacteria from non lactose-fermenting bacteria. lactose-fermenting bacteria such as E. coli often appear dark with a green metallic sheen, Enterobacter aerogenes produces characteristic dark-centered colonies. While non lactose-fermenting bacteria appear colorless (Alexander and Strete, 2001).

EMB agar was prepared by suspending 35.96g in 1000 ml distilled water and mixing until suspension is uniform. It is heating to boiling to dissolve the medium completely and sterilizing by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Avoid overheating, then cooling it to 45-50°C and shaking the medium in order to oxidizing the methylene blue and to suspend the flocculent precipitate, mixed well and dispensed it aseptically in sterile Petri dishes, this medium was storied at 4^{o}C.

3.1.4.5. Brain Heart Infusion Agar Medium

The 52g of powder was suspended in 1 liter of distilled water. It was then boiled shortly to dissolve completely. It was distributed into screw cup, corked firmly and then sterilized by autoclaving at 121^{0}C for 15 minutes and this medium was storied at 4^{o}C. The medium was prepared according to the manufacturer company construction.

3.1.4.6. Brain Heart Infusion Broth

This medium was prepared according to the manufacturer protocol (LAB/UK) by dissolving 37g from the powder in one litter of distilled water which was heated to be dissolved completely, distributed into culture tubes and then autoclaved at 121ºC for 15 minutes. This medium was storied at 4^{o}C.

3.1.5. Reagents

3.1.5.1. Oxidase reagent

It is consisting of dissolving 1g of tetra-methyl p-phenylene-diamine in 100 ml distilled water.

3.1.5.2. Catalase reagent

It is consisting of 3% Hydrogen peroxide.

3.1.6. Gram stain

It is used in the identification of bacterial shape and in the typing of bacteria into the Gram-positive and Gram-negative bacteria.

3.1.7. Statistical Analysis

The analyzed results were expressed as percentages for the descriptions of thedistribution of site infection cases according to age, sex, organisms, antibiotics, etc.

The information were coded, checked, entered and analyzed using SPSS file using SPSS version 18 software computer package. Chi square (X2) and validity tests were used for analysis, differences in proportions were assessed by Chisquare test, P values <0.05 were considered statistically significant.

3.2. Methods

3.2.1. Specimens Collection

Eighty *A. baumannii* isolates were identified from a total of 730 samples, were collected from different human infections including 400 burn, 170 wound surgeries, 130 urine and 30 ear swabs from Central Health Laboratory and Burn and Plastic Surgery Hospital/Emergency in Sulaimani city, during the periods between June 2014 and June 2015. Those samples were taken according to the methods suggested by (Collee et al., 1996).

In burn and wound exudates, swabs were taken from the depth of burn or lesion, a dry swab must first be moistened with a little amount of Brain-Heart Infusion broth or saline. In ear infection, swabs were taken after cleaning the external auditory canal from pus with a sterile swab moistened by (70%) ethanol, while in UTIs, urine samples were collected by taking the mid-stream urine in sterile wide mouth containers with tightly fitted lids. All swabs and specimens were transported to the lab without delay. Samples were transported to the laboratory and processed immediately or refrigerated at 4ºC as soon as possible. All samples were cultured on the suitable bacterial media as soon as they arrived to the lab.

3.2.2. Sterilization of media and materials

3.2.2.1. Autoclaving

All media were sterilized by autoclaving at 121°C and under 1.5 bar for 15-20 minutes, except for sugar containing media which were sterilized for 10 minutes.

3.2.2.2. Dry sterilization

All glasswares were washed with detergent and rinsing with water and allowed to dry, then the glass wares were sterilized in a hot air oven at 180°C for two hours.

3.2.3. Storage of *A. baumannii* strains

Isolates were stored for 1-3 months in glycerol at 4^{o}C according to (Prescott 2002), who described the short term maintenance (generally between one to three months) of aerobic bacteria.

3.2.4. Isolation of *A. baumannii*

3.2.4.1. Gram stain

The preparation and screening of a Gram-stained smear is a necessary part of the laboratory process. by rolling the swab of specimens over the clean glass slide or Placed one loop drop of well mixed uncentrifuged urine on a clean free glass slide. After fixation with heat, it was stained with Gram stain. The stained smear was screened carefully under an oil-immersion lens for the presence or absence of bacteria bacteria and their Gram reaction, shape, size and arrangement. *A. baumannii* was shown as short, plump, Gram negative bacteria.

3.2.4.2. Bacterial culture

The suspected isolates were platted onto dried plates of MacConkey agar and 5% sheep blood agar, EMB, and Nutrient agar (which prepared Previously) at one and same time by rolling the swab over the agar to make a primary well and then streaking from the primary inoculum using a sterile bacteriological loop to form secondary, tertiary and quaternary streak lines. These plates were incubated overnight at 37°C, and isolates showing the colony characteristics were noted Medium sized, smooth, convex colonies with a slightly pinkish tint on MA, *A. baumannii* grown on supplemented SBA were recognisable by non hemolytic opaque creamy colonies on blood agar. whereas the plates with no growth were discarded.

3.2.5. Identification of *A. baumannii*

3.2.5.1. Conventional Methods

3.2.5.1.1.Direct microscopic examination

asmear was made on a clean grease free glass slide. Smears of positive and negative controls were made on either side of the test smear. After fixation with heat, it was stained with Gram stain. The stained smear was screened carefully for presence of bacteria and their Gram reaction, shape, size and arrangement.

3.2.5.1.2. Biochemical tests

Preliminary identification was done with the help of the following methods: The process starts with primary rapid enzymatic tests like oxidase and catalase test.

3.2.5.1.2.1. Catalase test

Catalase test was used to determine the organism′s ability to breakdown hydrogen peroxide (H_2O_2) into oxygen and water by action of the enzyme catalase. With a loop a small amount of bacterial colony of pure growth was picked from MacConkey agar (preferably not more than 24 hours old) was transferred on to the surface of a clean glass slide then immediately a drop of freshly prepared 3% hydrogen peroxide (3% H_2O_2) was placed on to apportion of colony on the slide. The evolution of bubbles of gas indicates a positive result (Forbes *et al*., 2007).

3.2.5.1.2.2. Oxidase test

A speck of colony (preferably not more than 24 hours old) was rubbed onto a filter paper impregnated with freshly prepared 1% tetramethyl p-phenylene-diamine-dihydrochloride. An intense deep purple hue appearing within 5 – 10 seconds indicates a positive reaction. Colour appearing between 10 – 60 seconds was considered delayed positive result and any colour appearing after 60 seconds was considered as negative reaction (Kiser *et al*.,2011).

3.2.5.1.3. Growing at 44^{o}C

The suspected *A. baumannii* isolates were inoculated into brain heart infusion broth, incubated at 44^{0}C for 24 hours. *A. baumannii*growth was observed after incubation (Lennette *et al*., 1974).

3.2.5.2. Automated Identification Method by (Vitek 2 Compact System)

The process starts with primary rapid tests for identification of bacterial colony by direct examination, microscopic observation of slid smear with Gram staining, rapid enzymatic tests like catalase oxidase test etc. Rapid tests are followed by simultaneous transmission of desired colonies on a special suitable strip or on those culture media for *A. baumannii* colonies individually.

After isolation of bacterial colonies on the conventional culture media, isolates were identified by automated method was identification with Vitek2® compact auto analyzer system manufactured by BioMérieux®. The results produced by the machine were analyzed using Vitek2®compactsoftware.

3.2.5.2.1. Identification Process with Vitek2

A sterile stick applicator used to take pure colonies from culture media and transfer a sufficient number of them to plastic test tubes. Test tubes contain about 3.0 ml of sterile saline to suspend the bacteria. Concentration of bacterial suspension in saline (aqueous 0.45% to 0.50% NaCl, pH 7.0) in a 12 x 75 mm clear plastic (polystyrene) test tube, was adjusted to become equivalent to a McFarland No. 5 which had colony forming unit equal to 1.5×108. was checked by densitometer and was adjusted between the tolerances ranges before introducing the sample to the analyzer. Density means “turbidity in photometrical concept” here and it doesn’t use as a physical concept of gravity. After mixing by shaker in order to produce a homogenous suspension of bacteria, the turbidity of suspension was

adjusted by adding proper amounts of saline or bacteria. The density (turbidity) of the suspension was checked by using a calibrated turbidity meter called the DensiChek.

3.2.5.2.3. reagent (ID-GNB)card and Antimicrobial sensitivity testing (AST-N262)card

The identified isolates were confirmed with the automated VITEK-2 compact system by using Identification Nonenteric Gram-Negative Bacilli(ID-GNB) cards, according to the manufacturer's instructions The reagent cards have 64 wells that can each contain an individual test substrate. Substrates measure various metabolic activities such as acidification, alkalinization, enzyme hydrolysis, and growth in the presence of inhibitory substances. Susceptibility tests with the VITEK 2 system were performed with AT-N262 cards, according to the manufacturer's instructions. The 64-well AT-N262 card contains the following antimicrobial agents of 16 antibiotics were amikacin, Ampicillin/sulbactam, cefipime, cefoparazone-sulbactam, ceftazidime, ciprofloxacin, colistin, gentamicin, imepenem, levofloxacin, meropenem, netilmycin, piperacillin, tetracycline, tigecycline, trimethoprim/sulfamethoxazole An optically clear film present on both sides of the card allows for the appropriate level of oxygen transmission while maintaining a sealed vessel that prevents contact with the organism-substrate admixtures. Each card has a pre-inserted transfer tube used for inoculation (described below). Cards have bar codes that contain information on product type, lot number, expiration date, and a unique identifier that can be linked to the sample either before or after loading the card onto the system. Figure 2 shows the GN card.

3.2.5.2.2. Inoculum Preparation

Procedure:

1. The VITEK 2 Gram-negative identification (ID-GNB) and the VITEK 2 Gram-negative susceptibility (AST-NO29) cards were taken out the refrigerator and allowed to achieve room temperature.
2. A Smart Carrier containing room for 14 tubes and 14 cards were placed.
3. The identity numbers of the bacterial strains were manually entered in the "Accession number" field.
4. Colonies from an overnight agar plate was suspended in 3 ml 0.45 % NaCl (bioMérieux, France) and the turbidity was measured by a densitometer and adjusted to 0.50 – 0.63 McFarland.

5. A control spread to check for contaminants was performed.
6. The inoculum tubes were placed at the first positions while empty tubes were placed at the following positions.
7. The ID-GNB card barcode labels were scanned and the cards were placed in the card positions next to the inoculum tubes.
8. The AST-NO29 card barcode labels were scanned and the cards were placed in the card positions next to the empty tubes.
9. The Smart Carrier containing the inoculum tubes, empty tubes, ID-GNB cards, and AST-NO29 cards were placed in VITEK 2 where each test card was automatically filled with a bacterial suspension and automatic identification and susceptibility testing was performed by kinetic fluorescence measurement every 15 min. The software then analyzed the data and reported the results. All isolates introduced to the computer before processing and inoculated. The time interval between suspension preparation and card filling was less than 30 min to avoid changes in turbidity.

Inoculated cards were passed by a mechanism, which cut off the transfer tube and sealed the card prior to loading into the circular, incubator were incubated at 35.5 + 1.0ºC. Each card was removed from the incubator once every 15 minutes, transported to the optical system for reaction readings, and then returned to the incubator until the next read time. Data were collected at 15-minute intervals during the entire incubation period.

The total process to determine the MIC (Minimum Inhibitory Concentration) results by AST-N262 (bioMerieux, France) cards need to 18 hours.

Gram negative were processed for identification and antibiotic sensitivity tests by the Vitek 2 Compact system (BioMe´rieux, Marcy l'Etoile, France), following CLSI guidelines.7

3.2.5.2.3. Card Sealing and Incubation

Inoculated cards are passed by a mechanism, which cuts off the transfer tube and seals the card prior to loading into the carousel incubator. All card types are incubated online at 35.5 + 1.0ºC. Each card is removed from the carousel incubator once every 15 minutes, transported to the optical system for reaction readings, and then returned to the incubator until the next read time. Data are collected at 15-minute intervals during the entire incubation period.

Vitek2® GN cards were set up according to instructions given by BioMérieux®. All reagents and equipment needed for processing supplied by Manufacturer Company. All isolates introduced to the computer before processing and inoculated cards were processed in the instrument within 30 min of inoculation.

GN cards were loaded (inoculated) with bacterial suspensions using a vacuum chamber in machine. Test tubes containing the samples were placed into a cassette (a special test tube rack) and the identification card was placed in the neighboring place while inserting the transfer tube into the corresponding suspension tube. The cassette could accommodate up to 10 test tubes. The filled cassette was placed into a vacuum chamber station inside the vitek-2 analyzer machine. The vacuum was applied then the air was recharged into the station, the bacterial suspension was forced through the transfer tube into micro-channels that filled all the test wells.

Inoculated cards were passed by a mechanism, which cut off the transfer tube and sealed the card prior to loading into the circular incubator. The incubator could accommodate up to 30 cards. All card types were incubated at 35.5 + 1.0ºC. Each card was removed from the incubator once every 15 minutes, transported to the optical system for reaction readings, and then returned to the incubator until the next read time. Data were collected at 15-minute intervals during the entire incubation period.

3.2.5.2.4. Optical System

A transmittance optical system allows interpretation of test reactions using different wavelengths in the visible spectrum. During incubation, each test reaction is read every 15 minutes to measure either turbidity or colored products of substrate metabolism. In addition, a special algorithm is used to eliminate false readings due to small bubbles that may be present.

3.2.5.2.5. Identification Levels

An unknown biopattern is compared to the database of reactions for each taxon, and a numerical probability calculation is performed. Various qualitative levels of identification are assigned based on the numerical probability calculation. Strain identification at the species level was divided into four groups based upon the probability of accurate identification as follows: excellent (probability of accurate identification, _96%), very good (93 to 95%), good (89 to 92%), and acceptable (85 to 88%).

3.2.5.2.5. Importance of Pure Sample

Isolation of pure bacterial colonies in microbiology has a significant importance. It is the primary precondition for successful identification of microorganism. In automated systems it is even more important to get a huge amount of fresh and pure isolated samples. These bacteria are needed for making of high concentration of active exponential phase bacterial suspensions which prevents environmental contaminations. Since sterile working condition couldn't reach properly during inoculation of cards, old colonies of bacteria or low concentrations of inoculants could be corrupted by contaminations. Sometimes it takes more time to get proper density of desired bacterial colony otherwise low discrimination & misidentification of bacteria occurs during analyze process. For purity checking of the feeding samples, a subculture from applied suspension was done during inoculation of cards for every sample. The results of subcultures were helpful in the low discrimination cases reported by machine.

4. RESULTS AND DISCUSSION

4.1. Incidence of *A. baumannii*

The study showed that 80 isolates of *Acinetobacter* out from the total number of 730 positive cultures, from the Central Health Laboratory and Burn and Plastic Surgery Hospital/Emergency in Sulaimani city, between June 2014 and June 2015. In this work, the numbers and percentages of *A. baumannii* strains isolates from different specimens as shown in table (4.1) and figure (4.1).

The majority of samples that have been identified phenotypically via the VITEK-2 system as positive for *A. baumannii*were collected from burn, which totalled 50 samples, or 12.5% of the total burn samples, 18 (10.60%) isolates from 170 septic surgical wound exudates and pus specimens, 10 (7.70%) isolates from 130 tissue samples and 2 (6.67%) isolate from 30 urine samples. The total *A. baumannii* strains which were found nearly 11% of the total samples.

Table 4.1 Numbers and percentages of *A. baumannii* isolates from different infection sources

Result samples	**Burn**	**Wound, surgery and Pus**	**Urine**	**Ear**	**%**
Total samples	400	170	130	30	730
A. baumannii	50 12.5%	18 10.59	10 7.7	2 6.66	80 10.96
othe organisms	350 87.5	152 89.41	120 92.3	28 93.33	650 89.04

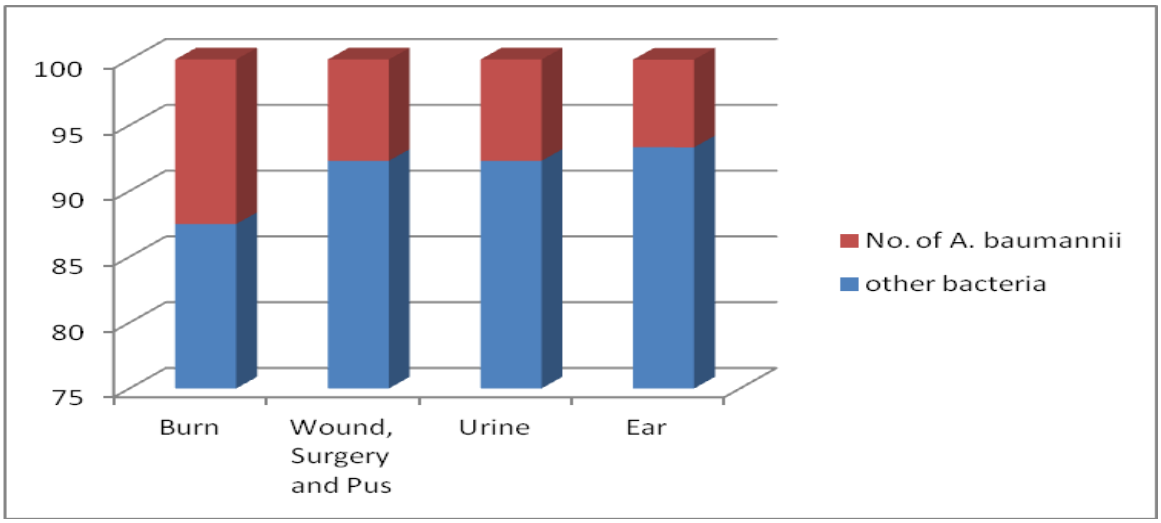


Figure 4.1. The *A. baumannii* isolates from different infection sources

The majority of samples identified as positive for *A. baumannii* were collected from burn, totaling 50, or 62.5% of the total positives isolates. This was followed by samples collected from wound, totalling 18, or 22.5% of the total positive isolates. The third most prevalent source was from tissue samples, totaling 10, or 12.5% of the total positive isolates, the fourth prevalent source was from urine samples, totaling 2, or 2.5%.

In this study, the prevalence of *A. baumannii* was 11% this result rather agree with who found that incidence of *A. baumannii* in Al-Diwaniya city was 10.3% Al-Hamadani *et al*.(2012).This result has correlated well with the another study conducted by Patwardhan *et al*.(2008). from developing countries like India where the rate were 11%. in a study conducted by Jafari and Karbasizade in Mashhad of Iran (2014), the frequency of *Acinetobacter* sp. in burn were determined about 10% and Arora *et al* 2003 reported it to be 8.4% which are lower than that in the current study. In Japan the prevalence rate was 18% (Endo *et al*., 2012), in Kuwait 22.1 (Abdallah *et al*., 2010), and in Saudia Arabia 31.7 (Al Johan *et al*., 2010), which are higher than that in our study. However Al Johani *et al*. (2010) in Saudi Arabia had reported a much higher rate of isolation of *A.baumanii* (31.7%)among the Gram negative and (21.13%) of the total case. In all these studies *A.baumanii* was the second commonest isolate after *P.aeruginosa*.

It is noted that incidence of *Acinetobacter spp*. differs from region to another even in the same city or hospital; this may be due to time and study conditions in addition to number of collected samples, however, the incidence of *Acinetobacter* infections has risen

significantly and continuously worldwide, this bacterium became important nosocomial pathogens.

4.2. Incidence of *A. baumanni* in Relation to Sex Groups

In this study, we also deduce that the percentage of males and females positivefor *A. baumannii*. Isolates were recovered from 80 patients including, 42 males (52.5%) and 38 females (47.5%) as shown in table (4.2) and figure (4.2). Our results were nearly agreed with the some of the other results, in a study conducted by Al-Hasan. (2011), showed that the percentage of male and female patients with positive *A. baumannii* equal to (50.9%), (49.1%) respectively, but disagreement with those obtained by Kirkgoz and Zer. (2014) reported that male (66 %) and female (44 %).

Table 4.2. Frequency *A. baumannii*of according to the gender.

Gender		**Culture results**		**Total**
		Positive	**Negative**	
Male	No.	42	308	350
	%	12	88	47.6
Female	No.	38	347	385
	%	9.9	90.1	52.4
Total	No.	80	655	735
	%	10.89	(89.11)	100%

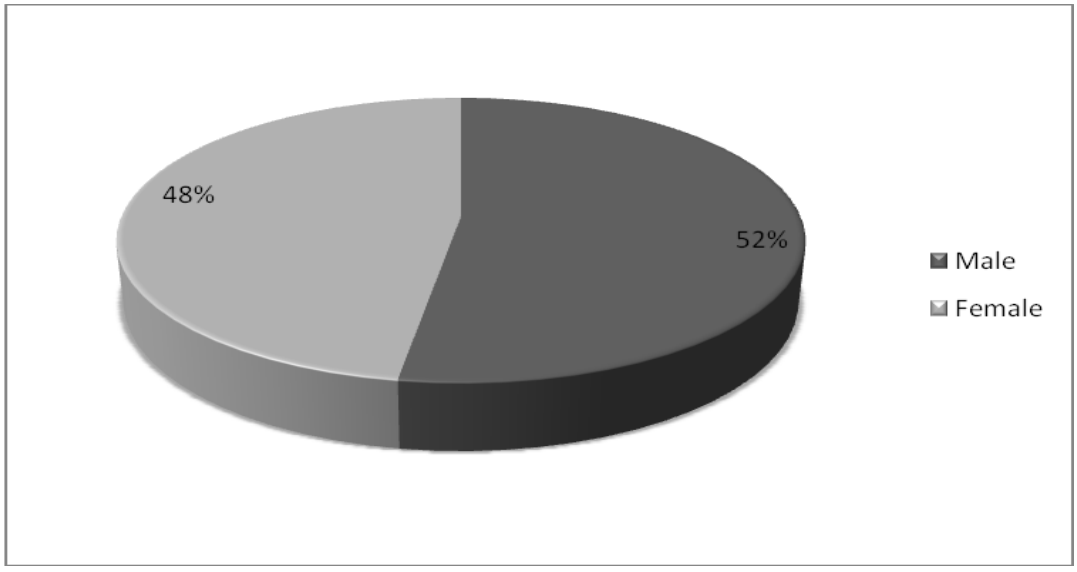


Figure 4.2. Frequency of A. baumannii according to the gender.

4.3. Incidence of A. baumannii in Relation to Age Groups

The distribution of *A. baumannii* isolates among different age groups are presented in table (4.3) and figure (4.3). in which 9(11.25%) of the patients were below 10 years, 20 (25%) of the patients were within the 11-20 years old, 23 (28.75%) of the patients were within the 21- 30 years old and 18 (22.5%) of the patients were within the 31- 40 years old and 3 (3.75%) of the patients were within the 41-50 and 51-60 years old and 4 (5%) patients were above 60 years old. However, there were no statistical significant differences regarding the distribution of A.bauman-nii isolates among the different age groups (Pvalue = 0.269). This results almost in agreement with Zhanel *et al*. (2013), also reported highest incidence of infection with *A. baumannii* was in the 21-30 years old group and the lowest in the 41-50 and 51-60 years old group. This could be explained by the fact that impaired immunity with the aging predisposes to many infections.

The mean and median age of the study population were 42 ± 19.4 years, (the range of age varied from less than 1 to 80 years).

Table 4.3. The distribution of *A. baumannii* isolates among different age groups

Age group	**No. of samples**	**Culture samples**	
		Positive No.	**Percentage %**
<10	140	9	11.25%
11-20	165	20	25%
21-30	170	23	28.75%
31-40	130	18	22.5%
41-50	50	3	3.75%
51-60	35	3	3.75%
61-and above	45	4	5%
Total	735	80	100%

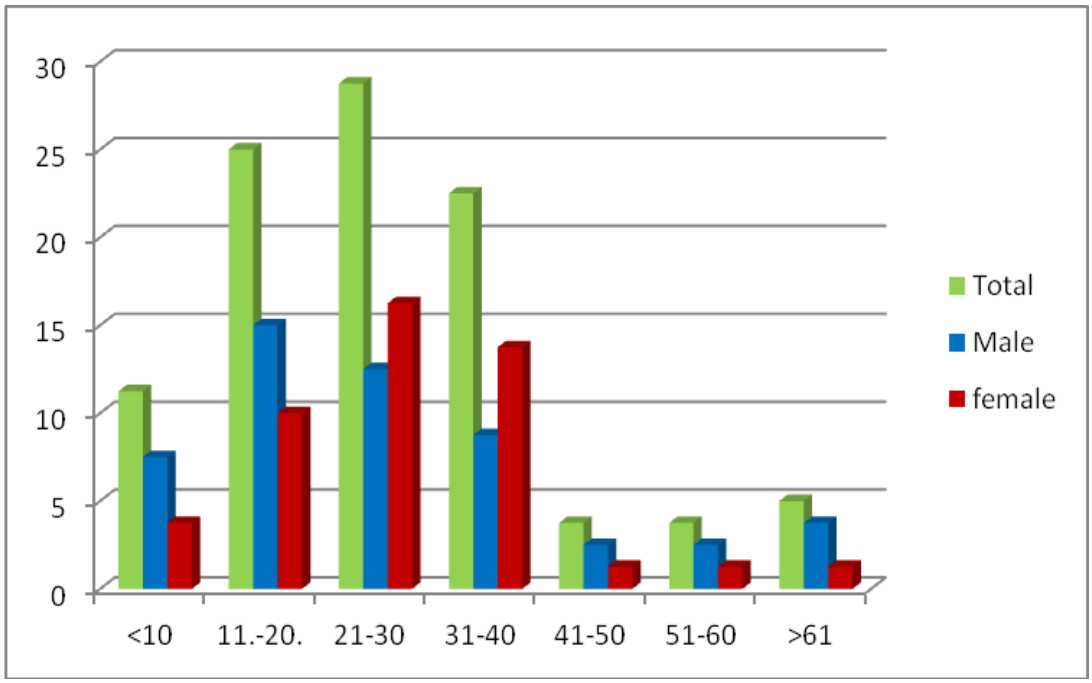
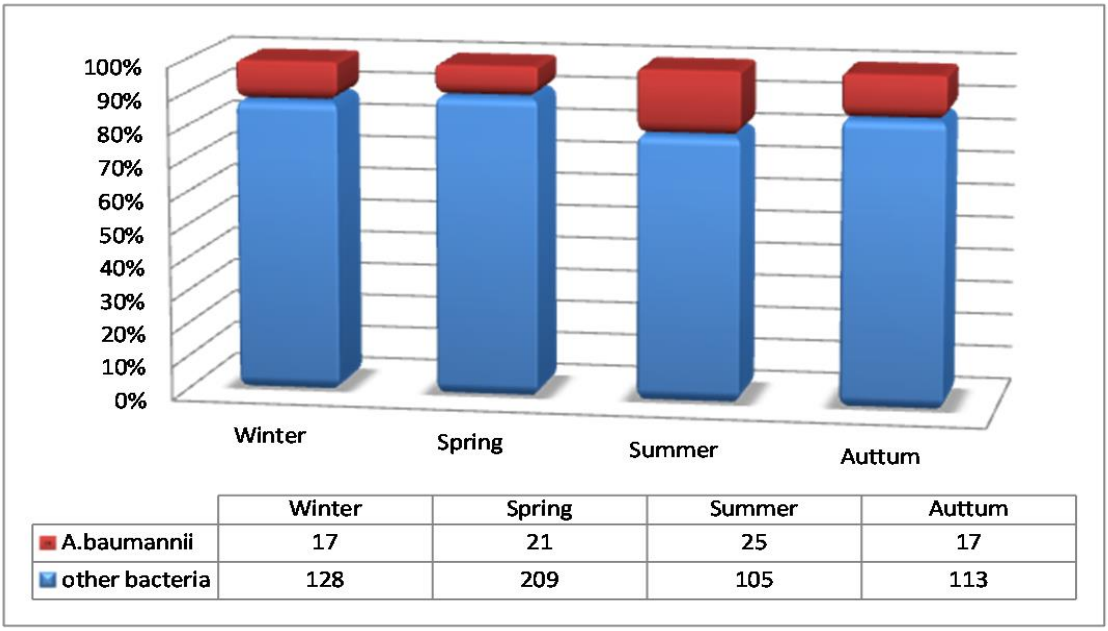


Figure 4.3. *A. baumannii* isolates among age group.

4.4. Seasonal Variation of A. baumannii

The present study shows that the seasonal increase in incidences of *A. baumannii*infections, which was maximal in the summer months(31.25%) than during the winter month(21.25%), spring months(25%), auttum months(22.5%), Although the correlation between season and frequency was not statistically significant as shown in table (4.4) and figure (4.4)

Reported data concerning this field of study regularly shows high incidence of *A. baumannii*infections with increases in late summer months for all major infection sites (McDonald *et al*., 1999). thisphenomenon has also been observed in other areas of the world that have similar temperature increases during the summer months, we consider that this pattern should be further investigated (Fukuta*et al*., 2012).



	Winter	Spring	Summer	Auttum
A.baumannii	17	21	25	17
other bacteria	128	209	105	113

Figure 4.4. Seasonal incidence density of A. baumannii

The high incidence of A. baumannii in in the summer might be as a result of a variety of factors. Rather, seasonal variation in Acinetobacter infections is probably the result of changes in climate.

According to a time-series analysis reported in a study conducted at the University of Maryland Medical Center, the increase in the monthly *A. baumannii* infection rate was 17% for each 10 8F increase in outdoor temperatures (Perencevich*et al*., 2008).

It has been suggested that increased ambient temperature may promote a biofilm "bloom" of *Acinetobacter* species in hospital tap water (Christie *et al*., 1995).

Although heating, changes in outdoor humidity can affect moisture within the hospital environment. Investigations in which *Acinetobacter* species isolates have been recovered from aerosols and the air of hospitals suggest that airborne dissemination has a role in nosocomial transmission (McDonald *et al*., 1999; Jawad *et al*., 1998; Beck-Sague *et al*., 1990).

4.5. Susceptibility tests

The isolates were tested by the VITEK 2 system, according to the manufacturer's instructions were performed with AT-N0262 cards against different classes of antibiotics including carbapemens, cephalosporins, aminoglycosides, penicillins, fluoroquinolones andglycylcyclines, polymycin E(colistin), .

The 64-well AT-N0262 card contains the following antimicrobial agent at the indicated concentrations: piperacillin, 4, 16, 32 and 64 mg/ml; ampicillin/sulbactam, 4, 16, and 32 mg/ml; ceftazidime, 1, 2, 8, and 32 mg/ml; cefepime, 2, 8, 16 and 32 mg/ml; imipenem, 1, 2, 6, and 12 mg/ml; meropenem, 0.5, 2, 6 and 12 mg/ml; amikacin, 8, 16 and 32mg/ml; gentamicin, 4, 16, and 32 mg/ml; netilmicin, 4, 16 and 32mg/ml; cefoperazone/Sulbactam, 8, 16 and 32 mg/ml; ciprofloxacin, 0.5, 2, and 4 mg/ml; levofloxacin, 0.25, 0.5, 2, and 8 mg/ml; tetracycli-ne, 2, 4, and 8 mg/ml; tigecycline, 0.75, 2 and 4 mg/ml; colistin, 4, 16 and 32 mg/ml; trimeth-oprim/Sulfamethoxazole, 1, 4 and 16mg/ml.

The result of antimicrobial susceptibility test from table (4.4) and figure (4.4) show that all strains100% (n = 80) were resistant to Piperacillin (MIC≥128), Ampicillin/sulbactam (MIC≥32), while 97.5% (n = 78), 96.25% (n = 77), 96.25 %(n = 77), 95%(n = 76), 93.75% (n = 75), 91.25%(n = 73), 91.25% (n = 73), of the strains showed resistant to Ceftazidime(MIC≥64), Gentamicin(MIC≥16), Ciprofloxacin (MIC≥4), Trimethoprim/Sulfamethoxazole(MIC≥320), Ce-fepime(MIC≥64), Imipenem(MIC≥16), Meropenem(MIC≥16) respectively, whereas, 87.5% (n = 70), 78.75% (n = 63), 75%(n=60), 66.25%(n=53) of the strains showed resistance to Tetracycline (MIC≥16), Levofloxacin (MIC≥8), Cefoperazone/Sulbactam, Netilmicin (MIC≥32) respectively,

Amikacin (MIC≥64), exhibited the high activity against the isolates in comparison to above antimicrobials 43.75 %(n=35) of strains were resistant, Also colistin(MIC<=0.5) exhibited the highest activity against the isolates in comparison to all antimicrobials 100%(n=80) of strains susceptible, followed by tigecycline(MIC<=1) 88.75%(n=71) of strains were susceptible.

This study shows an alarming rate of *A. baumannii* resistance to sixteen antibiotics. The greatest increase in antimicrobial resistance rate is observed in beta-lactamase inhibitors + penicillin, extended-spectrum cephalosporins, Folate pathway inhibitors, carbapenems, aminoglycoside and floroquinolones categories

Table 4.4. Antimicrobial susceptibilities of 80 *A. baumannii* isolates to 16 antibiotics

Antibiotics		**MIC(µg/ml)Range Concen-tration**	**Interpretive Break points MIC range (µg/mL)**		**Sensitive(S) NO %**		**Intermediate(I) NO %**		**Resistant(R) NO%**		
Piperacillin	IPIP	4-64	S<=4	R>=128	-	-	-	-	80	100	=
Ampicillin/Sulbactam	AM	4/2-32/16	S<=2	R>=32	-	-	-	-	80	100	
Ceftazidime	CAZ	1-32	S<=1	R>=64	-	-	2	2.5	78	97.5	
Cefepime	FEP	2-32	S<=1	R>=64	5	6.25	-	-	75	93.75	
Imipenem	IMP	1-12	S<=0.25	R>=16	5	6.25	2	2.5	73	91.25	
Meropenem	MEM	0.5-12	S<=0.25	R>=16	5	6.25	2	2.5	73	91.25	
Amikacin	AN	8-64	S<=2	R>=64	40	50	5	6.25	35	43.75	
Gentamicin	GM	4-32	S<=1	R>=16	2	2.5	1	1.25	77	96.25	
Netilmicin	NET	4-32	S<=1	R>=32	21	26.25	6	7.5	53	66.25	
Cefoperazone/Sulbactam	SEP	8-32	S<=8	R>=64	17	21.25	3	3.75	60	75	
Ciprofloxacin	CIP	0.5-4	S<=0.25	R>=4	3	3.75	-	-	77	96.25	
Levofloxacin	LEV	0.25-8	S<=0.12	R>=8	0	0	17	21.25	63	78.75	
Tetracycline	TE	2-8	S<=1	R>=16	8	10	2	2.5	70	87.5	
Tigecycline	TGC	0.75-4	S<=0.5	R>=8	71	88.75	-	-	9	11.25	
Colistin	CS	4-32	S<=0.5	R>=16	80	100	-	-	-	-	
Trimethoprim/Sulfamethoxazole	SXT	1/19-16/320	S<=20	R>=320	4	5	-	-	76	95	

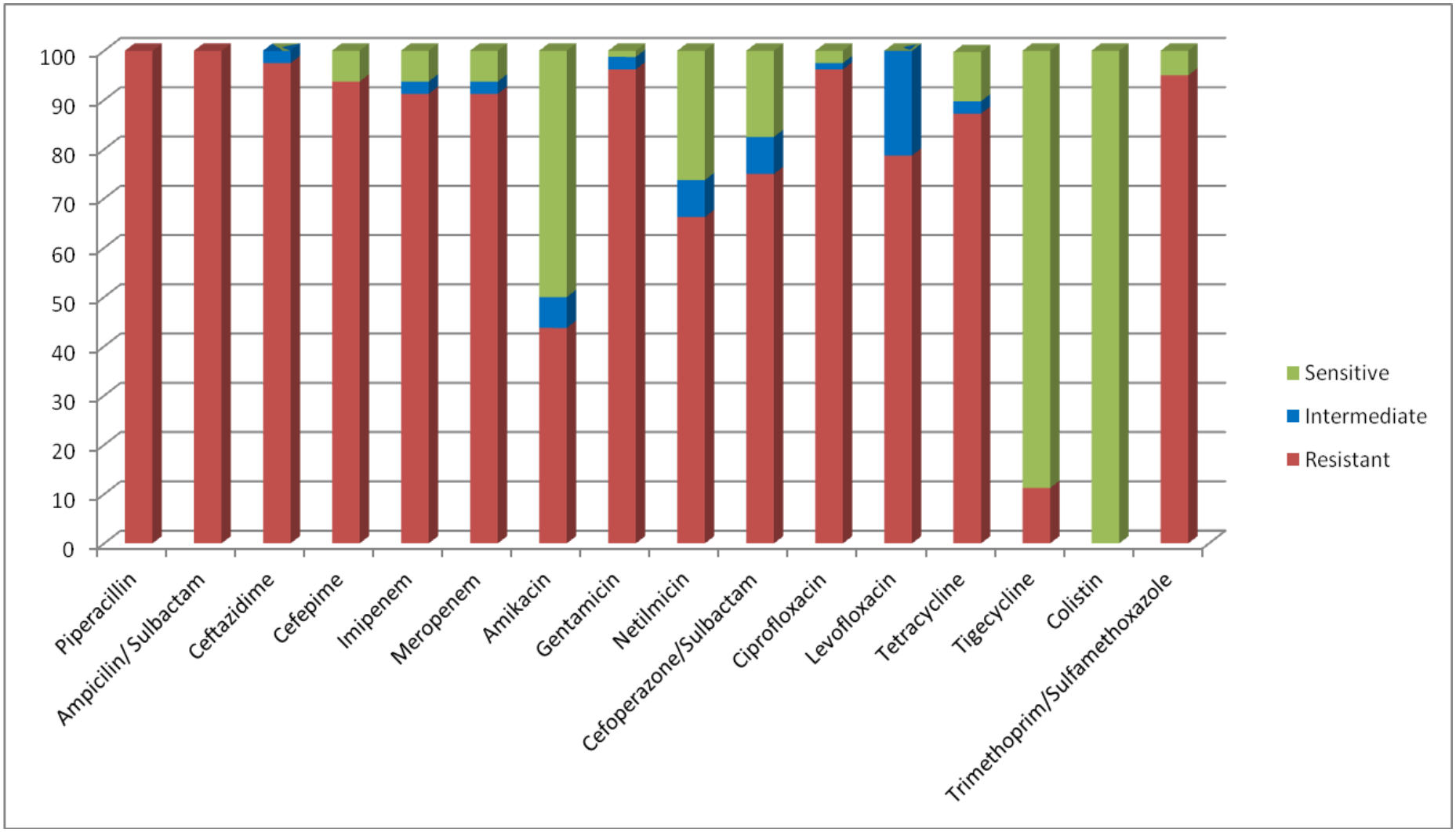


Figure 4.5. Antimicrobial Susceptibilities Of 80 A. baumannii Isolates Against 16 Antimicrobial Agents.

4.5.1. Resistance to Carbapenem

Of the 80 isolates, 73 (91.25) isolates were found to have Carbapenem Resistance *A. baumannii*(CRAB) in there clinical samples. A high levelof resistance were found against carbapenems (imipenem and meropenem) 73(91.25%) , 2 (2.5%) were intermediate and 5(6.25%) were sensitive to Carbapenem As shown in the table(4.5) and figure (4.5).

However, the present study showed that there was a significantly associated with imipenem and meropenem resistance (p value < 0.005).

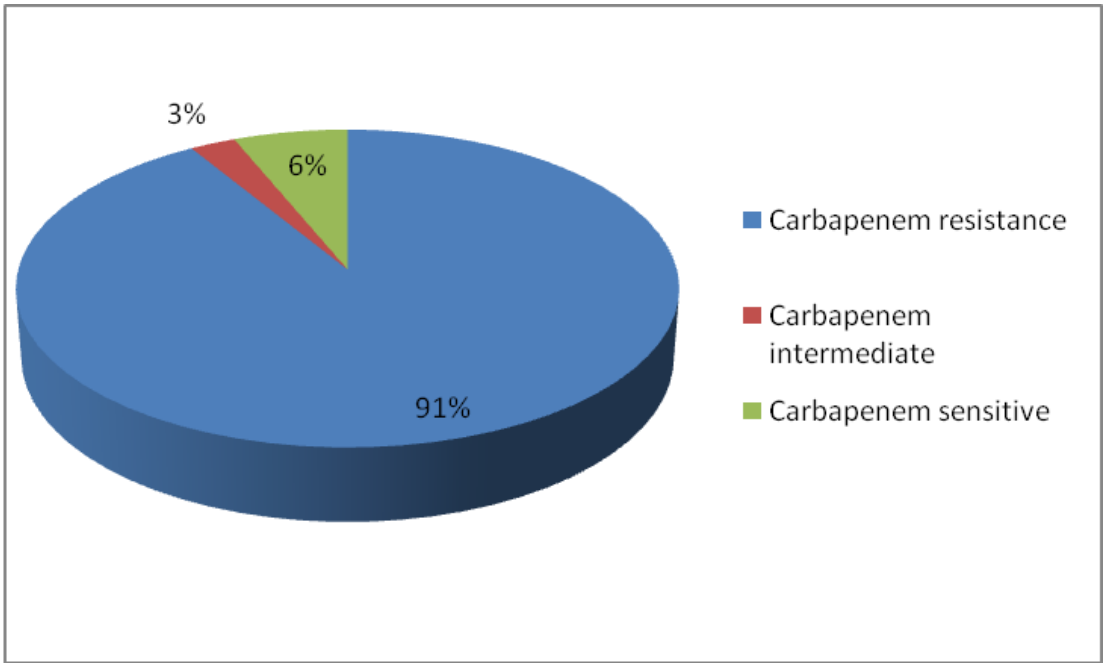


Table 4.6. Percentage of carbapenems susceptibility.to A. baumannii

Our study demonstrated high resistance to carbapenems in *A. baumannii*isolates thatwere collected from clinical specimens in Central Health Laboratory and Burn and Plastic Surgery Hospital/Emergency in Sulaimani city during the period from July, 2014 to Jun, 2015.

The increase in CR-AB is becoming a worldwide phenomenon, but with some variation in the carbapenem resistance numbers from one geographical area to another. There have also been reports of an increase of 6% in 1998 which increased to approximately 29% in 2005 of Meropenem resistant *A. baumannii*(Perez *et al*,. 2007).

Our results agreed with Another studies was reported that the resistance rate was 91.5% is increasing to meropenem (Goudarzi*et al*.,2013) and 91.30% among *A. Baumannii* isolated from two intensive care units (ICUs) of two Algerian University hospitals. another

results was reported that the resistance rate in tehran 91.7% to meropenem, 91.7% to imipenem(Noori *et al*., 2014). Also in some studies the resistance rate 86% in Araq-Iran lower than our study (Japoni-Nejad *et al*.,2013).

In some studies the resistance rate to meropenem and imipenem was reported 81% (Japoni-Nejad *et al*.,2013) and 60% (Feizabadi *et al*., 2008; Mirnejad *et al*., 2012) in Iran, which is in contrariety with the findings of Hujer *et al*. at 2006 who reported a resistance of about 20% to the mentioned antibiotics.

The resistance to carbapenems isolates from Sulaimani city-Iraq increased dramatically like other the world. In a study conducted by Shali.2012 the imipenem-resistance was 57.1% in Sulaimani city during the period from February, 2010 to March, 2012, but after five years ago in our study become 91.25%.this indicated that the rapid changes in the susceptibility to carbapenems in our study and another study make a problem to treat infections caused by this microorganism.

Even though the trend of carbapenem-susceptibility is clear in the regional countries. It is necessary to get more information about this, including the study of more medical centres and more isolates. This is because the evolution of the carbapenem resistance is clearly rapid which makes it necessary to take immediate action, otherwise these potent drugs will become useless within a few years.

In astudy confermed by Kırkgöz & Zer. (2014) in Gaziantep ahigh level of resistance was founded against carbapenems (98% for imipenem, 99% for meropenem) than our result. The effectiveness of betalactam antibiotics was found to be very low, and while only one isolate (1%) was susceptible to cefepime, none of the isolates showed susceptibility to ceftazidime, or piperacillin among CR-AB isolates. Moreover, there were not any isolates showing susceptibility to antimicrobial combinations containing beta-lactamase inhibitors (e.g. ampicillin/sulbactam and ampicillin/sulbactam).

In studies conducted in the authors' hospital, Karsligil *et al*. found carbapenem resistance at a rate of 9.6% in 2004, whereas it was 53.5% in Özgür Akın *et al*.'s study in (2009) the significant increase in the resistance rate is remarkable. It was reported that CRAB infections commonly occur in many hospitals. In fact, variable rates of antibiotic resistance in different hospitals are not surprising. However, a striking increase was observed in antimicrobial resistance as in the case for carbapenems(Zarrillii R *et al*., 2004 and Poque JM *et al*., 2013).

Also in a study conducted by Moradi *et al*.(2015) in Iran,observed that the resistance rate there was an increase in resistance to carbapenems (imipenem and meropenem) between 2001-2013 .Resistance to carbapenems was low at the study start point (51.1% imipenem, 64.3% meropenem) and increased by the end of the study (76.5% imipenem, 81.5% meropenem), thus demonstrating the most drastic increase in resistance rate.

For 2013, 26 countries reported 4084 isolates with AST information for carbapenem resistance. The percentages of resistant isolates in countries that reported ranged from 0 % in Finland and Norway to 90.6 % in Greece (De Kraker *et al*., 2013).

In another report of the SENTRY ProGram published in 2012 the susceptibility to carbapenems in isolates from Chile decreased dramatically. The imipenem-resistance has been significantly changing in Chile, while during the 1997- 1999 period, the imipenem-resistance was 0.0%, changing during the 2003-2005 period, when the imipenem-resistance was 6.2% and finally the rates of imipenem-resistance rose to 50.0% during 2010-2012 (Gales *et al*., 2012).

Surveillance studies indicate that the percentage of carbapenem-resistant isolates gradually increased over the ten years in Europe, Latin America and North America(Peleg *et al*., 2008). Numerous outbreaks of carbapenem-resistant *A. baumannii* were reported from hospitals in Northern Europe (Spain, the United Kingdom, Portugal, France, Netherlands, Poland, Czech Republic) (Dijkshoorn *et al*., 2007 and Coelho *et al*., 2006).also increased in Southern Europe and the Middle East particularly in Turkey, Bulgaria, Greece, Italy, Iraq, Iran, United Arab Emirates, Lebanon and Israel (Zarrilli R., 2007; Zarrilli R *et al*., 2004; Iacono M *et al*., 2008; Poirel L *et al*., 2006; Pournaras S *et al*., 2006; Zarrilli R *et al*., 2008; Meric *et al*., 2008; D'Arezzo *et al*., 2009 and Feizabadi *et al*. 2008), also in North America and Latin America cepecially in Chile, Colombia, Argentina and Brazil reported by (Villegas *et al*., 2007 and Merkier *et al*., 2008),in Tunisia and South Africa reported by (Marais *et al*., 2004 and Poirel *et al*., 2008), in China, Japan, South Korea, Taiwan, Singapore and Hong Kong reported by (Hsueh P-R *et al*., 2002 and Mendes RE *et al*., 2009) and Australia (Peleg *et al*., 2006). In the majority of cases, one or two epidemic strains were detected in a given hospital. Transmission of such strains was observed between hospitals in the same city and also on a national scale (Da Silva *et al*., 2004; Schulte *et al*., 2005; Hujer *et al*., 2006 and Scott *et al*., 2007) and a direct epidemiological link was established in several cases (van den Broek *et al*., 2006; Wybo, 2007; Schulte *et al*., 2005;

Hujer *et al*., 2006). The inter-hospital transfer of colonised patients was demonstrated during multi-facility outbreaks that occurred in the Netherlands, South Africa, Italy, and Tunisia (Poirel *et al*., 2008). The international transfer of patients colonised by CRAB was also reported (Schulte *et al*., 2005; Peleg *et al*., 2006 and Wybo *et al*., 2007).

The data also showed that the most drastic increase in resistance is associated with this antibiotic class, possibly due to its frequency of use in health care units. This is an alarming find-ing that strongly suggests the possibility of treatment failures in life-threatening *A. baumannii* infections due to carbapenem-resistance strains (Moradi *et al*., 2015).

The recent resistance or reduced susceptibility to carbapenems, is considered a serious clinical problem due to the role of first choice therapy that these drugs have had until now. *A. baumannii* isolates resistant to various classes of antibiotics are emerging worldwide.

Carbapenems were the most successful β-lactam antibiotics in evading bacterialresistance (Lee *et al*., 2005 and Livermore *et al*., 2000). Carbapenem sresistance, mediated by acquired carbapenemase genes, has been increasingly reported particularly for clinical isolatees of *P. aeruginosa* and *A. baumannii spp*. (Peymani *et al*., 2012: Towner *et al*., 2009 and Jacoby *et al*., 2005),The presence of bla-OXA-51-like beta-lactamases in Acinetobacter *baumannii* has a major role in the resistance to carbapenems (Shali.2012).

These beta-lactamases which have a role in carbapenem resistance appear to be globally spread (Poirel and Nordmann, 2006a), and recent studies also included Middle Eastern countries such as Iraq (Scott *et al*., 2007), Kuwait (Al-Sweih *et al*., 2011), Saudi Arabia (AlSultan *et al*., 2009), Bahrain (Mugnier *et al*., 2009) and the United Arab Emirates (Mugnier *et al*., 2008). These results provide evidences that detection ofbla-OXA-51-like can be used as a simple and reliable wayfor identifying *A. baumannii*. It has been found thatbla OXA-51-like exists in all isolates of *A. baumannii* and those strains that show carbapenem resistance arealmost possess bla OXA-51-like and bla OXA-23-like genes.

ESBL prevalence in *A. baumannii* also a significant in carbapenems resistance. This could be because of a lowering of infection control standards or the entry of multidrug resistant *Acinetobacter* from other hospitals.

Table 4.5. The distribution of antimicrobial susceptibility among CR-AB isolates

Antimicrobial agent	Sensitive(S) NO %		Intermedi-at(I)NO %		Resistant(R) No %	
AN	4	46	5	6.7	34	47
GM	-	-	1	1.4	72	98.6
NT	19	26	4	5.5	50	68.5
CIP	-	-	-	-	73	100
LEV	-	-	15	20.5	58	79.5
CAZ	-	-	-	-	73	100
FEP	-	-	-	-	73	100
SAM	-	-	-	-	73	100
SEP	3	17	3	4	58	79
PIP	-	-	-	-	73	100
IMP	-	-	-	-	73	100
MEM	-	-	-	-	73	100
TE	6	8	2	2.7	66	90.4
SXT	2	2.7	-	-	71	97.3
TGC	64	8.7	-	-	9	12.3
CS	73	100	-	-	-	-

Note: **AN**, Amikacin; **GM,** Gentamicin; **NT,**Netilmicin; **CIP,**Ciprofloxacin **LEV,** Levofloxacin; **CAZ,** Ceftazidime; **FEP**, Cefepime; **SAM**, Ampicillin/Sulbactam; **SEP**, Cefoperazone/Sulbactam; **PIP**, Piperacillin; **IMP**, Imipenem; **MEM**, Meropenem; **TE**, Teteracycline; **SXT**, Trimethoprim/sulfamethoxazole; **TGC**, Tigecycline; **CS**, Colistin

4.5.1.1. Effectiveness of betalactam antibiotics and extended spectrum cephalosporin on carbapenem resistance A. baumannii

In our result the effectiveness of betalactam or extended spectrum cephalosporin antibiotics was found to be very low, and while only five isolate (6.25%), was susceptible to cefepime, two isolate (2.5%) was intermediate to ceftazidime. all of them below the CRAB, and none of the isolates were showed susceptibility to piperacillin. In the present study high relationship observed between the non-resistance of cefepime and ceftazidime susceptiblity *A.baumanni* isolates among carbapenem non-resistance isolates.

Moreover, there were twenty (25%), four (5%),of isolates showing non-resistant(sensitive and intermediate) to antimicrobial combinations containing beta-lactamase inhibitors which cefoperazone/sulbactam and trimethoprim/sulphamethoxazole recepectivly also not any isolates showing susceptibility to ampicillin/sulbactam, Resistance rate against cefoperazone/sulbactam staticaly nonsignificant ($p>0.05$).but trimethoprim/sulphamethox azole and ampicillin/sulbactam were staticaly significant ($p<0.05$). in the present study Significant relationship not observed beeween the non-resistance of cefoperazone/sulbacta-

m, trimethoprim/sulphamethoxazole and ampicillin/sulbactam susceptiblity *A.baumanni* isolates among carbapenem non-resistance isolat-es (P > 0.05).

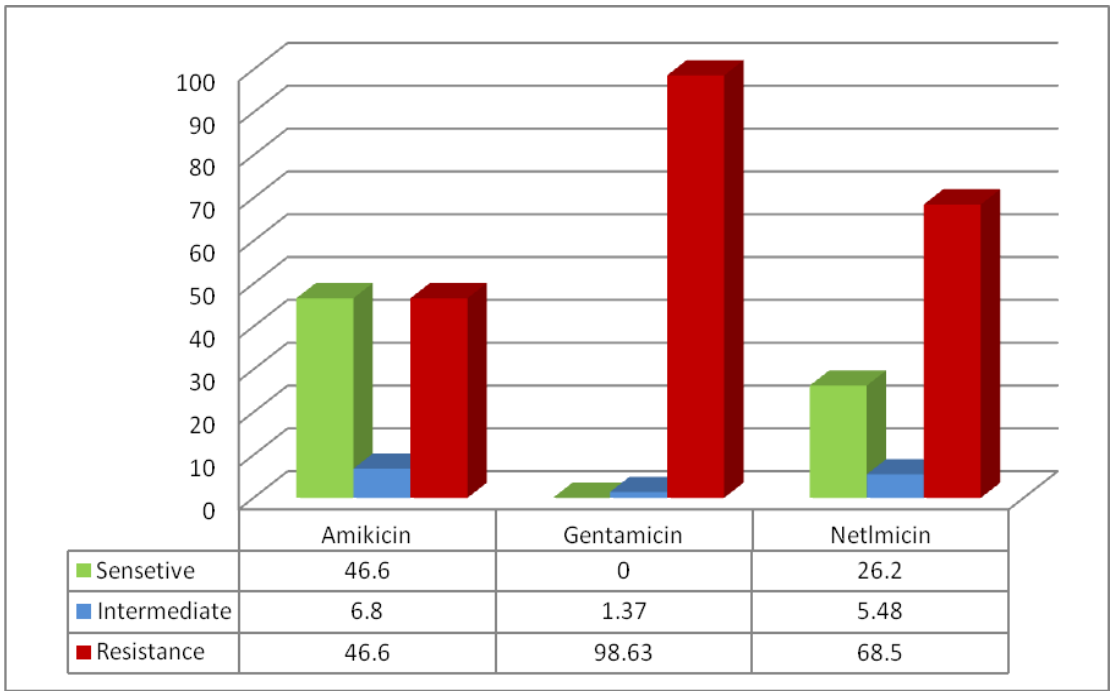
Among the 80(100%) *A. baumannii* isolates, 73 (91.25%) were found to be carbapenem resistance, while two (2.5%) isolates were found to be intermediate and five (5%) isolates of all *A. baumannii* showed sensitivity to carbapenem,

One of the main mechanisms of resistance to antibacterial agents is the production of β-lactamase enzymes. In most cases, β-lactamase causes bacteria to get resistant to a broad spectrum of antibiotics like fluoroquinolones, aminoglycosides and trimethoprim (Poole, 2004).

4.5.1.2. Aminoglycoside susceptibility amonge CR-AB

The activities of the aminoglycosides against the CR-AB isolates were limited, with Amikacin being the most active agent tested after colistin and tigecyclin. Our study shows higher activity of amikacin compared to other aminoglycosides, which is 47% of resistant strains, there was no statistically significant difference between amikacin susceptibility among the CR-AB isolates and to total *A. baumannii* isolates (P > 0.05). The levels of categorical agreement of the results obtained by Vitek 2 instruments. Aminoglycoside resistance matched the predicted phenotype in 72 of 73 isolates (98.65%) for gentamicin, 51of 73 isolates (69%) for netilmimicin, and 35 of 74 isolates (47%) for amikacin within the CR-AB.

İn another study the percentage of resistance to gentamicin increased to 94% (Gonzalez *et al*., 2000), indicating an important reduction of the activity of this drug.



	Amikicin	Gentamicin	Netlmicin
Sensetive	46.6	0	26.2
Intermediate	6.8	1.37	5.48
Resistance	46.6	98.63	68.5

Figure 4.7. Aminoglycoside susceptibility amonge CR-*A. baumannii*

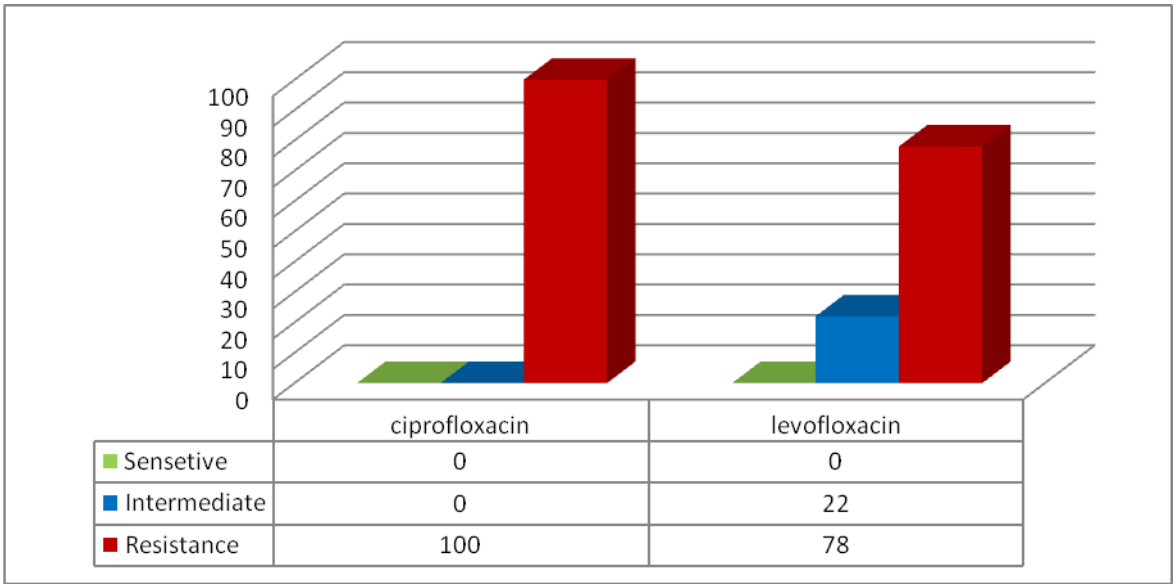
In astudy carried out by Ratemo *et al*.(2014) in Kenya showed resistance to amikacin was 40%.he explained by the fact that this molecule is little used in this hospital. Data from the literature postulate that this molecule loses over the years its effectiveness on the strains of *A. baumannii*(Memish *et al*., 2012; Patel *et al*., 2013 and Xu *et al*., 2013).

Co-resistance with other antibiotics like cefoperazone-sulbactam were more common in amikacin-resistant isolates which is significantly higher comparing to amikacin sensitive among the carbapenem resistant isolates (92.3%, 36/39 resistance vs. 3%, 1/34 sensetive, $P < 0.05$), all of the ciprofloxacin, piperacillin, Ceftazidime, Cefepime, Ampicillin/Sulbactam and gentamicin (100%, 39/39 resistance vs. 0%, 0/34 sensitive, $P < 0.005$), levofloxacin (100%, 39/39 vs. 3%, 1/34, $P<0.05$) and tetracycline (92.3%, 36/39 vs. 3%, 1/34, $P < 0.05$). (Figure 4.2).

The gentamicin resistance was nearly universal, even though it was the least used amin-oglycoside overall. These discrepancies suggest that aminoglycoside resistance in *A. baumannii* is mediated by complex and multifactorial mechanisms.

4.5.1.3. fluoroquinolones susceptibility amonge CRAB.

The fluoroquinolone, in this study ciprofloxacin showed 73(100%) resistance and levof-loxacin was 57 (78%) resistance . Similar results were obtained in Slovakia (Hos-tacka & Kloko-cnikova, 2002).



	ciprofloxacin	levofloxacin
Sensetive	0	0
Intermediate	0	22
Resistance	100	78

Figure 4.7. Antimicrobial susceptibility of fluoroquinolones among CRAB.

The sensitivity of *Acinetobacter* to ciprofloxacin dropped dramatically over the years in "Israel" (Simhon *et al*., 2001). The United States also experienced great reduction of susceptibility of ciprofloxacin against both *Pseudomonas aeruginosa* and *A. baumannii* in both ICU and Non-ICU patients (Karlowsky *et al*., 2003).

4.5.1.4. Cephalosporins susceptibility amonge CR-AB.

Cephalosporins are among the antimicrobials that showed the lowest activity against CR-AB as shown in table 4.5. The resistance rates were 100% for both antibiotics ceftazidime and cefepime these results are in agreement with other investigators (El-Shiekh et al., 2011).

4.5.1.5. Lipopeptides susceptibility amonge CRAB.

As mentioned previously, our data show that resistance to lipopeptides is lower compared with that of other antimicrobial groups. One of the antibiotics of this class is polymyxin E, which, despite previous concerns regarding its toxicity, has been implement-ed in treatment more frequently. Another lipopeptide antibiotic is colistin, which has prov-en effective in the treatment of wound, urinary tract, and bloodstream infections , although

its nephrotoxicity is a disadvanta-ge to its use. Although use of this antibiotic class has limitations due to toxicity, they are often used for the treatment of life-threatening infections (Moradi *et al*., 2015).

4.5.2. Resistotype patterns of *A. baumannii* isolates

The Antibiotic susceptibility profiles of MDR PDR-AB isolates which revealed 18 eighteen different resistotype patterns desig nated arbitrarily from **A-R.**

Table 4.6. Resistotype patterns of *A. baumannii*

Isolate code-number	**Isolates numbers**	**MDR**	**PDR**	**Antibiotic resistant pattern by vitek 2**	**No. %**
A	N6, N8, N10, N16, N23, N26, N29, N32, N39, N40, N46, N50, N51, N54, N55, N57, N59, N69, N72,	+	+	Resistant to all antibiot-ics but sensitive to CS and TGC only	19 23.7%
B	N3, N4, N7, N9, N11, N12, N17, N18, N20, N21, N25, N34, N42,N45, N58, N60, N61, N63,N65, N67, N70, N80	+	_	resistant to all antibiotics but sensitive to CS, TGC and AN only	22 27.5%
C	N24, N31, N66, N71, N73	+		resistant to all antibiotics but sensitive to CS, TGC, Intermedi-ate(non susceptible) to AN only	5 6.25%
D	N28, N33, N35,N41, N74	+	+	resistant to all antibi-otics but sensitive to CS, SEP, Intermedi-ate(susceptible) to LEV only	56.25 %
E	N48, N78	+	_	resistant to all antibiotics but sensitive to CS, SEP and NET only	2 2.5%
F	N14, N36	+	+	resistant to all antibiotics but sensitive to CS, TGC, and Inter-mediate(susce-	2 2.5%

				ptible) to LEV only	
G	N43	+	–	resistant to all antibiotics but sensitive to CS, TGC, AN and Intermediate to LEV only	1 1.25%
H	N19, N64	+	+	resistant to all antibiotics but sensitive to CS, TGC, and Intermediate (nons-usceptible) to SEP only	2 2.5%
I	N1, N2, N27, N49	+	–	resistant to all antibiotics but sensitive to CS, TGC, NET and AN only	4 5%
J	N13, N62, N68	+	–	resistant to all antibiotics but sensitive to CS, TGC, AN and Intermediate to NET only	3 3.75%
K	N5	+	+	resistant to all antibiotics but sensitive to CS and TGC, Intermediate (non-susceptible)to GM and NET only	1 1.25%
L	N52	+	–	resistant to all antibiotics but sensitive to CS, TGC, NET and TE only	1 1.25
M	N15, N22	+	–	resistant to all antibiotics but sensitive to CS, TGC, AN, NET, SEP and TE only	2 2.5%
N	N38, N44, N76	+	–	resistant to all antibiotics but sensitive to CS, TGC, NET, SEP, TE and inter-mediate only to LEV	3 3.75%
O	N47, N77	+	–	resistant to all antibiotics but sensitive to CS, SEF, NET, and Intermediate to LEV, TE	2 2.5%

P	N53, N79	+	–	resistant to all antibiotics but sensitive to CS, TGC, AN, ,TE and intermedia-te to IPM, MEM, NET and LEV only	2 2.5%
Q	N37, N75	+	–	resistant to all antibiotics but sensitive to CS, TGC, AN, ,GM, IPM, MEM, SEP and FEP only	2 2.5%
R	N30, N56	+	–	resistant to all antibiotics but sensitive to CS, TGC, AN, IPM, MEM, FEP and intermediate to CAZ only	2 2.5%

Most isolates (n=22, 27.5%) were grouped as resistotype B and were susceptible to colistin, tigecycline, and amikacin. Second largest group (n=19, 23.75%) was resistotype A, which were susceptible to colistin and tigecylcine. Resistotype C accounted for 5 *A. baumannii* isolates that were susceptible to colistin, tigecylcine and intermediate to amika-cin. Resistotype D included 5 *A. baumannii* isolates that were susceptible to colistin, cefoperazone/sulbactam and inter-mediate to levofloxacin. Four(5%) *A. baumannii* isolates showed Resistotype I, being susceptibl-e to colistin, tigecylcine,netilmicin and anikacin. three isolates (3.75%) were susceptible to colistin, tigecycline, amikacin and intermediate to netilmicin alone, and were grouped under resistotype J. Resistotype N involved 3(3.75%) *A. baumannii* isolates that were susceptible to colistin, tigecycline, netilmicin, sefoperazone/sulbactam tetracycline and intermediate to levoflo-xacin. only. Resistotype E covered 2(2.5%) *A. baumannii* isolates that were susceptible to colistin, sefoperazone/sul-bactam and netilmicin. Two(2.5%) isolates were grouped under resisto-type O being sus-ceptible to colistin, netilmicin, sefoperazone/sulbactam and intermediate to levofloxacin, tigecycline only. Two (2.5)*A. baumannii*isolatesshowedPattern**F**,beingsuscepti-ble to col-istin, tigecycline and intermediate to netilmicin only. Three isolates.

(3.75%) were susceptible to colistin, tigecycline and intermediate to sefopera-zone/sulb-actam alone, and were grouped under resistotype **H**. Two(2.5%) isolates were

grouped under resistotype **M** were susceptible to colistin, tigecycline, amikacin, netilmicin, sefoperazone/sulba-ctam and tetracycline only. Two (2.5%) *A. baumannii*iso-latesshowedResistotype **p**, beingsusceptible to colistin, tigecylcine, amikacin, tetracycline and intermediate to imipenem, merope-nem, netilmicin and levofloxacin. Two isolates (2.5%) were susceptible to susceptible to colistin, tigecylcine, amikacin, gentamicin, imipenem, meropenem, cefepime sefoperazone/sulbactam alone, and were grouped under resistotype **Q**. Two(2.5%) *A. baumannii*isolates were grouped under resistotype **R** were susceptible to colistin, tigecylcine, amikacin, imipenem, meropenem, cefepime and inter-mediated to ceftazidime only. Oneisolate (1.25%) was susceptible to suscepti-ble to col-istin, tigecylcine, amikacin and intermediate to levofloxacin alone, and was grouped under resistotype **G**. Oneisolate (1.25%) was susceptible to susceptible to colistin, tigecylcine, intermediate to gentamicin and netilmicin alone, and was grouped under resistotype **K**.finally One isolate (1.25%) was susceptible to susceptible to colistin, tigecylcine, netilmicin and tetracy-cline only and was grouped under resistotype **L**.

4.5.3. Multi-Drug Resistant (MDR) And Pandrug-Resistance (PDR) *A. baumannii*.

In this study ,the resistance profile of isolates showed that 100% (80/80) were mul-tidru-g-resistance (MDR) *A. baumannii* was defined as those resistant to 3 or more differ-ent antimicr-obial categories while pandrug-resistance (PDR) *A. baumannii* was defined as the isolates that were resistant to all tested antibiotics except colistin and tigecycline, were found 40% (32/80) listed in Table 5

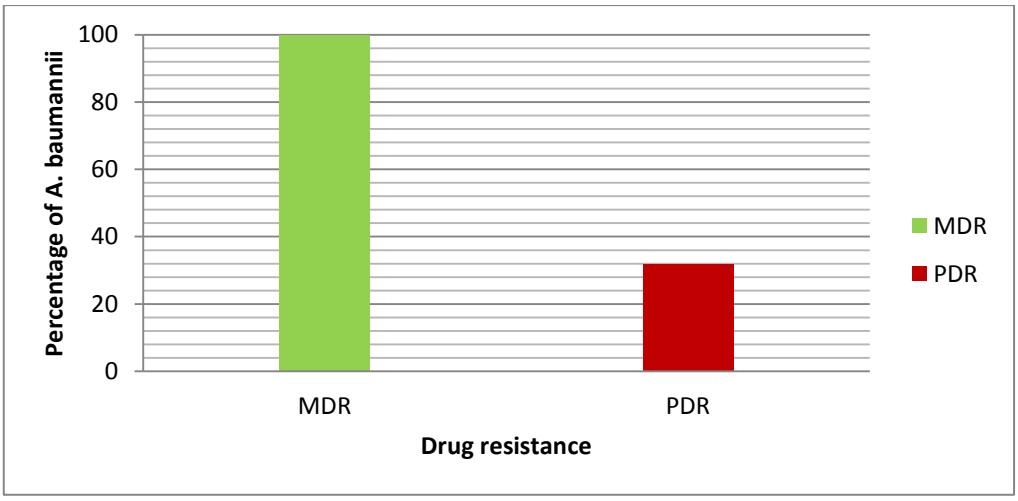


Figure 4.8. The percentage of MDR-AB and PDR-AB.

It is commonly known that MDR and PDR strain rates are high in nosocomial *A. baumannii* infections (Deveci et al., 2012) Joung et al.(2010) found the MDR and PDR resistance rates to be 60.3% and 15.5%, respectively

In comparison to studies performed in Colombia, Brazil, Iraq, Algeria, Thailand and china 17, 18, 19, 23, 26, a high resistance to imipenem was seen in our study.

In comparison to studies performed in Thailand. Aimsaad et al.(2009) reported 67.5% and 21.1%, a high prevalence of MDR-AB nd PDR-AB respectively. In a study conducted in turkey, Eser et al.(2009) reported the MDR *Acinetobacter* antibiotic resistance rate to be 41%. The resistance profile of the *A. baumannii* isolates in astudy conducted by Omer et al. (2015) in sudan showed that 97% were MDR This is considered high resistance rate which is simelar when compared to our study.

A review of resistance distributions of antibiotics used in our study over a period of five years showed increasing rates of MDR and PDR strains. Particularly, the 4.7% PDR strain rate in 2007 was found to be 20.9% in 2011. These resistance rates are considered indicators of a gradual increase in difficulties treating *Acinetobacter* infections (Çelik et al., 2014).

The emergence of PDR-AB represents a major problem in health care settings because the colistin and tegecycline are the only therapeutic option available for treatment of inf-ections caused by PDR

4.6. Effect of Tigecycline and Colistin against MDR-Ab and PDR-Ab

4.6.1. Effect of Tigecycline against MDR-Ab and PDR-Ab

It is clear that new drugs are required to be replaced for the treatment of MDR and PDR *A. baumannii*. Tigecycline was found to be active in 88.75% and 85.3% of the MDR and PDR *A. baumannii* in our study as shown in the figure (4.8.). Interestingly, all Tigecycline resistant isolates were susceptible to Colistin and Cefoperazone/sulbactam. This is very important for treating serious infections caused by Tigecycline resistance isolates. However, this combination still needs to be validated in animal model and clinical trials.

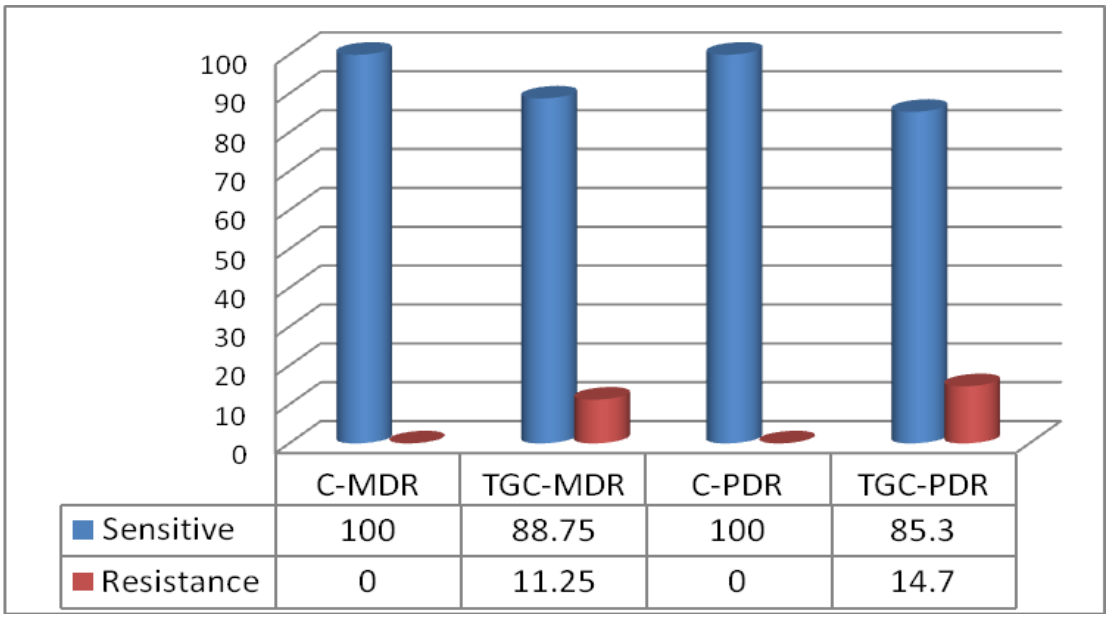


Figure 4.9. Activities of tigecycline and Colistin against MDR or PDR strains.

Note: C-MRD: Susceptibility of Colistin on Multi-Drug Resistance, TGC-MRD; Susceptibility of Tigecycline on Multi-Drug Resistance, C-PRD; Susceptibility of Colistin on Pan-Drug Resistance, TGC-PDR; Susceptibility of Tigecycline on Pan-Drug Resistance.

Tigecycline was found to be active in 86.7–93.3% of *Acinetobacter* species in different studies (Henwood et al., 2002). Tigecycline resistance rates ranging from 9.5% to 66% were reporated in *A. baumannii* from the Arabian Gulf region, the Far East and South America.

A. baumannii was sensitive to tigecycline in 99.47% cases in Sharma et al. (2015) study which correlates well with the study by Yilmaz F. et al. (2015) where 3.57% isolates were resist-ant to tigecycline. However studies by Behara.et al. (2009) in India have shown only 42% susceptibility in *A. baumannii* isolates to tigecycline (Siegel, 2007). Since therapeutic options are limited for MDR-*Acinetobacter* infection, the development or discovery of new therapies, well controlled clinical trials of existing antimicrobial regimens, combi-

nations, and greater emphasis on the prevention of health care-associated transmission of MDR-*Acinetobacter* infection are essential.

100% of the MDR and PDR *A. baumannii* isolates in our study were susceptible to colistin. In a similar study conducted by Hannan et al., (2014), in Pakistan all the strains of *A. baumannii* investigated were susceptible to colistin. In a recent study by Çelik et al. (2014) reported that 98% of *Acinetobacter* strains were susceptible to colistin under in vitro conditions.Reports from multiple investigators have also illustrated a 100% susceptibility to MDR-*A. baumannii* strains (Kuo et al., 2012b; Lim et al., 2011).

4.6.2. Effect of colistin against MDR-Ab and PDR-Ab

In contrast to our findings, Colistin resistance has been reported from various regions of the world. *A. baumannii* colistin resistance rates of 1% have been reported from Taiwan (Tan and Ng., 2007) also In Spain, colistin resistance was found to be 40.6% (Arroyo et al., 2009), and 12% in Kuwait (Al-Sweih et al., 2011) and 27.9% from Korea(Lee et al., 2009). In a study reported by Chang et al., (2012), 10.4% colistin resistance was documented. In another study, colistin resistance has been reported as 37% which is considered high when compared to other reports like the recent study from Malaysia carried out by Soo-Sum Lean et al., in which the resistant rare was (25.9%). Therefore, rationale use of colistin is highly recommended whenever possible to reduce the emergence of resistance to such antibiotic (Omer et al., 2015).

In another study by Rodriguez et al. (2010), colistin resistance was found to be 7.1%. Although the frequency of colistin resistance is low globally, it has been substantiated through in-vitro experiment that the rate of development of resistance among *Acinetobacter* to colistin is rapid (Tan et al., 2007). Colistin is now used in the treatment of infections caused by MDR and PDR Gram-negative organisms such as Pseudomonas and *A. baumannii* due to its relatively low neurotoxicity and nephrotoxicity as compare to Polymyxin B.

Susceptibility to colistin was reported as 91.2–100% in various studies (Dizbay et al., 2008 and Hernan et al., 2009), and it seems to be a good option in the treatment of MDR A. bau-mannii, but adverse reactions, has limited use of this agent. Colistin-resistant isolates have been recently identified in several Gram-negative species, such as *A. baumannii*, K. pneumonia and P. aeruginosa (Park et al., 2011).

Although studies conducted in countries, have shown that decreased susceptibility to colistin and tigecycline among *A. baumannii* isolates has been reported but they can be used as effective drugs for treatment of *A. baumannii* infections, but dissemination of *A. baumannii* resistant to colistin is worrying (Lolans et al., 2006).

In conclusion, the broad-spectrum in vitro activity of tigecycline and colistin may make them suitable candidates to be used in the empiric treatment of serious infections. Of course, there is a need to establish a severe hospital infection control policy and continuous surveillance of bacterial resistance to antimicrobial agents' should be also measured.

All the isolates were screened and identified via the VITEK-2 System (BioMerieux, MarcyL'Etoile, France), accordance to the manufacturer's instructions. This is a phenotypic type of identification which depends on biochemical reactions to identify the isolates.

Strain identification at the species level was divided into four groups based upon the probability of accurate identification as follows: excellent (probability of accurate identification, 96%), very good (93 to 95%), good (89 to 92%), and acceptable (85 to 88%). We considered ide-ntification with low selectivity between two subspecies of the same species, e.g., Achromobacter xylosoxidans subsp. xylosoxidans and denitrificans, to represent identification to the species level. Identification with low selectivity between two or more species of the same genus was regarded as identification to the genus level; low selectivity between species belonging to

more research and greater emphasis on the prevention of HAI due to MDR Acinetobact-er infection are essential. As of now, colistin may be the most useful agent active against MDRA and PDR infections.

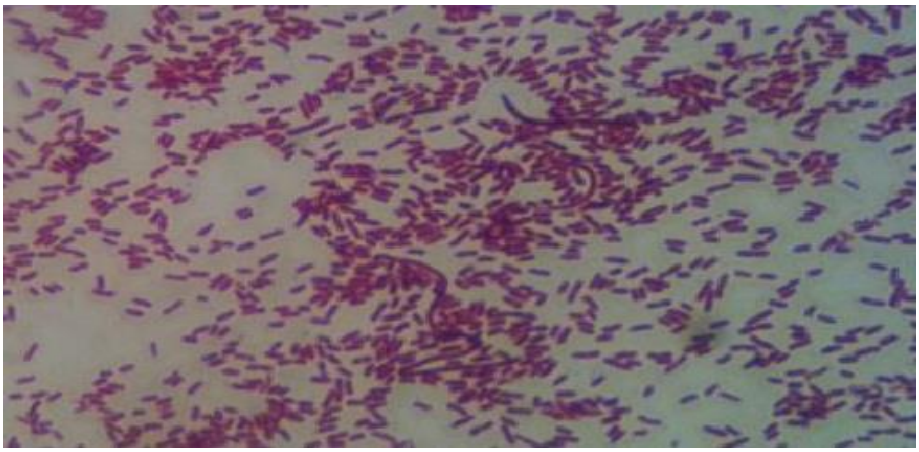


Figure 4.11. Morphology of Gram stained *A. baumannii* under light microscope using oil immersion (100X).

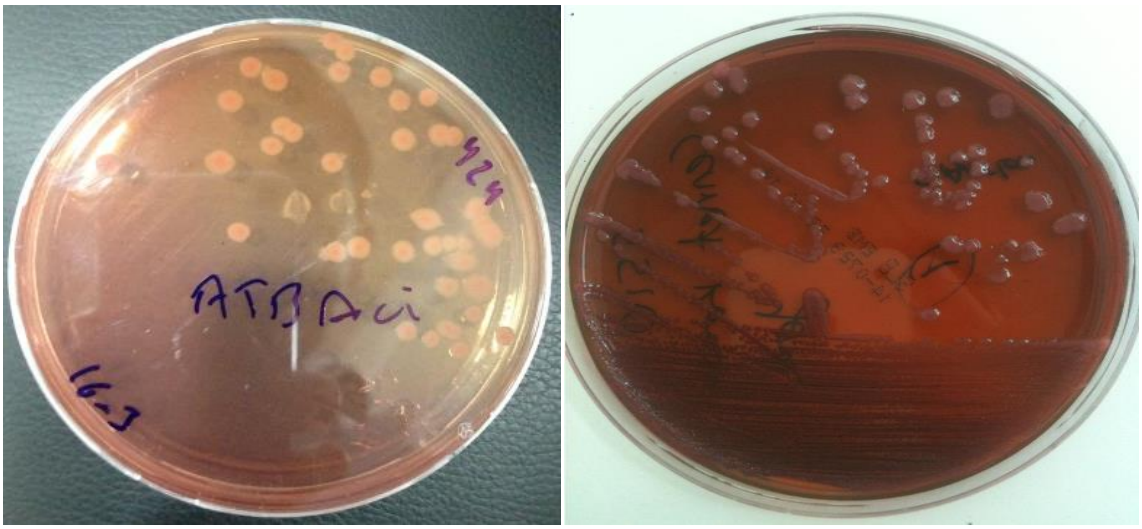


Figure 4.11. The shown colonies of A. baumannii on MacConkey agar and Eeosin methelin blue.

Figure 4.12. ID-GN and AST 262.

5. CONCLUSION AND SUGGESTION

5.1. Conclusion

According to the result of this study *A. baumannii* was significantly observed in clinical isolateand one of frequently nosocomial pathogens in hospitals, burn exudates, surgical wounds exudates and urine samples were the commonest source of *A. baumannii*infections followed by ear discharges and the most significant microbial challenges of the current study

1. in this study was high incidence of *Acinetobacter* strains in burned patients because their skin lose a first protective barrier, putting the patients at high risk for multiple infections, this problem remembers the need to think about strategies required to control the rapid spread of the bacteria in hospital environments, especially in burn centers.

2. The rate of infection according to the age group was high for the group (21-30) years old.

3. While the percentage of males infection found to be more predominant than females infection

4. Prevalence of drug resistance among *A. Baumannii* against most of the antibiotics and management of *Acinetobacter spp*. infections is difficult due to the emergence of isolates with multipledrug resistance

There was a high resistance rate to available and common antibiotics and also carbapenems. It seems that infection control strategies may help to control the evolving problem of *A. Baumannii* infections and prevent an epidemic nosocomial life threatening infections.

5. We concluded that a high resistance of cephalosporins, fluoroquinolones, carbapenems, betalactamase inhibitors+penicillin,folate pathway inhibitors and aminoglycosides(exception of amikacin).

The present study showed data on the rates of antimicrobial resistance observed in *A. baumannii* in those two Hospitals. Amikacin was the most effective antibiotic against *A. baummannii* isolates after tigecycline and colistin.

Our review reveals that various definitions have been used for the term MDR-AB and even more importantly for the term PDR-AB isolates in the relevant publications during recent years. We believe that the relevant professional societies and authorities responsible for the surveillance and prevention of bacterial resistance to antimicrobial agents

should try to formulate definitions for both terms, in order to enhance the communication between researchers and clinicians around the world. We acknowledge that it is probably difficult to establish a definition for MDR-AB and PDR-AB that would be widely accepted by investigators and clinicians worldwide. However, we believe that a widely accepted definition for those two term should be uniformly used.

6. Carbapenem resistance amongst *Acinetobacter spp*. has been increasing in the last decade, and outbreaks due to CR-AB have been identified worldwide. the rate of resistant *A. baumannii* to carbapenems were significantly observed ($p<0.05$) in this study.

7. The resistance profile of isolates showed that 100% (80/80) were MDR-AB while PDR-AB isolates were found 40% (32/80) of total isolates to 16 antimicrobial agents tested

8. According to our results, colistin remains a more effective antibiotic against PDR-AB and/or MDR-AB. These results can be a concern for physicians because 100% of the strains were sensitive to colistin and 88.75% to tigecycline only

9. The VITEK 2 compact system is an automated microbial identification system that provides highly accurate and reproducible results as shown in multiple independent studies. With its colorimetric reagent cards, and associated hardware and software advances, the VITEK 2 offers a state of the art technology platform for phenotypic identification methods. Thus it could be concluded that vitek2 automated system is an economic and trustable system to replace API Microsystems in clinical lab.

A reference method for susceptibility testing and MIC breakpoints should be established to better monitor trends of resistance.

It is also important to decide the evaluation criteria to determine the antibiotic susceptibility properly. Interpretive breakpoints for susceptibility reporting by clinical microbiology laboratories were previously set for an antimicrobial agent with no consideration of bacterial species differences.

5.2. Suggestions

We suggest the following;

1. In the present study, a high percentage of isolates had multi drug resistance. Some strategies should be designed and developed to control the spread of multidrug-resistant strains of *Acinetobacter sp.*. Correct management of dealing with hospital infections, especially burn infections seems very essential. More attention to the development and use of new non-drug therapies such as phage therapy seems more effective.
2. Further molecular epidemiology study recommended to observe any MDR clone spreading in our region and to compare our MDR clone to any international clones that are spreading worldwide.
3. Evaluate data over time for the facility and/or specific units to characterize prevalence or transmission rates
4. Identify clusters in transmission in risk populations and/or units to determine if enhanced interventions may be appropriate
5. Judicious use of antibiotics by making an attempt to distinguish colonization from true infections and treatment should be only given to the clinically confirmed infections and not colonization.
6. National surveillance of antibiotic resistant organisms and increasing awareness among the population to the hazards of inappropriate antimicrobial use through public health education campaigns.
7. Continuous monitoring and updating the antibioGram of bacteria in the hospitals.
8. Applying standardized infection control policies to minimize the spread of multi-drug resistant bacterial infections.
9. Tigecycline Therefore we suggest that in the current situation it can be a suitable drug for the treatment of highly resistant nosocomial infections and it should not be used for empirical treatment. For future, we recommend that to deal with the ever increasing antimicrobial resistance, it is necessary to monitor resistance patterns carefully and continuously.
10. Antimicrobial susceptibility testing be carried out on isolates of different infection source before chemotherapy to avoid selection of drug resistant strains.

11. Our study suggests that continuous evaluation of antibiotic policy in hospitals should be done on a routine basis, to avoid irrational prescribing of antibiotics and to treat different infections according to their antibiotic susceptibility profile.
12. Hand hygiene is considered worldwide to be the cornerstone of nosocomial infection prevention.
13. Several studies have been done on the harboring of pathogens by artificial nails. There is evidence that wearing artificial nails can result in carriage of Gram-negative organisms and yeast. It is recommended that persons giving patient care not wear artificial nails or extenders. Natural nails should be kept short.
14. We suggest the usage of Vitek2 compact auto analyzer system because it has more accuracy to determine the identification of bacteria and antibiotic profile
15. Chose a suitable antimicrobial susceptible card AST which contain those antibiotics can kill the pathogen

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