BAŞKENT UNIVERSITY
INSTITUTE OF HEALTH SCIENCES
DEPARTMENT OF MEDICAL MICROBIOLOGY
MASTER'S PROGRAM

SUSCEPTIBILITY OF *ACINETOBACTER BAUMANNII* TO SEVERAL DISINFECTANTS

BY

FATMA ALI AREIBI AL-ARABI

MASTER'S THESIS

ANKARA 2023

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THESIS SUPERVISOR

Prof. SEYYAL ROTA M. D.

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INSTITUTE OF HEALTH SCIENCE

This study, which was prepared by Fatma Ali within the framework of the Department of Medical Microbiology master's Program, was accepted as the Master's Thesis by the following jury.

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APPROVAL

Prof. Dr. F. Belgin ATAÇ
Institute Director
Director Date: ... / ... / .....

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Student Name, Surname: FATMA ALI AREIBI AL-ARABI

Student Number: 22020138

Department: Medical Microbiology

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Supervisor's Title /Name, Surname: Prof. SEYYAL ROTA M.D.

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ÖZET

Al-Arabi F. Acinetobacter Baumannii'nin Çeşitli Dezenfektanlara Duyarlılığı, Başkent Üniversitesi, Sağlık Bilimleri Enstitüsü, Tıbbi Mikrobiyoloji Ana Bilim Dalı, Tezli Yüksek Lisans Programı, 2023

A.baumannii, ciddi nozokomiyal ve toplum kökenli enfeksiyonlara neden olma yeteneği ile bilinen gram-negatif bir bakteridir. Son yıllarda, *A. baumannii'*nin çok ilaca dirençli suşlarındaki artış, enfeksiyon kontrolü ve hasta yönetimi için önemli zorluklar ortaya çıkarmıştır. Dezenfeksiyon, A. *baumannii*'nin sağlık hizmeti ortamlarında bulaşmasını önlemede önemli bir rol oynar. Bu patojenin yaygın olarak kullanılan dezenfektanlara duyarlılığı, spesifik dezenfektan, dezenfektan konsantrasyonu, temas süresi ve biyofilmlerin varlığı gibi birkaç faktöre bağlıdır. *A. baumannii*'nin dezenfektanlara duyarlılığı, genellikle atım pompalarının üretimi, dış zar geçirimsizliği ve direnç mekanizmalarının varlığı gibi asıl özelliklerinden etkilenir. Bu faktörler, dezenfektanlara duyarlılığın veya direncin azalmasına katkıda bulunarak geleneksel dezenfeksiyon yöntemlerini daha az etkili hale getirebilmektedir.

Bu çalışmada *A. baumannii* suşlarının Ankara Başkent Üniversitesi Hastanesi'nde kullanılmakta olan iki tür dezenfektana (Oxivir excel foam®, Chlortab®) duyarlılığının sıvı mikrodilüsyon yöntem ile minimum inhibitör konsantrasyonlar (MICs) ve minimum bakterisidal konsantrasyonlar (MBCs) belirlenerek değerlendirilmesi amaçlanmıştır.

Sonuçlarımıza göre tüm suşlar test edilen dezenfektanlara hassastır. Kullanılan iki dezenfektan MBC/MIC oranı 4'ün altında olan bakterisittir.

A. *baumannii*'nin hastanede halihazırda kullanılan biyositlere duyarlılığı periyodik olarak belirlenmelidir. Bu strateji enfeksiyon kontrolü yönetiminde başarılı olacak ve dezenfektanlara karşı direncin olup olmadığını belirlememizi, duyarlılıkta azalma varsa dezenfektanların değiştirilmesini sağlayacaktır.

Anahtar Kelimeler: *A. baumannii*, biyosit duyarlılığı, sıvı mikrodilüsyon.

Bu çalışma Başkent Üniversitesi Tıp ve Sağlık Bilimleri Araştırma Kurulu tarafından onaylanmış (Proje no: KA22/464) ve Başkent Üniversitesi Araştırma Fonunca desteklenmiştir.

ABSTRACT

Al-Arabi F, Susceptibility Of *Acinetobacter Baumannii* To Several Disinfectants, Başkent University, Institute Of Health Sciences, Department Of Medical Microbiology, Master's Program With Thesis, 2023

Acinetobacter baumannii is a Gram-negative bacterium known for its ability to cause severe nosocomial and community acquired infections. In recent years, the rise in multidrug-resistant strains of *A. baumannii* has posed significant challenges to infection control and patient management. Disinfection plays an important role in preventing the transmission of *A. baumannii* within healthcare settings. However, the susceptibility of this pathogen to commonly used disinfectants depend on several factors such as the specific disinfectant, concentration, contact time, and the presence of biofilms. The susceptibility of *A. baumannii* to disinfectants is often influenced by its intrinsic characteristics, such as the production of efflux pumps, outer membrane impermeability, and the presence of resistance mechanisms. These factors can contribute to reduced susceptibility or resistance to disinfectants, rendering traditional disinfection methods less effective.

This study aimed to evaluate the susceptibility of *A. baumannii* strains to two kinds of disinfectants (Oxivir excel foam®, chlortab®) that are currently in use at Başkent University Hospital Ankara, by determining minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) using broth microdilution method.

According to our results all strains were susceptible to tested disinfectants. According to the MBC/MIC ratio which were less than 4, both of disinfectants showed bactericidal activity.

The susceptibility of A. *baumannii* to biocides which are currently used at hospitals should be determined periodically, this strategy will be successful in the management of infection control, and it will allow us to identify whether there is resistance to disinfectants or not, allowing change the disinfectants if there are decreases in susceptibility.

Key Words: *A. baumannii*, biocides susceptibility, broth microdilution method

This study was approved by Baskent University Institutional Review Board (Project no: KA22/464) and supported by Baskent University Research Fund.

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LIST OF ABREVIATIONS

Acb	*Acinetobacter calcoaceticus–baumannii*
APIC	Infection Control and Epidemiology
ASP	Antimicrobial Stewardship Programs
Ata	Trimeric autotransporter
Bap	biofilm-associated protein
BCDA	bioluminescence-based carbapenem susceptibility detection assay
CaOC	calcium hypochlorite
CDC	Center for Disease Control
CLSI	Clinical and Laboratory Standards Institute
CRAB	carbapenem-resistant *A. baumannii*
DAP	1, 3-diaminopropane
ESKAP	*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, A. baumannii, Pseudomonas aeruginosa, and Enterobacter spp*
EtO	ethylene oxide
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FIBA	fluorescence Identification of B-Lactamase Activity
Fur	ferric uptake regulator
GA	glutaraldehyde
GNB	Gram negative bacteria
HAIs	hospital-acquired infections
HCWs	health care workers
HLD	high level disinfectant
HPGP	hydrogen peroxide gas plasma
HPV	hydrogen peroxide vapor
ICUs	intensive care units
KOCl	potassium hypochlorite
LiOCl	lithium hypochlorite
LOS	lipooligosaccharide

LPS	lipopolysaccharide
MALDI-TOF	matrix assisted laser desorption ionization time of flight
MATE	multidrug and toxin extrusion family
MBC	minimum bactericidal concentration
MDR	multi-drug resistant
MFS	major facilitator superfamily
MHA	muller Hinton Agar
MHB	muller Hinton Broth
MIC	minimum inhibitory concentration
MS	mass spectrometry
NaDCC	sodium dichloroisocyanurate
NaOCl	Sodium hypochlorite
NCSI	N-chlorosuccinimide
OMPs	outer Membrane Proteins
OMVs	outer Membrane Vesicles
OXA	oxacillinase
PVP	polyvinylpyrrolidone
QAC	quaternary ammonium compound
RND	resistance-nodulation-cell division superfamily
SMR	small multidrug resistance family
T2SS	type II secretion system
T6SS	type VI secretion system
TCICA	trichloroisocyanuric acid
TCS	two component regulatory systems
UTIs	urinary tract infections
VAP	ventilation associated pneumonia
WHO	World Health Organization

1. INTRODUCTION

There is a significant danger posed to healthcare systems by hospital-acquired infections (HAIs), particularly those caused by Gram-negative germs, which have a propensity to develop resistance to several drugs. Acinetobacter baumannii is an opportunistic human pathogen that is a rod-shaped Gram-negative bacteria that does not produce spores and is found everywhere in nature [1]. Infections caused by *A. baumannii* are more frequent in critically ill patients who are hospitalized for a long period. These bacteria are able to quickly develop resistance to antimicrobials [1, 2], which leads to infections that are difficult to cure and may require longer treatment period with higher dosages of drugs that are more toxic [1]. The Centers for Disease Control and Prevention (CDC) states; *A. baumannii* cause immediate risk because they can become resistant to many antibiotics [1, 3, 4]. The World Health Organization (WHO) has shown that infections caused by carbapenem-resistant *A. baumannii* (CRAB) are very dangerous in terms of death, health care costs, and costs to the community as a whole [2]. *A. baumannii* is able to survive in dry and humid conditions. It displays resistance to disinfectants, complete drying out, may create biofilms that promote bacterial attachment to tissues as well as diverse environmental surfaces, equipment, etc. It is hypothesized that the presence of these features have contributed to the fast endemic spread of *A. baumannii* in hospitals and in many intensive care units (ICUs) across the world [5].

Due to the worldwide increase of antibiotic-resistant *A. baumannii*, selection of appropriate biocides and disinfectants is necessary for prevention of nosocomial infections by minimizing spreading of the bacteria. Improper and incorrect use of biocides has also been cited as factors development of resistance to antimicrobials [6].

As a result, it is essential to evaluate the susceptibility of bacterial pathogens to the disinfectants that are already in use. This may be done in a variety of ways, such as by calculating the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) [7].

The goal of our study is to assess the susceptibility of *A. baumannii* strains to the biocides that are currently in use at Başkent University Hospital Ankara. For this purpose, we determined minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs).

2. GENERAL INFORMATION

2.1. Acinetobacter baumannii

2.1.1. History and taxonomy of Acinetobacter baumannii

A. baumannii first isolated from soil in 1911 was named as *Micrococcus calcoaceticus* [8, 9, 10, 11]. The same bacteria were recovered numerous times over the course of the following 50 years and was given various names, including *Moraxella lwoffi*, *Micrococcus calcoaceticus, Alcaligenes hemolysans, Herellea vaginicola*. In 1968, *Acinetobacter* was placed as a single genus by Baumann et al. [9, 11, 12], Further this genus was subdivided into 12 groups based on DNA similarity. [8, 9, 13]. They are now grouped as γ-proteobacteria, *Moraxellaceae* family, order *Pseudomonadales* [9].

A total of over fifty *Acinetobacter* species have been identified, and majority are non-pathogenic. The *Acinetobacter calcoaceticus–baumannii* (Acb) complex have particular importance in clinical services, comprising five pathogenic species (*A. baumannii, A. nosocomialis, A. pittii, A. seifertii and A. dijkshoorniae*) and one non-pathogenic species (*A. calcoaceticus*). Among these, *A. baumannii* is the most clinically relevant [12, 13].

2.1.2. Phenotypic characteristics

It is a Gram-negative coccobacillus observed as pairs of 1 - 1.5 μm (Fig. 2.1) [9]. Sometimes it is observed as Gram-positive cocci due to incomplete removal of Gram dye [9, 12]. It is aerobic, non-fastidious, non-fermenter and non-motile, indole negative, catalase-positive, oxidase-negative, citrate positive bacterium [3, 12, 13, 14].

Optimum temperature for growth of the *A. calcoaceticus-baumannii* complex is 35^0C–37^0C. Certain environmental isolates can grow at 20^0C– 30^0C. *A. baumannii*, which is the only species of this genus has the capability to grow at 44^0C [6, 9].

A. baumannii grows optimally on media; blood, chocolate and MacConkey agar. On blood agar, it appears as colorless, non-hemolytic, smooth and glossy colonies (Fig. 2.2). [9, 13].

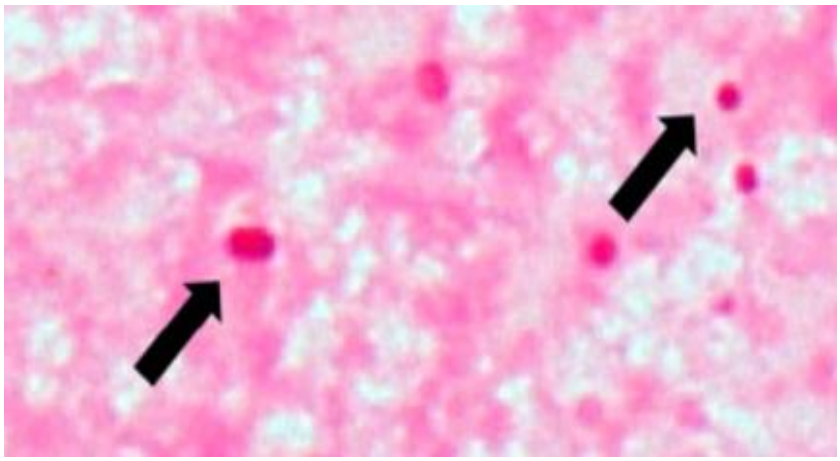

Figure 2. 1. Gram staining of *A. baumannii*

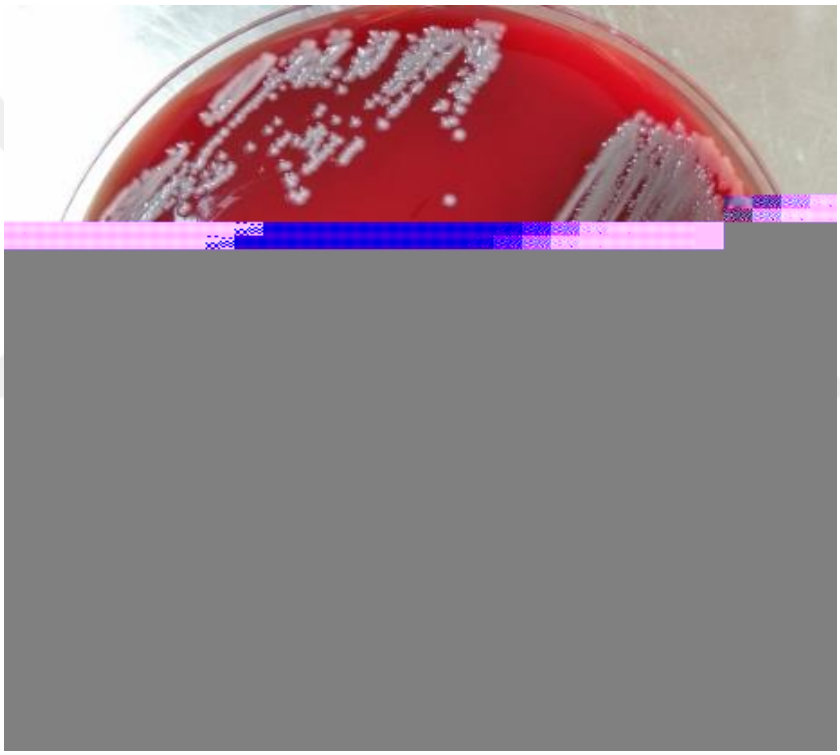

Figure 2. 2. *A. baumannii* on blood agar

2.1.3. Virulence factors

A. baumannii is able to cause disease as a result of various virulence factors. Investigation of these factors and involved pathogenesis leads to creating new treatments for the control of the disease [14].

2.1.3.1. Porins/Outer Membrane Proteins (OMPs) and Outer Membrane Vesicles (OMVs)

The outer membrane of *A. baumannii* facilitates a higher level of transmembrane transport than the cytoplasmic membrane. Mainly porins are responsible. They can constitute up to 50% of the outer membrane. These structures enable the passage of solutes and are associated with antimicrobial resistance and virulence [16].

***1. A. baumannii* (AbOmpA):**

The AbOmpA of *A. baumannii* is a major surface-bound protein which has significant properties, including its role in signal processing, pathogenesis, attachment, invasion, and the onset of apoptosis at the onset of infection [9,13,14]. OmpA can promote cell apoptosis through mitochondrial and nuclear targeting [9,13,16,17,18]. Furthermore, OmpA has been reported as a threatening virulence factor due to its capacity to induce host cell apoptosis as well as its porin transport function, biofilm formation, dissemination into the circulation, and interaction with epithelial cells, mostly through the use of host fibronectin [13, 16, 18].

2. Omp34

It is another porin which is a channel for water. Adheres to lung epithelial cells [16, 18]. Also it is linked to carbapenem resistance. Its expression has been associated with the virulence of *A. baumannii* and fitness [13].

3. Carbapenem-associated OMP (CarO)

In *A. baumannii,* CarO is shown to be a factor which is responsible for carbapenem resistance [12, 13, 16]. It possibly operates similarly to other porins, also facilitates host cell adhesion [13, 16].

4. Outer membrane vesicles (OMVs)

These are small, spherical vehicles and have been suggested to serve as a form of bacterial defense, potentially by facilitating signaling, host-pathogen interaction modulation, and immune evasion [18]. These OMVs have been identified as secreting OmpA or other possible virulence factors to host cells. In clinical isolates *of A. baumannii*,

OMVs are employed as vehicle for plasmids dissemination with genes that cause carbapenem resistance [14, 18], and are also responsible for increasing biofilm formation on non-living surfaces [9].

2.1.3.2. Cell Envelope factors (lipopolysaccharide LPS, and Capsule)

All bacterial outer membrane (OM) components—lipopolysaccharide (LPS), lipooligosaccharide (LOS), and capsule— are moved to outer membrane of cell envelope after synthesized in cytoplasm. *A. baumannii* does not produce LPS or O-antigen but produces LOS [18].

LPS are composed of lipid A, O-antigen and core oligosaccharides while LOS has extended core oligosaccharides but does not have the O-antigen [11, 18]. *A. baumannii,* like *Neisseria meningitidis* and *Moraxella catarrhalis,* has been found to be able to survive in the absence of lipid A [18].

Capsule:

Capsule protects *A. baumannii* from environmental conditions such as dryness, disinfection, some antimicrobials and immune system reactions like phagocytosis [9, 13, 17, 18].

2.1.3.3. Enzymes:

Two lypolytic enzymes are encoded by *A. baumannii;* phospholipase C, phospholipase D. The transcriptional regulation is achieved by ferric uptake regulator (Fur). These enzymes have hemolytic activity against human erythrocytes and provides its iron [13, 18].

2.1.3.4. Biofilm:

Biofilms are clusters of bacteria that are attached to biotic or abiotic surfaces and are surrounded by an extracellular matrix containing nucleic acids, carbohydrates, proteins, and other structures [1, 14, 17]. They exhibit limited metabolic activity [8, 9, 19], and

create a protective barrier for bacteria, allowing their survival in harsh circumstances. Additionally, biofilms have been associated with antimicrobial resistance [6, 14, 16, 19]. Biofilm production by *A. baumannii* is an important virulence factor, increasing its adaptability [13, 16].

Due to its hydrophobic properties, *A. baumannii* can bind to objects such as plastic parts of medical apparatus like catheters, ventilators and survive on dry materials for minimum three days and up to 5 months [8].

A. baumannii can cause soft tissue and skin-related diseases due to its ability to form biofilms in wounds and on occlusive dressings. It was reported that all catheter-related urinary or bloodstream infections caused by *A. baumannii* were produced by biofilm-forming strains [12].

2.1.3.5. Protein secretion systems:

The proteins secreted by the protein secretion systems which are present in *A. baumannii* enable the pathogen to interact with its surroundings and hosts which plays a role in its virulence and describes the targets for treatment [13].

There are 5 protein secretion systems in *A. baumannii*.

Trimeric autotransporter (Ata) was firstly described secretion system (T1SS) in *A. baumannii*. The attachment of Ata to human matrix components such as collagen and its involvement in biofilm formation and maintenance of virulence have been documented [13, 16, 18].

Type II secretion system (T2SS) secretes effector proteins; lipase, elastase, alkaline phosphatase, phospholipases which are important for the virulence of *A. baumannii* [18]. T2SS enables *A. baumannii* to survive in vivo [16], exporting multiple effector proteins such as CpaA, LipA, and LipH. Lipases, LipA and LipH are necessary in utilization of exogenous lipids. Metallo-endopeptidase CpaA functions in the degradation of fibrinogen, factor V resulting in inhibition of blood clotting pathways [13].

The Type IV secretion system (T4SS) is related with plasmids, conjugative transfer of DNA, other mobile genetic elements. The transfer of genetic material is important especially for antibiotic resistance determinants [18].

Type V secretion system (T5SS) are autotransporters which are related with biofilm formation, *in vivo* virulence and bacterial cell killing [18].

Type VI secretion system (T6SS) is multi-component system which is related with delivering of protein toxins into the other bacteria and influences polymicrobial infections [13, 16, 18].

2.1.3.6. Micronutrient Acquisition Systems:

Bacteria require iron for DNA replication and energy production in order to remain alive and for multiplication [3]. *A. baumannii* obtains iron from its host using two different mechanisms: the siderophores-mediated iron acquisition systems [3, 9, 14, 17, 18], and the iron-dependent repressor known as Ferric Uptake Regulator (FUR) [3]. Siderophores are secreted and bind ferric ions, allowing *A. baumannii* to acquire iron in an iron-deficient environment [9, 14, 17]. Bacterial cells acquire Fe^{3+} and heme-loaded siderophores via protein receptors [14].

2.1.3.7. Motility

As *A. baumannii* has no flagella it has been thought to be non-motile, [13]. *A. baumannii* strains move in two distinct patterns. One is twitching motility; which is the expansion and retraction actions that shift the organisms through their environment [13, 16], and depends on type IV pili. *A. baumannii* can also move on biotic and abiotic surfaces also by surface-associated motility, which is related with the production of 1,3-diaminopropane (DAP), quorum sensing, and lipooligosaccharide [12, 13, 16].

2.1.3.8. Two component regulatory systems (TCS):

The two component regulatory systems having two sets of regulatory proteins as sensor kinase which is membrane-bound and a DNA-binding transcriptional regulator, so it

can respond to environmental situations and cell stress. These sensors are sensitive to environmental physical conditions like pH and osmotic pressure [16, 18]. For an optimal function of these systems a feedback mechanism is used to response for the organism's demands depending on the environmental conditions [16].

In *A. baumannii* TCS, seven systems were identified that are thought to contribute to virulence [18]. One of these is BaeSR which indirectly regulates the expression of major efflux pumps; while BfmRS affects morphology, resistance, expression of biofilm, attachment to abiotic and biotic surface and also to eukaryotic cells [16, 18].

2.1.3.9. Efflux pumps

Beside the other systems efflux pumps also contribute to the survival of the bacteria. These membrane spanning pumps are substrate specific and used to expel potentially toxic compounds, antimicrobials [18]. In *A. baumannii*, the overproduction of chromosomally encoded efflux pumps is one of the causes of multidrug resistance [6, 9]. Several efflux pump families are defined, which are**:** ATP binding cassette superfamily, major facilitator superfamily (MFS), multidrug and toxin extrusion family (MATE), the resistance-nodulation-cell division superfamily (RND), small multidrug resistance family (SMR) [6, 18] and the proteobacterial antimicrobial compound efflux family [18]. The expression of efflux pumps might be augmented after antimicrobial exposure by changing gene regulators such as marA, soxS [6,20]. These multi-drug efflux pumps, called Qac (Quaternary ammonium compound) proteins, are encoded by QAC genes (*qac*), which can be transmitted to other bacteria using plasmids [6].

Acinetobacter has been found to resist biocides and aminoglycosides through the activities of AceI and AdeABC efflux pumps, respectively [18].

Table 2. 1. Virulence Factors in *A. baumannii* and their roles in pathogenicity

Virulence factor	Role in pathogenesis
Outer membrane protein A (OmpA)	Adhesion and invasion of epithelial cells, formation of biofilms, resistance to serum, surface motility [19], cytotoxicity inducer [16, 18, 19].
Omp34	Induce death in lung epithelial cells, bacterial pathogenesis, adherence [16, 18] carbapenem resistance [13].
Biofilm-associated protein (Bap)	Biofilm formation, intercellular adhesion [16, 19].
Lipopolysaccharide (LPS)	Employs escape from immune response of host, resistance to cationic antimicrobial peptides, diminished TLR4 signaling, desiccation survival [16, 19], adhesion [16].
Outer membrane vesicles (OMV)	Delivery of genes of virulence to the host cell cytoplasm, genetic material transfer among bacterial cells [16, 18, 19].
Phospholipase D	Existence in vivo, immunity tolerance, bacteria dissemination [16, 19].
Phospholipase C	Hemolytic activity, assists iron uptake from human erythrocytes [18,19].
Acinetobactin or Siderophore mediated iron acquisition mechanism	To survive in vivo, cell death [16, 19].
Capsule	Assisting the pathogen in surviving infections and growing in serum, and antimicrobial resistance [9, 13, 17, 19].
The trimeric autotransporter (Ata)	Biofilm formation, maintenance of virulence [13, 16, 18].
Two-component regulatory system, BfmRS	Expression of Csu pili chaperone-usher assembly system, development of biofilm, morphological integrity of cells [16, 18, 19], virulence organizer [16].
AbaI autoinducer synthase	Regulation of biofilm formation [13, 16, 19].
T2SS	To survive in vivo [16].
T6SS	Killing of competing bacteria, host colonization [13, 16, 18], motility, biofilm production, gene transfer [13].
Csu pili	Cell attachment [13], biofilm formation [13, 18].

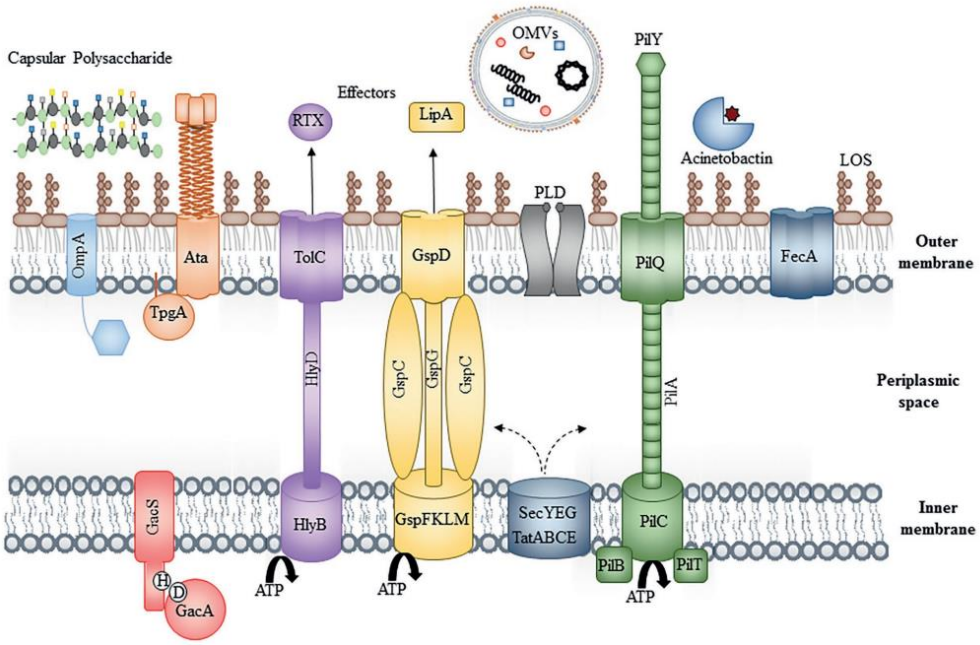

Figure 2. 3. Virulence factors

2.1.4. Natural habitats of *Acinetobacter baumannii*

They are recovered from water, soil, animals and humans so they are assumed to be abundant in nature [9, 12, 21]. *A. baumannii* prefers hospital environment, especially in adults and neonatal intensive care units, neurosurgery, surgery, oncology and burn_unit [12].

In hospitals *A. baumannii* can be observed in a wide range of sites as furniture, medical devices and supplies, elevator buttons, hand sanitizers, tap water sinks, items of medical staff [6, 8, 9, 12, 22]. On wet or dry surfaces *A. baumannii can* persist for one month. It is related to incidence of hospital-acquired infection [6, 23]. In healthy individuals, *A. baumannii* has been detected in different sites such as hands, nose, trachea, conjunctiva, vagina [6, 9].

A. baumannii is usually observed in lesions on tissues, skin, respiratory system, circulation, central nervous system. Also, it is found to be related to surgical wound, skin, and tissue infections, as well as catheter-associated urinary tract infections [8, 12].

2.2. Clinical Importance of *Acinetobacter baumannii*

Besides growing number of reports of community-acquired infections, *A. baumannii* is one of the most effective pathogens in the current healthcare system causing hospital acquired infections (HAI) [3, 22].

2.2.1. Community-acquired infections

A. baumannii, can lead to community-acquired infections, like bacteremia and pneumonia. About more than 80% of *A. baumannii* community-acquired infections is pneumonia [12]. Male gender and pre-existing factors, including alcoholism, smoking, diabetes mellitus and chronic obstructive pulmonary disease can be potential risk factors. Furthermore, *A. baumannii* pneumonia acquired in the community has been seen to be more difficult to manage than nosocomial pneumonia. These kinds of infections have a high mortality rate. In hot and humid countries, the clinical manifestations of community-acquired infections are distinct and severe [12, 13, 18].

2.2.2. Nosocomial infections

A. baumannii is an important nosocomial infection pathogen. The features that it displays such as attachment to abiotic surfaces, resistance to desiccation, also multidrug and disinfectant resistance are the contributing factors for its pathogenicity [24]. *A. baumannii* has a unique ability to stick to and thrive on abiotic surfaces of materials like door knobs, medical devices (catheters, respirators). This characteristic allows the bacterium to be present in healthcare services that are under highly desiccated and harsh conditions, which kills other bacteria. As a result *A. baumannii* is transmitted by contact with lifeless, non-living objects and for immunocompromised patients it is a continuing threat [13, 24].

A. baumannii is resistant to antibiotics and the related infections have high mortality rate.

Patients who have been admitted to a hospital and those who are particularly susceptible to disease are more likely to be infected with *A. baumannii*, because of its capacity to enter through the skin and respiratory defects [12, 17].

A. baumannii commonly impacts those who are in ICU leading to skin, tissue and wound infections; urinary tract infections; endocarditis; meningitis; bacteremia; ventilation associated pneumonia (VAP) [5, 6, 12, 16, 17, 21]. Mortality rate for *A. baumannii* blood stream infections is 28–43%. VAP, a common infection acquired in ICUs, often requires prolonged antibacterial treatment and hospital stay, and mechanical ventilation. Unfortunately, it is associated with high mortality rate 40–70% [13, 17].

CDC has noted an increase in *A. baumannii* nosocomial infections, as 0.6% of infections at the surgical wound, 1.2% of catheter-related urinary tract infections, 2.2% of central line-related bloodstream infections, and 8.4% of VAP [12].

This bacterium is also a major cause of burn infections because of its resistance to antibiotics, making chemotherapy difficult to implement. Burn infections caused by *A. baumannii* are rather prevalent among military staff [13, 17].

2.3. Risk Factors for Infection with *A. baumannii*

A. baumannii infection is associated with risk factors such as; recent severity of illness, severe burns, invasive procedures or surgery, prolonged hospital stays, admission to ICU, mechanical ventilation, intravascular devices, clinical use of third generation cephalosporins, carbapenems, broad-spectrum penicillins. Being elderly or immunocompromised, low birth weight, prematurity are also among the risk factors [6, 8, 12].

2.4. Modes of Transmission

Transmission can occur through air droplets, contact with skin colonized patients, as well as through contact with hospital staff's hands (fig 2.4). Those who are infected may not show symptoms for days to weeks until the agent is isolated in clinical specimen [8, 12, 21]. It was reported that the most frequent way of transmission is by direct hand-contact

among healthcare workers, staff, and visitors. When a patient infected or colonized with *A. baumannii* is admitted to hospital, the strain can survive on dry surfaces. During an outbreak, it can be recovered from patient's environment, as drapes, furniture, handwashing sinks, and medical devices like humidifiers, arterial pressure transducers, ventilators [8, 12, 21].

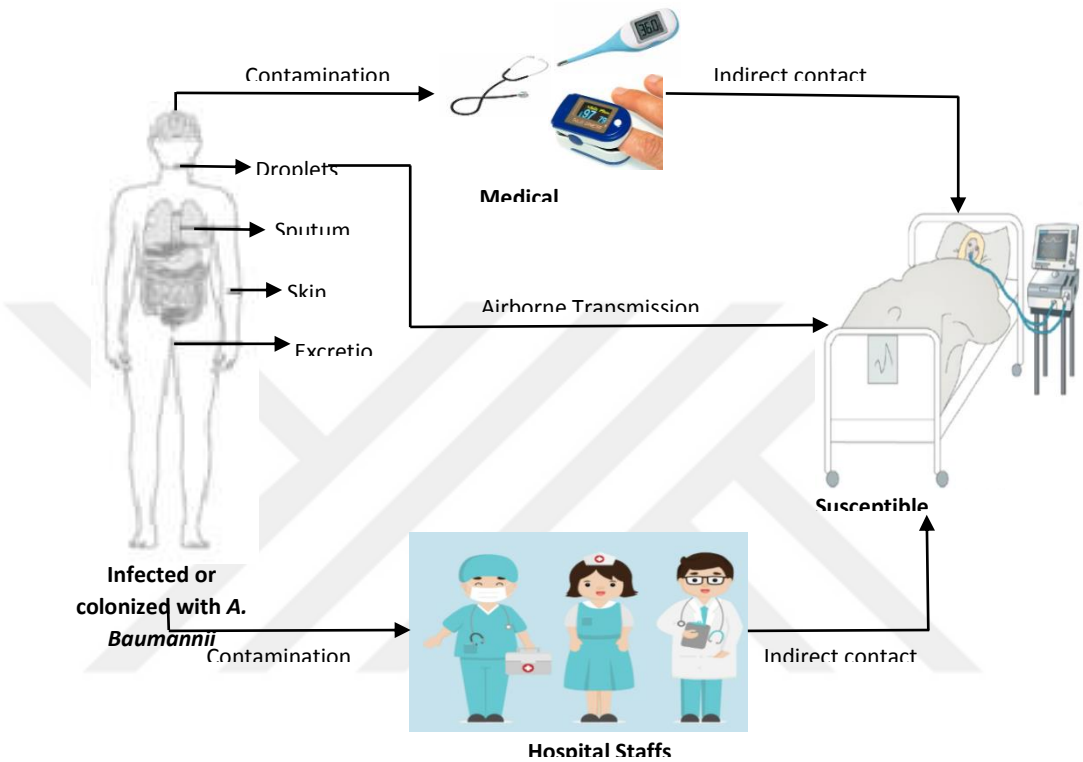

Figure 2. 4. Modes for transmission of nosocomial infections caused by *A. baumannii*.

2.5. Antibiotic Resistance and Morbidity and Mortality

A. baumannii is one of the five pathogens labeled by CDC, as ESKAPE *(Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, A. baumannii, Pseudomonas aeruginosa, Enterobacter spp.*) [6, 16, 21, 25, 26]. They can develop resistance to a variety of standard antibiotics. According to CDC, most clinically relevant strains of *A. baumannii* are multi-drug resistant (MDR) [3, 27].

MDR *A. baumannii* infections are resistant to three or more antibiotics of the quinolone, cephalosporin, β -lactam, aminoglycoside, and carbapenems [3, 24].

It was reported that, the prevalence of MDR among A. *baumannii* causing HAP and VAP were 100% in Croatia and 63% in Romania [1, 26, 27]. Whereas the percentage of MDR in Turkey was reported as 86% [27] and 91.6% in Greece [1, 27].

According to research, the overall mortality estimate pooled from 27 studies was 42.6%. Turkey, Egypt and Greece were the three countries with the highest mortality rates respectively 61.4%, 53.3%, 68.2% [27].

2.5.1. Mechanisms of antibiotic resistance

A. baumannii is one of the most resistant species in clinical treatment today. Resistance is the result of different systems working together at the same time. They are mainly: permeability deficits, multidrug efflux pumps, target site modification, expression of enzymatic drug degradation and acquiring of foreign resistant genes [28].

1. Permeability deficits

The antimicrobial agent transport into the cell is achieved by porin channels and OMPs. Mutation or reduced expression of porins is related with membrane impermeability [12, 21].

2. Multidrug efflux pumps (AbeABC, AbeFGH and AbeIJK)

Overexpressed efflux systems continually discard different antimicrobial drugs by pumping them out of the cell resulting with multidrug resistance [12, 14, 21].

3. Target site modification

Changes in penicillin-binding protein (PBP), a class of enzymes in the cytoplasmic membrane having role in the synthesis of peptidoglycan, cell division and morphological characteristics, is related with lower drug affinity due to PBP downregulation [12, 14, 21].

4. Enzymatic drug degradation

A. baumannii is known to produce carbapenemase, which is located on plasmids and is very transmissible. There are four main categories of β-lactamase enzymes in this organism: A, B, C and D [12, 14, 21].

5. Acquiring foreign resistant genes

Insertion sequences, gene cassettes, Integrons, integrative and conjugative elements (ICEs), bacteriophages, plasmids and transposons are associated with acquiring foreign resistance genes. Tn2053-like transposons are related to disinfectant resistance. It was documented that the resistance gene *qacC* is related with insertion sequence and resistance to antiseptics and disinfectants [6].

Gene cassettes which are short DNA strands having one or two genes without recombination sites generally integrate into integrons, so resistance genes move to special sites and recombination occurs. As a result, in highly-resistant strains, co-resistance both to disinfectants and antibiotics are detected. It has been reported that both disinfectant and antibiotic resistance of *A. baumannii* are often associated with class 1 integrons [6].

ICEs often harbour resistance genes. Also plays an important role in disinfectant-antibiotic resistant class 1 integrons mobilization [6].

2.6. Laboratory Diagnosis of *A. baumannii*

It is a Gram-negative non-fermenter, indole-negative, catalase-positive, oxidase-negative, citrate-positive and non-motile organism [3, 5, 12, 13]. It is observed as coccus to coccobacillus under light microscope and can easily grow on microbiological media such as sheep blood agar, tryptic soy agar, eosin methylene blue agar and MacConkey agar. On blood agar, bacterium grows as smooth colonies with a diameter of approximately 2mm [10].

1. Chromogenic media

MDR *A. baumannii* can be rapidly detected using chromogenic media, such as CHROMagar™ *Acinetobacter*. The media is a selective agar containing chromogenic substrate and chemicals that inhibit the growth of the majority of Gram-positive bacteria and also carbapenem-susceptible Gram-negative bacilli. As a result, *A. baumannii* and its MDR variants can grow on chromogenic media [12].

2. MALDI-TOF mass spectrometry (MS) systems

Matrix assisted laser desorption ionization time of flight (MALDI-TOF MS) systems are very efficient for species differentiation [10,12]. MALDI-TOF MS systems can accurately distinguish *A. baumannii* species. By this system *A. baumannii* isolates with and without carbapenemase can be differentiated [12].

3. Bioluminescence -based carbapenem susceptibility detection assay (BCDA)

A precise phenotypic method that enables identification of carbapenem-resistant organisms in a few hours from culture media [12].

4. Polymerase Chain Reaction

Using PCR, it is possible to detect and differentiate the three most common carbapenemases of *A. baumannii* (OXA-23-like, OXA-24-like, and OXA-58-like subfamilies) roughly in an hour [12].

5. Fluorescence Identification of β-Lactamase Activity (FIBA)

By using this method, it is possible to detect carbapenamase activity in several minutes [12].

2.7. Strategies for Intervention and Prevention

Multidimensional programs in hospitals can decrease outbreak frequency and probability, such as in endemic situations, by adopting infection control, taking surveillance cultures, and implementing an antimicrobial stewardship program [12, 29, 30].

2.7.1. Infection control precautions

1. Hand hygiene:

It is believed that 20–40% of hospital-acquired infections is related with contamination of hands of health care workers. Even with the use of gloves, healthcare staff caring for colonized patients have hand contamination at a rate of 4.5%. It is stated

that, touching a patient and a surface in the patient's room, healthcare workers' gloves or hands are contaminated in the same manner [8, 12].

It was shown that, 10% povidone-iodine and 70% ethyl alcohol combination has been proven to be effective in eliminating *A. baumannii* hospital strains. It is also essential to provide hand hygiene educational activities and feedback regarding hand hygiene compliance and colonization or infection rates to all hospital employees to emphasize how hand hygiene is important in controlling MDR *A. baumannii* outbreaks [8, 12].

Healthcare workers in burn units, who have a higher risk of exposure to MDR *A. baumannii*, should wear plastic aprons to reduce the risk of contamination [8, 12]. In addition, chlorhexidine skin disinfection and daily bathing with chlorhexidine gluconate-impregnated clothes can help prevent the spread of this pathogen [12].

2. Environmental cleaning

Hospitals should employ strict disinfection and environmental cleaning protocols in order to control MDR *A. baumannii* outbreaks. To terminate an outbreak, ICU wards may be closed in a year for several times. Moreover, after a colonized patient is released, all surfaces of ICU and rooms in adjacent wards must also be thoroughly cleaned [12,30].

Patients should be given their own medical items, and disposable products should be disposed when they are no longer necessary, and non-disposable products should be thoroughly cleaned [12].

Disinfectants with hypochlorite at a minimum concentration of 0.5% can be used to decontaminate non-disposable medical items. In hospitals for an efficient disinfection ethylene oxide or H_2O_2 free radicals can be used for medical devices which are contaminated with *A. baumannii* [12].

An individual who stays at a room that was inhabited by a patient infected with a MDR pathogen have a minimum of a 39% possibility of nosocomial infection [12]. This may be attributed to the *qacE* and *qacED1* genes, which are resistant to disinfectants. Because of the dry biofilms on the surfaces, elimination of *Acinetobacter* from surroundings is difficult, even after decontamination [8].

3. Patient and healthcare personnel cohort isolation

In order to restrict the outbreaks, effective strategic interventions should include single rooms with dedicated equipment and staff, if not possible, gathering patients who are infected with the same organism [12].

4. Surveillance cultures: patient and environmental cultures, rapid diagnostics

To decrease nosocomial infection rate, detection of colonization by surveillance of *A. baumannii* cultivation may be helpful [12].

According to the Association for Professionals in Infection Control and Epidemiology (APIC), cultures should be taken from multiple sites, as nose, throat, skin, rectum, open wounds, endotracheal aspirates [8, 12].

If there is a possibility for the source of outbreak being in inert environment, samples can be obtained from various surfaces like, water supplies, walls of hospital [12]. *A. baumannii* is isolated mainly from wheelchairs, pillows, and other items that are in patients' surroundings. It can also be isolated from places which have a lesser chance of direct patient touch but a higher chance of medical staff touch, like cabinets, medication drawers, keyboards [8, 12].

For the control of outbreaks, accurate identification of *Acinetobacter spp*. and differentiation of those belonging to the *A. baumannii* group is important [12].

Rapid diagnostic assays are also important as patient monitoring cultures in order to achieve accurate susceptibility test findings that would give healthcare professionals relevant therapy alternatives in a timely way [12].

5. Antimicrobial Stewardship Programs (ASP):

ASP are systems designed to optimize the use of antimicrobials to improve patient outcomes while minimizing the development of antimicrobial resistance. They involve the active monitoring of antimicrobial prescribing and use, and the implementation of interventions to ensure that the right drug is used at right dose, for right duration [12, 31].

2.7.2. Antibiotic used for *A. baumannii* infections

A. baumannii can rapidly gain resistance through inherent and acquired mechanisms so the treatment options for *A. baumannii* infections are getting progressively restricted [3].

Priority is given to carbapenems in the treatment of *A. baumannii* infections in areas with low resistance [1, 3, 27]. However, it is not recommended for use as a monotherapy in severe infections having resistance. Infections can be treated with a variety of antimicrobials if carbapenem resistance is predicted [1, 3].

1. β-lactamase inhibitors

Sulbactam, tazobactam, and clavulanic acid are β-lactamase inhibitors often used in CRAB infections [3, 12].

It is reported as sulbactam is the most effective and may provide an effective treatment choice for infections caused by MDR *A. baumannii* strains [3, 11, 12].

2. Tetracyclines (Minocycline and Doxycycline) and Glycylcyclines (Tigecycline)

When confronted with carbapenem and sulbactam-resistant *A. baumannii* strains, tetracycline therapy such as minocycline and doxycycline, as well as glycylcyclines are reported as good choice [11, 12].

Minocycline and doxycycline are very effective against VAP caused by these bacteria [11, 12].

A. baumannii strains that produce (RND)-type efflux pumps have been found resistant to tigecycline [11, 12].

3. Polymyxins

They induce cellular permeability and death by interacting with the outer membrane's lipid A and acting as a detergent by damaging the membrane phospholipids [3,11,12].

4. Trimethoprim–Sulfamethoxazole

The combination of trimethoprim and sulfamethoxazole is a successful treatment for *A. baumannii* infections [11, 12].

5. Cefiderocol

Cefiderocol, a new siderophore-conjugated cephalosporin, inhibits a wide range of MDR Gram negative bacteria [33, 34]. It is non susceptible to β-lactamases and has showed in vitro activity against Gram-negative bacteria such as *A. baumannii* [12, 33, 34].

2.8. Disinfection

Contaminated surfaces and instruments in hospitals aid in the transmission of nosocomial [8, 12, 23].

A patient is infected by microorganisms if they come in touch with contaminated surfaces in a healthcare center. Infection can be transmitted through secretions of the patient such as saliva, feces, urine or respiratory tract secretions [35]. Touching infectious surfaces in the patient's environment or direct handling with patients can cause contamination of health care worker's hands. [23]. Microorganisms on the hands can be transferred to various surfaces, from which they can re-infect other people, both patients and medical staff [8, 12, 23]. The bioburden refers to the number of germs present on a particular surface prior to disinfection or sterilization [35].

As a result, both microbiological observation of the hospital environment and surface cleaning or disinfection are necessary to avoid cross-contamination [23, 35]. One of the safeguards that helps to restrict the spread in health care facilities and general population is the use of disinfectants, hand sanitizers, as well as washing hands frequently using soap [36].

Disinfection is the process of eliminating or reducing harmful microorganisms except some spores from an environment, such as surfaces, water, or soil. Disinfection is typically accomplished by chemical or physical methods, such as the use of bleach, ultraviolet light, or heat [36].

This approach does not necessarily eliminate all bacteria but rather reduces them to levels that are safe for both human and environmental health [38]. In sterilization, all of the microorganisms together with the spores of bacteria are eliminated [37].

Antimicrobial agents used for disinfection are also utilized as sanitizing, sterilizing, and antiseptic agents, having a broad range that is adjusted to the demands of the user. Antiseptic and sanitation products can be used for the tissues directly for curing wounds, cleaning the hands and arms of the surgeons before surgery [38].

Usually, disinfectants used in the agricultural industry are less toxic and more diluted than those used in human and animal health, which are relatively toxic and potent antimicrobial chemicals that are applied to contaminated surfaces [20, 38].

2.8.1. Properties of an ideal disinfectant

To decide which disinfectant is appropriate, one must evaluate both the kind of surface to be disinfected, the type and contamination level. [23]

The main properties for being an ideal disinfectant are as follows: [23, 39]

1. Environmentally friendly formulation
2. Being hypoallergenic
3. Showing a rapid action
4. For easy preparation having a high water solubility
5. A broad range of activity spectrum for eradication of several microorganisms
6. Being odorless or have a nice or less unpleasant odor
7. Compatibility with the particular surface for being safe and effective
8. For time-saving and being cost-effective having both cleaning and disinfecting property simultaneously [23]
9. Remain active even on sputum, feces and other organic matters
10. Disinfection will be active at different pH values
11. Disinfectant should be affordable
12. Must be stable [23, 39].

2.8.2. Classification and uses of disinfectants

Disinfectants, often called as "biocides," are critical in prevention of the pathogens spreading. Using disinfectants by following the instructions, the transmission and spreading of the microorganisms causing nosocomial infections can be restricted [40].

Sanitizing agents, and disinfectants, have been used for prohibiting microbial growth in industries of pharmaceuticals and medical instruments, also in healthcare, drinking water, food and residential environments [20].

Disinfectants are categorized as disinfectants, sanitizers, antiseptics, and sterilizing agents depending on some properties as follows:

- Targeted objectives,
- Biocide composition and concentration,
- Contact time,
- Disinfected area [20, 38].

Disinfectants are composed of one or more substances having biocidal activity to destruct the pathogenic microorganisms by chemical or biological meanings [40]. These active substances range from simple inorganic molecules like sodium hydroxide to more complex molecules like polymerized quaternary ammonium compounds (QACs) [38, 41]. Disinfecting the surfaces is also important in addition to good hand hygiene of patients, healthcare professionals, and visitors for protection from the spread of diseases [40].

As there are different products in the market, selecting the proper disinfecting agents has become difficult for health workers [42].

2.8.2.1. Classification of disinfectants related with effectiveness

Depending on the items that are going to disinfected, the choice of cleaning, disinfection, or sterilization will be determined [37].

Disinfectants are categorized as: sterilant, high-level disinfectants, intermediate disinfectants, and low-level disinfectants.

1. Sterilant

A sterilant is capable of destroying all forms of organisms, including the bacterial spores [36]. It is used in the sterilization of critical items that need complete sterility before use, like surgical instruments, cardiac, vascular, and urinary catheters, implants, and various needles [35]. Ethylene oxide (EtO) gas sterilization, hydrogen peroxide vapor

(HPV), and hydrogen peroxide gas plasma (HPGP) sterilization is categorized as a sterilant [35].

2. High-level disinfection

It is effective in killing all microorganisms except some of bacterial spores. [36, 37]. This category includes glutaraldehyde, hydrogen peroxide, ortho-phthalaldehyde, and peracetic acid. Semi critical objects that are in contact with mucous membranes that are sensitive to high temperatures are disinfected by high level disinfectants [37]. This category includes some medical devices as certain endoscopes, laryngoscope handles and blades, probes of esophageal manometry, endocavitary and for prostate biopsy, catheters, cystoscopies, [37, 37].

3. Intermediate-level disinfectants

These disinfectants are effective on most viruses and most fungi excluding spores, vegetative bacteria and mycobacteria. Improved hydrogen peroxide, chlorine-based products, phenolics are examples for this group. This group is also used for the same purposes as low-level disinfectants, and is not especially suggested for special areas to be used. Some equipments such as ultrasound probes may have a contact with non-intact skin. If these type of equipments are going to be used this group of disinfectants are suitable [37].

4. Low-level disinfection

This group of disinfectants are used for noncritical patient care equipments like blood pressure cuffs or surfaces because of their ability to kill certain fungi and viruses, vegetative bacteria, except spores or mycobacteria [37]. Alcohol, sodium hypochlorite, iodophors, and phenols are examples of this type of disinfection [36, 37].

The disinfection should be performed following the instructions. If improper disinfection is applied, it may cause a risk of infection for both the patient and the health care workers. Also over-dose disinfection may cause waste of resources and reduction in the lifespan of equipment. Cleaning ultrasonic probes using alcohol-based disinfectants may permanently give harm, for instance to the transducer due to interaction with the rubber head [35].

2.8.2.2. Classification according to mechanism of action

In general, it is believed that biocides have a large number of target sites within the bacteria causing an extreme distress to these targets. Biocides can act on proteins, membranes, nucleic acids, cell walls of bacteria [6, 43].

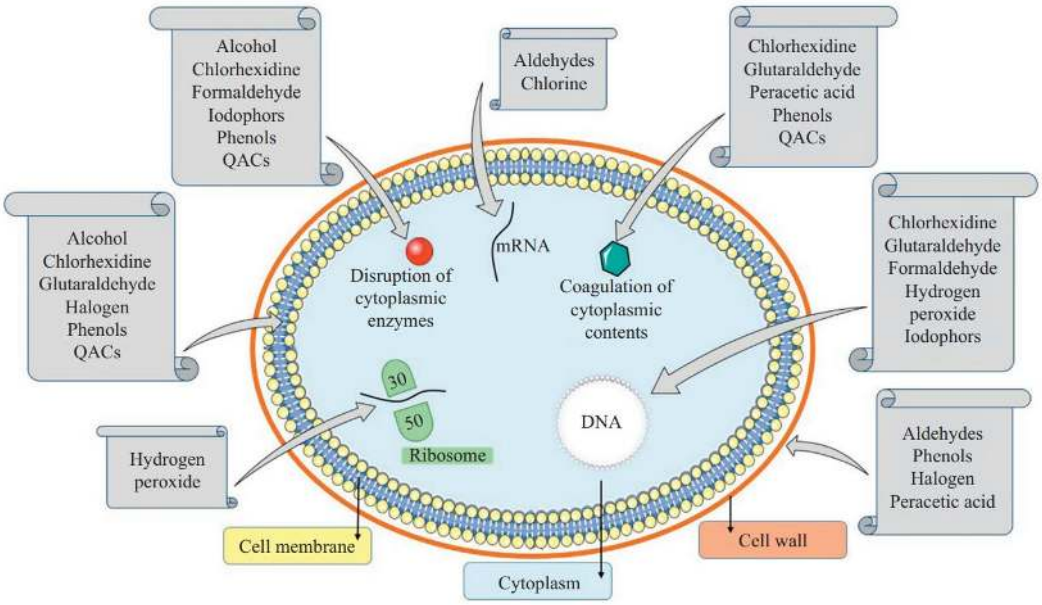

Figure 2. 5. Cellular targets of biocidal agents

2.8.3. Classification according to chemical structure

1. Alcohols

Alcohols, in particular isopropyl alcohol, and ethanol (60%-90%), act by the inactivation of a wide variety of microbiological agents through acting on proteins and cell wall. It is mainly used for antisepsis of the skin and disinfection of small medical items. Even though alcohols have been shown to be effective on killing infectious bacteria, they do not act on spores [44, 45]. So they are generally used after mixing with other biocides to make them more effective for disinfection [44]. Because of this, alcohol shouldn't be used as disinfectants both during sterile and invasive procedures and in intensive care units [45]. The antimicrobial activity mechanism of alcohol is not clarified. But it is assumed to be related with protein and membrane denaturation, inhibition of protein synthesis, uncoupling of mRNA acting on ribosomes and RNA polymerase [6, 40, 44].

2. Quaternary Ammonium Compounds (QACs)

QACs are positively charged cationic surface-active agents binding to the surface of most microorganisms that are negatively charged [6, 38, 44, 45].

Active chemicals like polymeric biguanides or chlorhexidine can be combined with QACs to be more effective against gram-negative bacteria. Also glutaraldehyde combination with QACs destroy microorganisms in a shorter time compared to solely glutaraldehyde does [38]. The mechanism of action is characterized by the destroy of the cell membrane, suppression of energy-producing enzymes, denaturation of important cellular proteins. It was demonstrated that they have a wide range of cidal effects on such as bacteria, fungus, enveloped viruses [6,38,45]. Because of the biofilms, QAC biocides are not always effective in clinical practice [46].

In home disinfectants cetyl pyridinium chloride, benzalkonium chloride, alkyl dimethylbenzyl ammonium saccharinate, didecyldimethyl ammonium chloride are a few examples for the mostly used QACs [44].

QACs are also used in the industrial sector as a biocide for the disinfection and prevention of the infection spreading. Benzyldimethyldodecylammonium chloride (BAC 12), benzyldimethyltetradecylammonium chloride (BAC 14) and benzyldimethylhexadecylammonium chloride (BAC 16) are utilized in food sector for decontamination of milk transportation tanks inside surface. For the application to the surface, they are used as wipes or sprays [46].

Since QACs do not generate free radicals, they are not carcinogenic or genotoxic [46].

3. Phenols:

Phenolic chemicals show their effect by acting on the cell membranes. Bacterial proteins are denaturated and the cell membrane lysis by high phenol concentrations [38,45]. Inactivation of important enzyme systems causing to the release of metabolites from the cell wall leading to the death of bacteria is achieved by low phenol concentrations and high molecular weight phenol derivatives [45].

The mostly used derivatives are alkyl, nitro, chlorinated and polyphenols [38]. Phenol is effective on different microorganisms, such as some fungi and viruses; but has a moderate level of success against spores [45]. Though phenol is one of the first antiseptics that was described is now not widely used because of its toxic and irritant properties. [38,45]. So concentrated phenol solutions is just used for disinfection of organic materials or equipment that are going to be disposed and not used for antisepsis except for certain purposes such as cauterization of infected sites [45].

4. Aldehydes

The aldehydes, mainly glutaraldehyde and formaldehyde denaturates the proteins and nucleic acids. They destroy, fungi and viruses [8, 45].

Aldehydes are used to disinfect the surfaces and devices. Formaldehyde is generally used in liquid form [45]. Formaldehyde can easily penetrate through the skin and cause skin and mucosal membrane irritation [38, 41, 45]. The most important feature of formaldehyde is its carcinogenic and mutagenic effects, so skin should be protected from direct contact [45]. The gaseous form cause ocular and respiratory mucosa irritation while when ingested it causes disorders of digestive and nervous systems. This property leads the disinfectant to be used for device and inert surface disinfection [38, 41]. Another area for formaldehyde use is the preparation of viral vaccines and disinfection of surgical devices [41].

Glutaraldehyde has at least three times higher biocidal activity compared to formaldehyde [38, 41] and is used for disinfection of medical devices for endoscopy, laparoscopy, hemodialysis, bronchoscopy. [41]. It may cause sensitization and irritation of the skin and mucosa by direct contact or inhalation. It may be toxic causing eye, nose, throat, lung irritation, and some other clinical findings [41, 45].

5. Chlorine-based compounds

These are the main compounds used for household and industrial disinfection. As these agents are quite inexpensive and can easily eradicate a broad spectrum of pathogenic microorganisms it is used to disinfect drinking water, swimming pool water and wastewater [38, 41, 45]. They act through exchange of atoms with other compounds, such

as the enzymes of microorganisms causing a structural change resulting with nonfunctional enzyme leading to the death of the microorganism [38].

Sodium hypochlorite (NaOCl) is the oldest and most well-known disinfectant containing chlorine that is effective against viruses and bacteria [38, 41, 45]. Potassium hypochlorite (KOCl) resembles to sodium hypochlorite in many ways and both are in liquid form while calcium and lithium hypochlorite (CaOCl and LiOCl) are available in solid form. Trichloroisocyanuric acid (TCICA), sodium dichloroisocyanurate (NaDCC), and N-chlorosuccinimide (NCSI) are alternate sources of chlorine in powder form. Due to their bactericidal and microbicidal properties, this category of chemicals can be used for high-level disinfection. In most cases, their minimal concentrations are sufficient [41]. It is important to prevent direct contact with sodium hypochlorite since it can cause skin irritation [41, 45].

Chlorine dioxide is a strong oxidant which kills and stops the growth of bacteria. It has bactericidal effect on non-tuberculosis mycobacterium, legionella, and gram-negative rods and also on human immunodeficiency virus. It can be used for decontamination of medical waste. Additionally, it is used for sterilization of medical devices [45]. It is susceptible to light, corrosive and irritating compounds. For high-level disinfection roughly 5 minutes is enough [41].

6. Biguanides

They have a wide range of activity, as killing bacteria, fungus, and spores [6,38,46]. For biguanides, alexidine and chlorhexidine at pH 5–7, whereas for polymeric biguanides pH 5–10 is the ideal pH for their activity [38]. Beside using for hands, chlorhexidine has a wide range of other uses, like antiseptic mouthwashing [46].

At low concentrations, binding to the bacterial wall, chlorhexidine increases permeability resulting with the filtration of cellular components together with potassium, causing bacteriostatic action [6, 38, 46], while causing precipitation of the bacterial cytoplasm and cell death meaning the bactericidal effect at higher concentrations [6,38].

7. Iodine-Based Disinfectants:

Iodine combined with a solubilizing agent is known as an "iodophor." Povidone-iodine (PVP-I) called as “Betadine” is the most well-known and the mostly used one. For

infection control and prevention it has been used as a powerful, wide spectrum antimicrobial for many years. It has cidal effects on bacteria, fungi, and virus [6, 41, 45], as well as on several bacteria capable of producing spores [6].

"PVP-I, is an iodophor solution composed of water-soluble iodine which is the microbicidal component and polyvinylpyrrolidon (PVP) which releases the iodine to the target. After the contact with the tissue free iodine is released [6,45], and membrane structure is disrupted, nucleic acids are denatured, and important intracellular systems as electron transport, cellular respiration, and protein synthesis are inhibited [6]. It is formulated in a variety of ways, so can be used as a disinfectant for different targets as skin, hands, and mucosal surfaces, in addition used for wound and eye care [6, 41, 45].

8. Other Disinfectants

A. Hydrogen peroxide (H_2O_2)

Both gaseous and liquid forms of hydrogen peroxide is a widely used antiseptic. For antisepsis of skin the liquid form is used at a concentration of 3% - 6% (v/v). For dental disinfection the concentration is 0.4 % - 1 %. It is also present as 3% in contact lens solutions. It is an antibacterial agent against a wide variety of microorganisms, including bacteria, viruses, yeasts and protozoa [6, 38, 45, 46]. By its oxidative activity damage in membrane structure, intracellular proteins and DNA caused by the radicals are some of the most important biocidal mechanisms [6, 40, 46].

Unstable structure of H2O2 causes problem for storage [46], and also is an irritant chemical [41, 45].

Beside these disadvantages being an environmentally friendly chemical because of degradation to water and hydrogen, it can be used for disinfection in some industrial areas such as food industry. Also it can be used for disinfection of medical devices and surfaces as 3–6% hydrogen peroxide in water for being non-toxic [46].

B. Peracetic acid (PAA):

PAA is a powerful oxidizing agent and shows its effect in a short time on a wide range of microorganisms [38, 41].

As it is highly reactive and weak acid it can be used instead of ClO_2. It can also be used for disinfection of medical, surgical, and dental devices. It is entirely efficient against all bacterial spores and pathogens, also it deactivates glutaraldehyde GA-resistant mycobacteria [41].

C. Ozone (O_3)

It is also an oxidizing agent acting on bacteria, viruses, protozoa and fungus [46]. The mechanism of its action is by the oxidation of bacterial cell membrane phospholipids and lipoproteins resulting with the lysis of the cell [46]. It can be used for high-level disinfectant, but it requires electricity and trained personnel for use and is expensive [41]. For the treatment of wastewater ozone eliminates organic micropollutants [41,46]. The exact toxicity level of O_3's is not defined and depends on different factors varying to the individuals [41]. The advantages of ozone; produced easily, have a 20-min half-life and applicable to places that are difficult to reach [46].

2.8.4. Efficacy of disinfectants

The efficacy of a disinfectant is determined by several aspects relating to the disinfectant and the microorganism.

Table 2. 2. Factors affecting the efficacy of disinfectants.

Factors	Comment
Disinfectant concentration	Concentration not sufficient kill the bacterium causes stress reaction, and resistance [6, 20].
Contact time	Insufficient contact time, cause survival microorganisms. Five minutes is the ideal contact time in general. For proper contact time, follow the manufacturer's instructions. [20, 38].
Nature of disinfected surface	Microorganisms can attach to irregular surfaces easier so the disinfectants are less effective [38]
Nature of microorganism	Disinfectants have varying effects on different microorganisms due to differences in their cell structure, content, and physiology [38]
The amount of organic material on the surface	Substances that act as a barrier reduce the effectiveness of disinfectants [20, 46].
Presence of biofilms	biofilms impair efficiency of biocidal agents[38] .
Bioburden	A higher amount of microbial agents needs higher concentrations of disinfectant [38]
The design of equipment	extremely porous or rough materials, which provide a more ideal surface for adhesion for organic residues and microorganisms [38].
Temperature	The effectiveness of many disinfectants, especially those containing QAC and aldehydes, is enhanced at higher temperatures (usually 20°C) [46], while they can be rendered ineffective at lower temperatures [20].
pH	Some disinfectants, including QACs and glutaraldehyde, have better antibacterial activity at higher pH, while others, like iodin, have less action at higher pH [20].

2.8.5. Resistance to biocides

The bacteria that are resistant to disinfectants raises some problems such as disease transmission in healthcare issues, food industry, sterile drug and medical device manufacturing industry [20].

In the past 10 years, in the incidence of antimicrobial resistance demonstrated a steady rise. In vitro researches demonstrate that bacteria possess a capacity to adapt to chemical stress arised from use of biocides through a variety of distinct ways [40, 46]. The overuse, misuse, and improper utilization of disinfectants are generally considered to be the primary contributors to the growing resistance [1, 40, 46].

When organisms are exposed to biocides below their ideal levels, clones that have developed resistance mechanisms to them are selected. Experimentally it was demonstrated that, low exposure is seen within biofilms, during extensive soiling, and improper decontamination due to wrong dilution, wrong formulation and short exposure time [47].

The definition of "biocidal resistance" is still in debate. While it is described as a reduction in susceptibility that is detected by increased in the minimum inhibitory concentration (MIC) by some researchers, the others accept it as to be resistant after application of biocidal at any concentration that can be practically applied [20].

Different terms are also used for biocides. Tolerance reduced or decreased susceptibility, insusceptibility, acquired reduced susceptibility are some examples that are used for biocidal resistance. "reduced susceptibility," is mainly evaluated by the result of MIC. Furthermore, a biocide or biocide product may still be effective even when used at the recommended concentration [48,49].

Mostly "resistance" was used to define a 2-10 folds of increase (87%) in MIC. Also some researchers evaluated "resistance" as an isolate with a MIC that was above the MIC for 90% - 99% of bacteria belonging to the same species [49]. The main problem is to decide what fold difference both in minimum inhibition concentration and MBC, points out the resistance or decreased susceptibility of the disinfectant in order to use it in the right concentration in practice [48].

Mechanism of resistance to biocides

Biocide resistance in bacteria may be result of the nature of the bacterium which is called as intrinsic, or having resistance genes as transposons or plasmids which is expressed as acquired [6, 20, 48].

Bacteria regulate and counteract the effects of disinfectants in several ways (table 2.3.). Reducing their permeability, production of efflux systems, degradation of the disinfectant by enzymes, shifting the disinfectant's target sites, formation of biofilms, as well alteration in hydrophobicity, ultrastructure, protein composition, fatty acid modifications on cell the surface are the factors that regulates the effects of the disinfectants [6, 40, 48]. For instance *A. baumannii* becomes resistant after genes responsible for production of lipooligosaccharides are inactivated**.** Biofilms may also be formed. Biofilms are resistant to disinfectants because of changes in bacterial growth due to impairment in nutrition inside the biofilm and also decreased binding of disinfectant to the biofilm [6, 40]**.** Efficient efflux system is also a significant contributor to bacterial resistance [6, 40, 47]. If the expression of efflux pump system is high the effect of many disinfectants is diminished which is demonstrated by increased MIC [20]. This was demonstrated for many disinfectants. *A.baumannii* which is a very resistant bacterium having two genes, as *quacA* and *quacB*, regulates the activity of efflux system which carries out the biocides and antibiotics. If biocides having a specific antimicrobial mechanism e.g QAC which develops resistance to disinfectants are used, cross-resistance to antibiotics are observed [6,40]. If the concentration of the agent as in the case of formaldehyde, chlorhexidine, and QAC is lower than the intended clinical use, less effective enzymatic degradation or inactivation of the disinfectant is seen. When disinfectant is used at minimal inhibitory concentration expression of neutralizing enzymes are increased which is important in biodegradation of disinfectants [40].

Table 2. 3. Bacterial resistance mechanisms for the most commonly used disinfectants.

Disinfectant	Resistance mechanism
Formaldehyde	Formaldehyde inactivation involves pterin cofactors, sugar phosphates, glutathione-dependent metabolic pathways. Conversion of formaldehyde to carbon dioxide requires three different enzymes as thioesterase, alcohol dehydrogenase, Gfa. Inactivator glutathione-dependent formaldehyde dehydrogenase is encoded by transmissible adhC gene on the plasmid [40].
Chlorhexidine (gluconate/ diacetate)	Efflux pump gene expression induction by upregulation and downregulation of coding genes, inactivation of active ingredient, changes in cell wall, increase in lipopolysaccharide or phospholipid of cell membrane, [20, 40]. biofilm formation [6], efflux pump [51].
Povidone Iodine	Thickening of cell walls and biofilm formation [40].
Hydrogen Peroxide	Production of catalases and peroxidases, which break down H_2O_2 and form thick biofilm [40].
Benzalkonium Chloride	Decreased porins, increased or modified efflux pumps (Mrdl, EmrE, and MdfA) with Mex system mutations, horizontal gene transfer of transposable elements (Tn6188), stress factors, formation of biofilm, biodegradation through dealkylation [20, 40].
Ethyl alcohol	The component of RNA polymerase, encoded by the *rpoB* gene, has been subject to changes in the central genome as a result of horizontal gene transfer, transformation, and transduction [40].

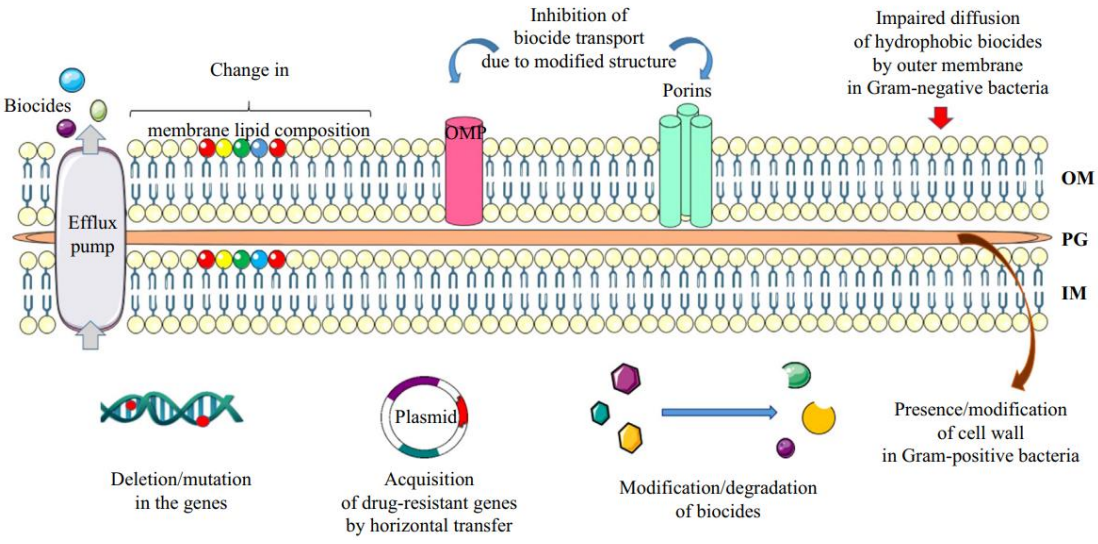

Figure 2. 6. Various mechanisms of biocide resistance in bacteria

OM, outer membrane; PG, peptidoglycan; IM, inner membrane

2.8.6. Laboratory techniques to study biocide resistance/susceptibility in bacteria

1. Microscopic techniques:

Structural differences among susceptible and resistant bacteria can be distinguished by transmission and scanning electron microscopy. In transmission electron microscopy heavily stained with negative staining cells are resistant and by transmission and scanning electron microscopy it is shown with a rough surface having amorphous substance surrounding it. It has been reported that the amorphous material contains exopolysaccharide [6].

2. Expression analysis:

While changes in the expression of OMP can be analyzed by PCR, SDS-PAGE and 2D gel analyses, changes in the expression of genes except OMP can be analyzed by proteomic, microarray, and PCR analysis. PCR-RFPL is used for demonstrating cross-resistance by analyzing genetic markers related with antibiotic resistance [6].

3. Nucleotide sequencing

It is stated that by CRISPR-Cas system which is a genome engineering technique can demonstrate the disinfection resistance genes in some organisms. These techniques can also be used for reverse disinfection resistance [6].

4. Measurement of Minimum Inhibitory Concentration (MIC)

For antimicrobial susceptibility testing, including Kirby Bauer disc diffusion, micro- and macro-broth dilutions, The Clinical and Laboratory Standards Institute and the European Committee on Antimicrobial Susceptibility Testing have detailed standardized protocols [6,50].

Up to day for reduced susceptibility no cut off value of MIC is defined for biocides [6,50].

In addition, several papers suggested various epidemiological cut-off values based on the statistical analysis of MIC. MICs can be used for biocides when they are used as a preservative depending on their bacteriostatic effect. If the susceptibility breakpoints of

biocides is known effective disinfectants can easily be chosen. This will be useful for following up how well the disinfection plan is working [6,50].

5. Measurement of Minimal Bactericidal Concentration (MBC)

The measurement of Minimum Bactericidal Concentration (MBC) is a critical tool in evaluating the effectiveness of biocides. It determines the concentration required to achieve bactericidal effect against specific bacterial species. This measurement is crucial because using sub-lethal concentrations of biocides can be ineffective [52].

Monitoring MBC values periodically can help to identify the development of bacterial tolerance to biocides, where elevated MBC values may indicate reduced susceptibility or the emergence of resistant strains [1]

3. MATERIALS AND METHODS

3.1. Bacterial strains

In this study 45 multi drug resistant *Acinetobacter* strains were obtained from bacterial archive of Medical Microbiology Department, Başkent Medical School

3.2. Laboratory tools

- Muller Hinton Agar (CondoLab, Spain)
- Muller Hinton Broth (CondoLab, Spain)
- Autoclave (Hirayama, Japan)
- Pasteur oven (Nüve, Turkey)
- Sensitive balance (SCALTEC, USA)
- Vortex (VELP, Italy)
- BD PhoenixSpec nephelometer (Fisher scientific ,USA)
- Incubator (Nüve, Turkey)
- Microplates 96wells (Thermo scientific, Denmark)
- Automatic pipette 0,5-10 µl (Eppendorf, Germany), 10-100 µl(GILSON-France)
- Pipette tips 5/10 µl, 200 µl (microcult, Turkey)
- Sterilized test tube
- Sterilized swab
- Sterilized loop
- Petri dish (Fıratmed, Turkey)
- Sheep blood agar plate

Muller Hinton Agar

Muller Hinton Broth

Pipette

Vortex

Microplates 96wells

BD PhoenixSpec nephelometer

Figure 3. 1. Materials that used in study.

3.3. Disinfectants

Disinfectants were obtained from Başkent University Hospital Ankara. These disinfectants are applied routinely in ICU. The basic compounds of each disinfectant are shown in Table 3.1.

Oxivir excel foam spray 750ml (Hydrogen peroxide 0.36%), (Diversey, Turkey)
Chlortab (50%NaDCC) effervescent tablet (ADA AQUA, Turkey)

Oxivir excel foam®. Chlortab®

Figure 3. 2. Disinfectants used in study

Table 3. 1. Conditions which the disinfectants are applied in ICU at Başkent University Hospital Ankara

Disinfectant	Active substance	Purpose of use	Usage	Contact time
Oxivir excel foam®	Accelerated Hydrogen peroxide 0.36%	Medical device disinfection	Use in spray bottle 750 ml	30 sec
Chlortab®	Active chlorine 50%	Floor-surface disinfection	2tablets dissolved in 1litre water=10,000ppm	15 min

3.4. Testing the MIC of Disinfectants

Due to the lack of published standard breakpoints for disinfectants against *A. baumannii*, we carried out the procedures according to the CLSI, we carried out the procedures according to the recommendations for the broth microdilution technique for BST that was given in reference [53], however a few modifications have been made:

3.4.1. Bacterial inoculum preparation

Each bacterial inoculum was prepared by the colony suspension method, overnight-grown subculture bacterial colonies were used, colonies of each bacterial strain were

picked up with a sterile cotton swab and transferred to 5 ml of MHB. After mixing perfectly by vortex, turbidity was measured with the McFarland device. After adjustment, the bacterial amount in the broth culture was (0.5 McFarland) approximately 1.5×10^8 colony-forming units per mL (CFU/mL).

3.4.2. Plate preparation and MIC determination

Into a sterilized 96-well plate 100µl of sterile MHB was added to each well starting from well 2 to well 12 in aseptic conditions. 200µL of testing disinfectant was added to well 1. The starting concentration for each antiseptic agent is listed in Table 3.2; subsequently, serial two-fold dilutions were prepared by transferring 100µl of the disinfectant from each first well to the next one. The process continued to well 9 (except for wells 10, 11, and 12). From well 9, 100µL was poured out, so the volume of all wells was adjusted to100µL. After preparing serial dilutions, 100µL of 0.5 McFarland adjusted fresh bacterial culture was inoculated into each well (except wells 11 and 12) and incubated at 37 C° for 16 - 20 hours. For each test plate, three controls were prepared: one (well 10) containing 100 µL medium plus 100 µL of bacterial inoculum (as a growth control), another (well 11) containing 100 µL medium plus 100 µL disinfectant, and the third one (well 12) containing 200 µL medium alone (acting as a sterility control). Then, the plates were examined for their turbidity by naked eyes. The lowest concentration of a disinfectant that inhibited the bacterial visible growth was considered as MIC; the experiments were performed in triplicate as in figure (4.2).

3.4.3. Minimum bactericidal concentration (MBC)

MBC testing was carried out to determine the bactericidal effect of each disinfectant that kill the growth of bacteria, 10 µl from each well of the microtiter plate (MIC assay) that showed visible, and no visible growth (from well 1 to 9 were further spotted on MHA) and incubated at 37˚C for 24 h and were observed for growth. The lowest concentration that does not show any bacterial growth was determined as the MBC [54].

Table 3. 2. The starting concentration for each disinfectant agent, and serial dilution

Disinfectant	Unit	Serial dilution								
		1	2	3	4	5	6	7	8	9
Chlortab	µg/ml	625	312.5	156.25	78.12	39	19.5	9.7	4.88	2.44
	ppm	625	312.5	156.25	78.12	39	19.5	9.7	4.88	2.44
	%	50%	25%	12.5%	6.25%	3.125%	1.56%	0.78%	0.39%	0.19%
Oxivir	%	0.36%	0.18%	0.09%	0.045%	0.022%	0.011%	0.0056%	0.0028%	0.0014%

4. RESULTS

According to our results all strains were susceptible to tested disinfectants, the concentrations of disinfectants utilized in hospitals were considerably higher than the MICs and MBCs.

The overall distribution of MICs and MBCs of studied disinfectants are listed in Table4.1., MICs were evaluated by naked eyes according to turbidity as in fig 4.1.

The Oxivir MICs showed ranges between 0.045%-0.09% with 4.4 and 95.5 percent, respectively.

The Chlortab MICs showed a range between 6.25%-12.5% with 62.2 and 37.7 percent, respectively.

The MBCs for all disinfectants were higher than or equal to their respective MICs, the Oxivir MBCs showed range between 0.18%-0.09% with 8.88 and 91 percent, respectively.

The Chlortab showed MBCs range between 6.25%-12.5% with 33.3 and 66.6 percent respectively.

Our results show that drug-resistance properties of bacterial strains do not automatically provide increased survival following exposure to widely used hospital disinfectants.

All our results have MBC/MIC less than 4 and considered as bactericidal for both evaluated disinfectants. Generally, a MBC/MIC ratio >4 indicates that the agent has bacteriostatic properties, while a ratio of <4 indicates the agent is bactericidal [1].

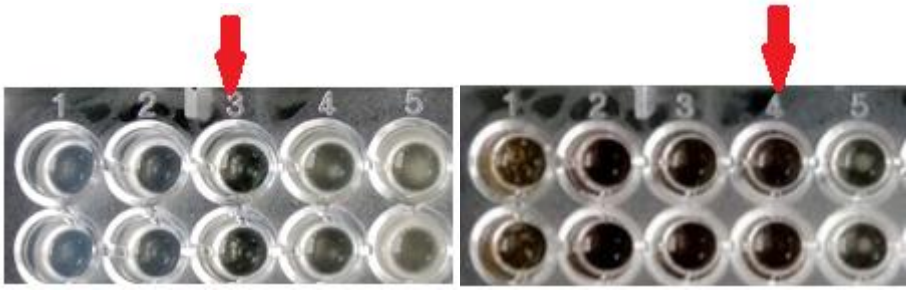

Figure 4. 1. Evaluation of plates according to turbidity

Wells no. 3 and 4 represent the MIC value because the solution in this well is clear, which indicates there is no growth of bacteria.

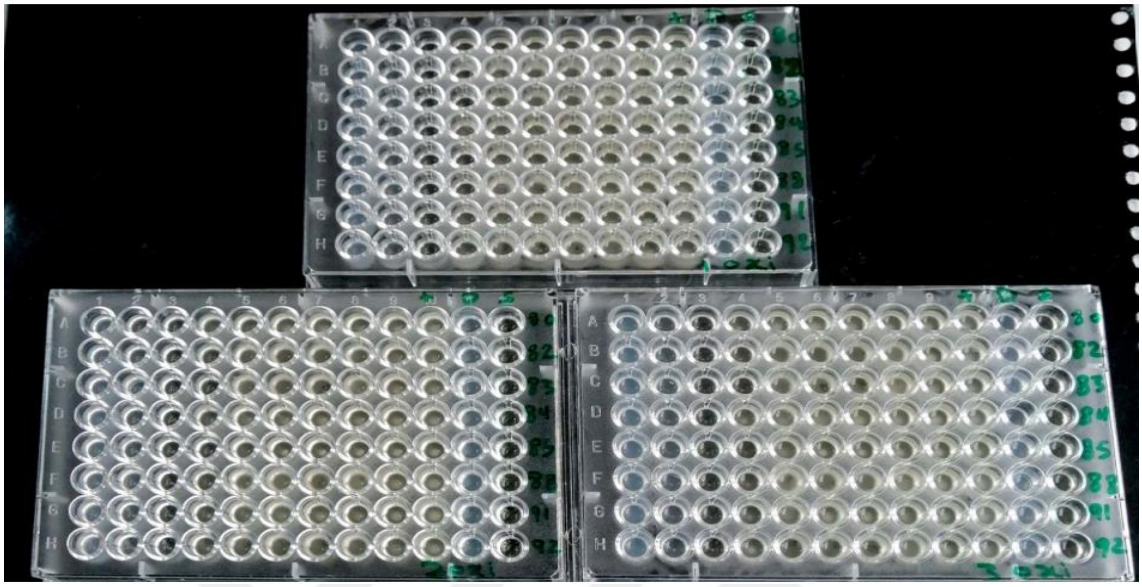

Figure 4. 2. Triplicate repetition for Oxivir disinfectant

+ for positive control (100µL bacteria +100µL media), D (100 µL medium + 100 µL disinfectant for negative control, S (200 µL medium alone acting as a sterility control), The numbers on the right side represent the bacterial strains.

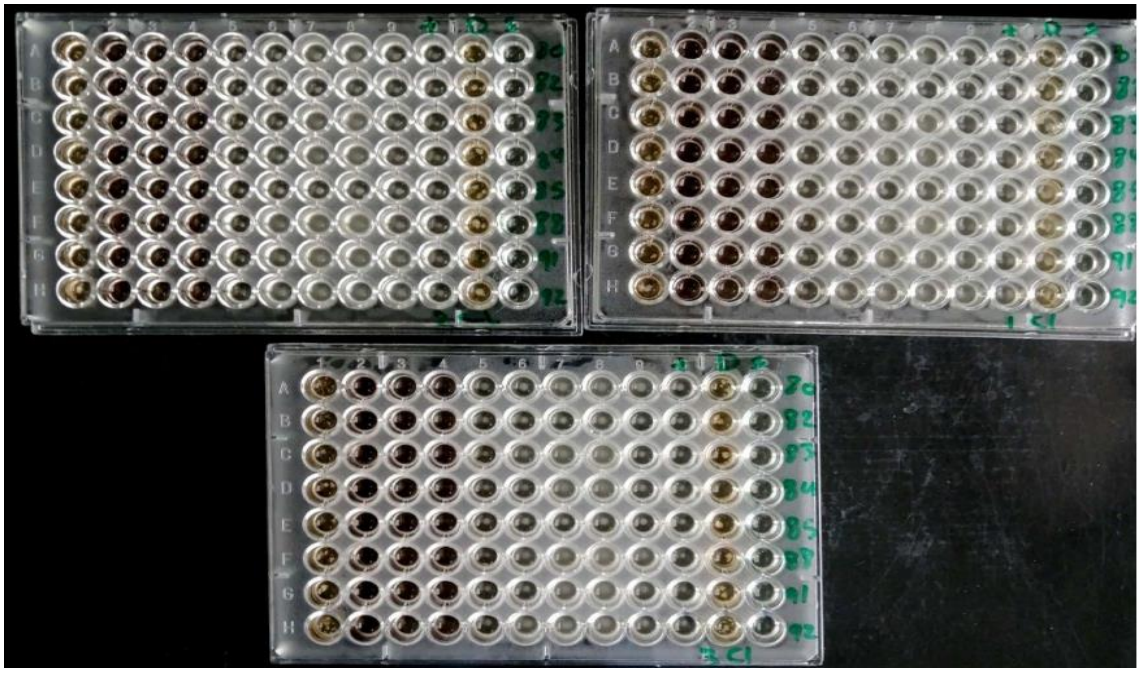

Figure 4. 3. Triplicate repetition for Chlortab disinfectant

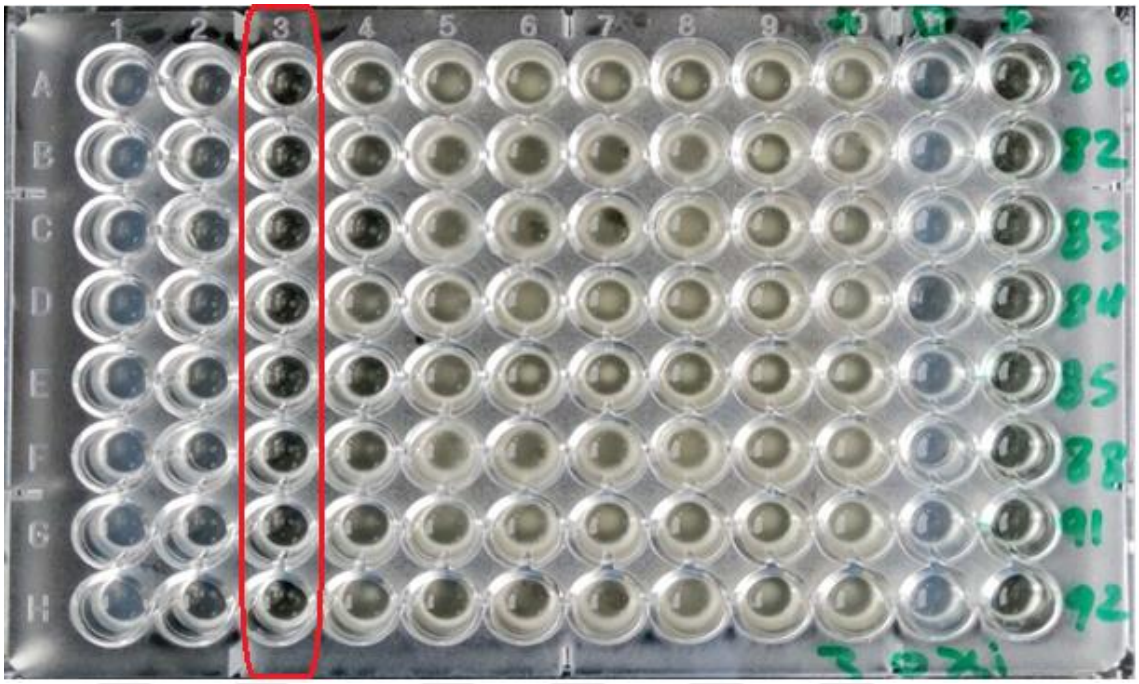

Figure 4. 4. MIC value for Oxivir at Well No. 3, which is equal to 0.09%

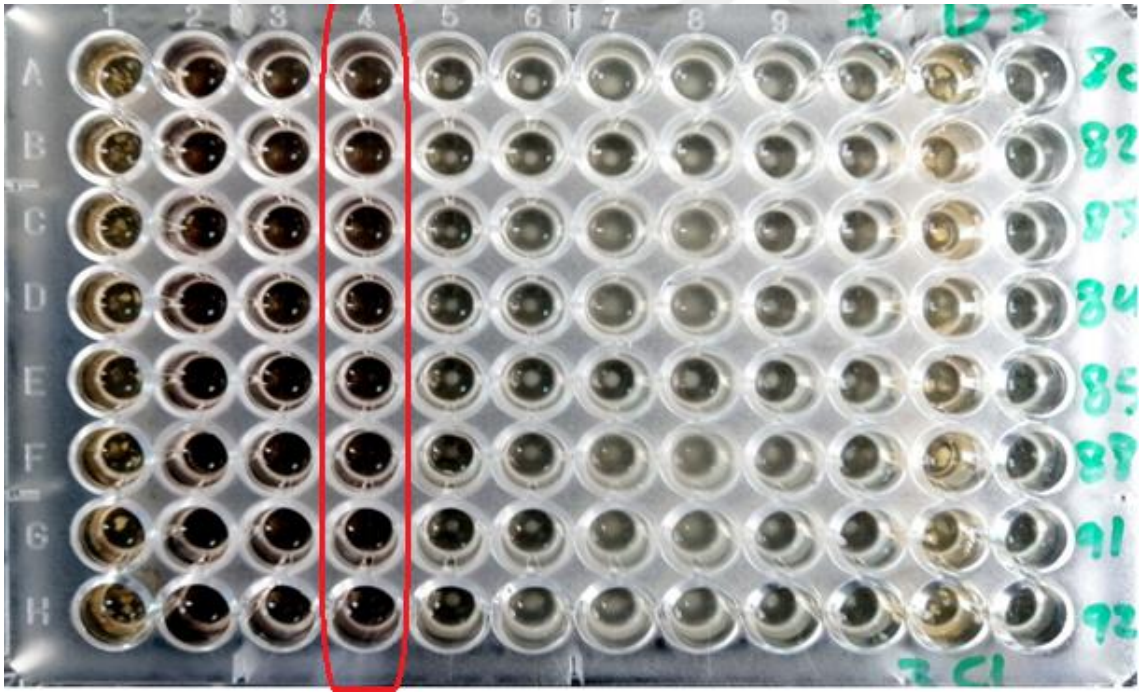

Figure 4. 5. MIC value for chlortab at Well No. 4, which is 6.25%

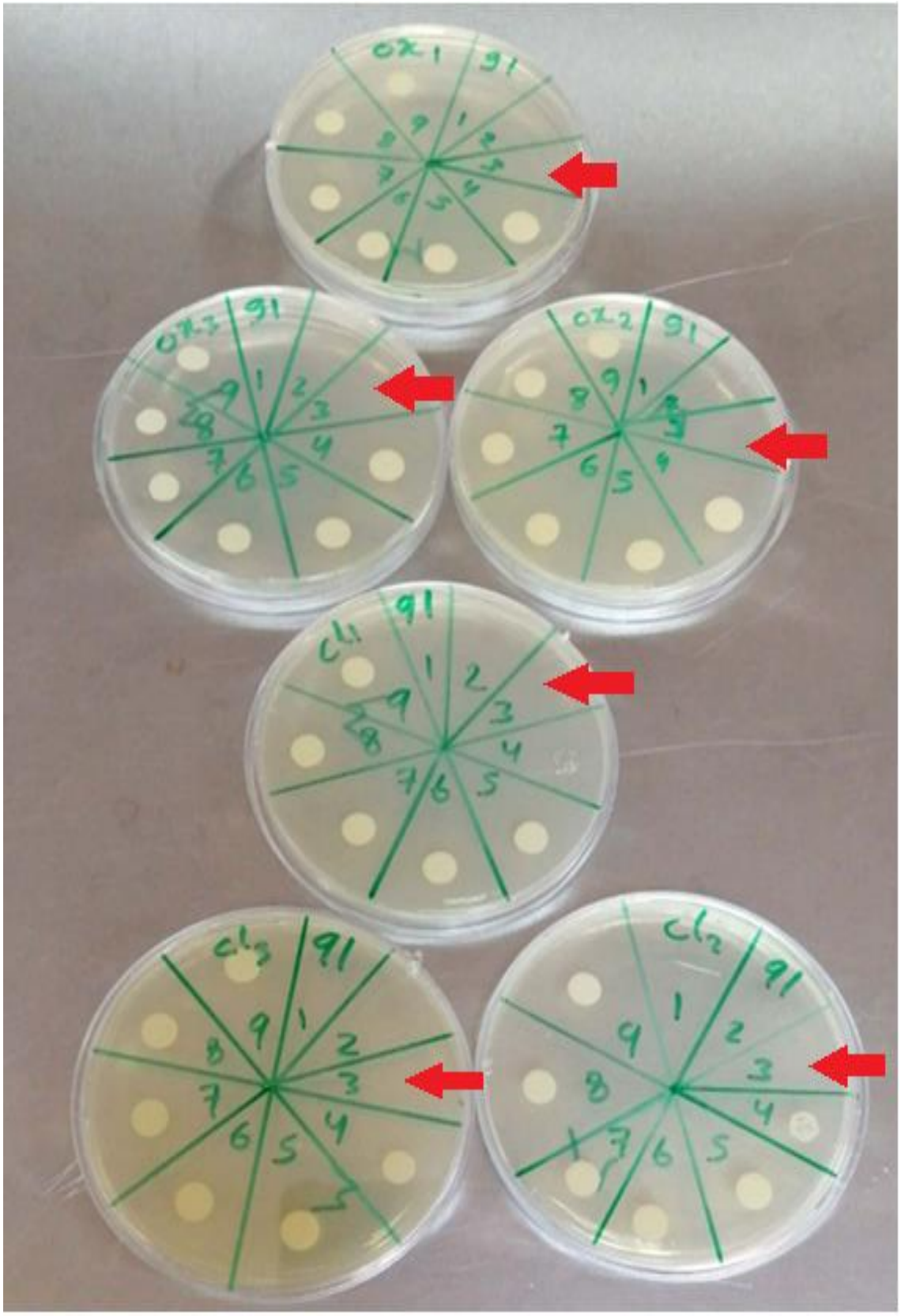

Figure 4. 6. Triplicate MBC values plates.

Numbers from 1 to 9 represent the corresponding serial dilutions in the microplate wells; spots in the plate indicate bacterial growth, 91 represent the strain number.

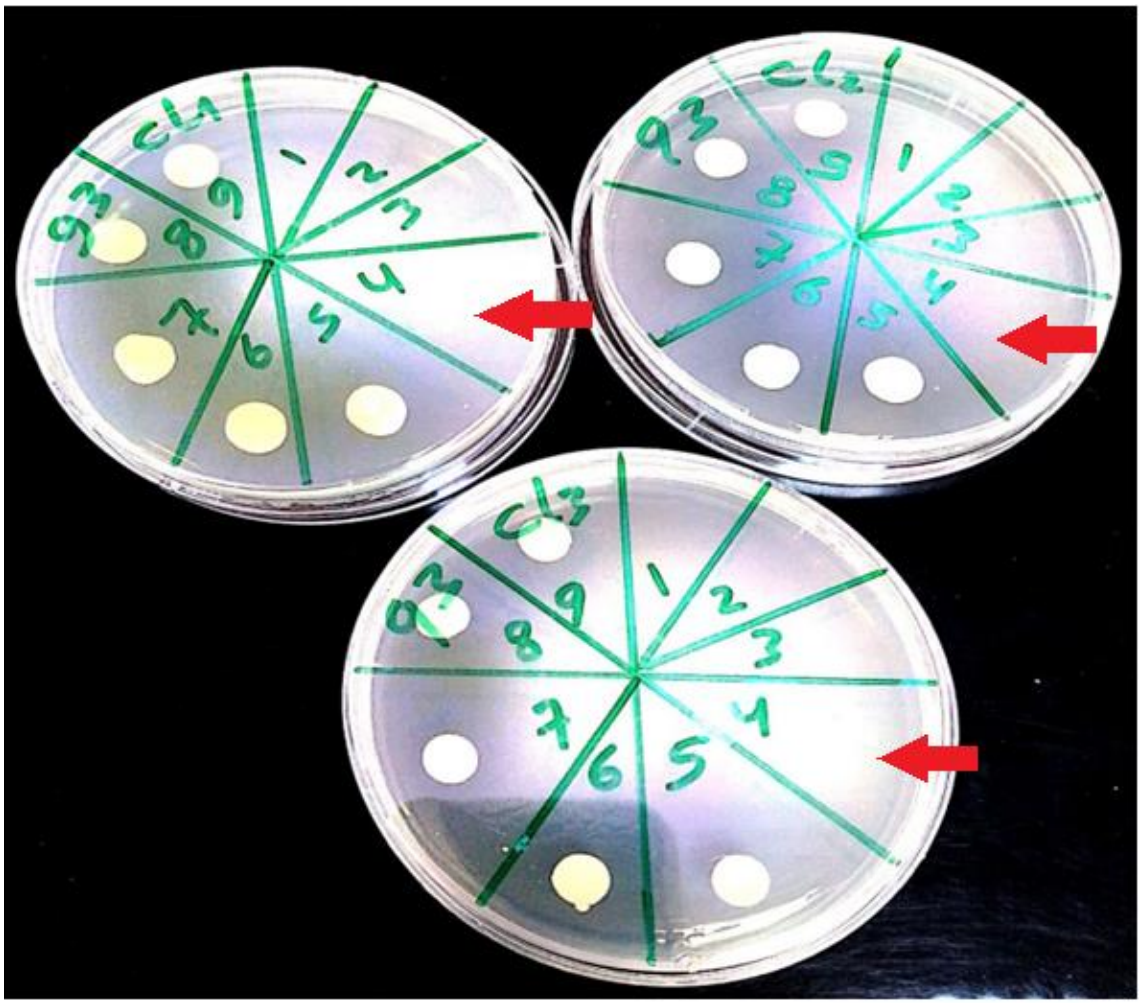

Figure 4. 7. Minimal bactericidal concentration (MBC) of disinfectants

Numbers from 1 to 9 represent decreasing concentrations from 1 to 9 (serial dilutions in the wells); spots in the plate indicate bacterial growth.

Table 4. 1. Distribution of MICs and MBCs of studied disinfectants

no	Strain number	Oxivir Disinfectant		MBC/MIC Ratio for oxivir	Chlor tablet Disinfectant		MBC/MIC Ratio for chlor tablet
		MICs values	MBCs values		MICs values	MBCs values	
1	**A1**	0.09%	0.09%	1	6.25%	6.25%	1
2	**A2**	0.09%	0.09%	1	6.25%	6.25%	1
3	**A3**	0.09%	0.09%	1	6.25%	12.5%	2
4	**A5**	0.09%	0.09%	1	6.25%	6.25%	1
5	**A6**	0.09%	0.09%	1	6.25%	6.25%	1
6	**A8**	0.09%	0.09%	1	6.25%	6.25%	1
7	**A10**	0.09%	0.09%	1	6.25%	12.5%	2
8	**A11**	0.09%	0.09%	1	12.5%	12.5%	1
9	**A12**	0.09%	0.09%	1	6.25%	6.25%	1
10	**A15**	0.09%	0.09%	1	12.5%	12.5%	1
11	**A16**	0.09%	0.09%	1	6.25%	12.5%	2
12	**A19**	0.09%	0.09%	1	6.25%	6.25%	1
13	**A21**	0.09%	0.09%	1	6.25%	12.5%	2
14	**A22**	0.09%	0.09%	1	6.25%	6.25%	1
15	**A26**	0.045%	0.09%	2	6.25%	6.25%	1
16	**A27**	0.09%	0.09%	1	6.25%	12.5%	2
17	**A28**	0.09%	0.09%	1	6.25%	12.5%	2
18	**AU32**	0.09%	0.18%	2	6.25%	12.5%	2
19	**A36**	0.09%	0.09%	1	12.5%	12.5%	1
20	**A37**	0.09%	0.09%	1	12.5%	12.5%	1
21	**A38**	0.09%	0.09%	1	12.5%	12.5%	1
22	**A43**	0.09%	0.09%	1	12.5%	12.5%	1
23	**A47**	0.09%	0.09%	1	12.5%	12.5%	1

24	**A51**	0.09%	0.09%	1	12.5%	12.5%	1
25	**A52**	0.09%	0.09%	1	12.5%	12.5%	1
26	**A55**	0.09%	0.09%	1	12.5%	12.5%	1
27	**A56**	0.09%	0.09%	1	12.5%	12.5%	1
28	**A57**	0.09%	0.09%	1	12.5%	12.5%	1
29	**A69**	0.09%	0.09%	1	12.5%	12.5%	1
30	**A70**	0.09%	0.09%	1	12.5%	12.5%	1
31	**A75**	0.09%	0.09%	1	12.5%	12.5%	1
32	**A76**	0.09%	0.09%	1	12.5%	12.5%	1
33	**A77**	0.09%	0.09%	1	12.5%	12.5%	1
34	**A80**	0.09%	0.09%	1	6.25%	6.25%	1
35	**A82**	0.09%	0.09%	1	6.25%	6.25%	1
36	**A83**	0.09%	0.09%	1	6.25%	6.25%	1
37	**A84**	0.09%	0.09%	1	6.25%	6.25%	1
38	**A85**	0.09%	0.09%	1	6.25%	12.5%	2
39	**A88**	0.09%	0.09%	1	6.25%	12.5%	2
40	**A91**	0.09%	0.09%	1	6.25%	12.5%	2
41	**A92**	0.09%	0.18%	2	6.25%	12.5%	2
42	**A93**	0.09%	0.09%	1	6.25%	6.25%	1
43	**A97**	0.09%	0.09%	1	6.25%	12.5%	2
44	**A100**	0.045%	0.18%	4	6.25%	6.25%	1
45	**A102**	0.09%	0.18%	2	6.25%	12.5%	2

5. DISCUSSION

A. baumannii is a member of ESKAPE group *(Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, A. baumannii, Pseudomonas aeruginosa, and Enterobacter spp.*). The pathogens in this group can escape from the effects of antimicrobials [6, 16].

A. baumannii, a Gram-negative, nonfermentative coccobacilli, is a predominant cause of nosocomial infections and can survive also on inanimate surfaces? for long periods of time [55].

A. baumannii can cause serious infections and antibiotic resistance of bacterium may lead problems in treatment. Disinfectants owing to their antimicrobial properties, have role in inhibiting the spread of infections [1].

A. baumannii has been reported as having resistance or reduced susceptibility to biocides. Bacterium owes its resistance to mechanisms such as permeability deficits, multidrug efflux pumps, target site modifications [28]. Furthermore, biofilms have been linked to the resistance of pathogens to antimicrobial agents [14, 16].

Improper use of disinfectants may lead resistance or reduced susceptibility to these chemicals. Bacteria displaying resistance to disinfectants may affect the transmission of bacteria, leading to spread of infection especially in hospitals. Effective disinfection and sanitation are important in preventing communicable disease transmission [20].

Nosocomial infections caused by *A. baumannii* are increasing in recent years, possibly related with the increase in strains having reduced susceptibility to disinfectants. Although resistance to antibiotics have remarkably attracted researchers' interest, this is not the fact with disinfectants [40]. Currently the researches are increasing on this topic.

Increased resistance of microorganisms to antibiotics and emergence of biocide-resistant bacterial strains have made it difficult to eliminate them. *A. baumannii* strains may display resistance or reduced susceptibility to biocides which can lead to transmission of infection in hospitals. In recent years researches are going on to determine the susceptibility of *A. baumannii* to disinfectants.

Disinfectants are effective in reducing infections, but the correct choice of disinfectants considering the wide range of efficacy against hospital pathogens is important and essential in this process [56].

Overuse and improper use of disinfectants can lead to disinfectant resistance, which may be important for daily life from compromising food security to threatening our healthcare systems [40].

We aimed to figure out the susceptibilities of 45 MDR *A. baumannii* strains which were obtained from ICU patients to some disinfectants. In the study we evaluated the efficacy of two disinfectants, Oxivir excel foam spray (Hydrogen peroxide 0.36%), and Chlortab effervescent tablet (50%NaDCC), which are currently in use at Başkent University Ankara Hospital. The susceptibility of bacteria were determined by MIC and MBC using broth microdilution method.

Oxivir excel foam® is used in medical device disinfection and contains hydrogen peroxide (0.36%) as the active substance. H_2O_2 is nontoxic, inorganic chemical and a high-level disinfectant [41, 44, 51]. H_2O_2 which is an oxidizing agent, having role in DNA, intracellular protein and membrane damage [46].

Chlortab used in floor-surface disinfection is effervescent tablet containing sodium dichloroisocyanurate. It is a chlorine releasing compound, oxidizing bactericidal and cytotoxic agent [45]. Compared to sodium hypochlorite, sodium dichloroisocyanurate has a higher disinfectant efficacy and is more tolerant to the presence of organic material [44].

According to our results our strains were susceptible to the disinfectants used at Başkent University Ankara Hospital. The MICs and MBCs of the biocides were less than concentrations that were recommended by manufacturer. However, it should be considered that the hospital environment contains infectious microorganisms other than *A. baumannii*, so it can be considered that the manufacturer's recommended concentrations used in hospitals are not specific for *A. baumannii,* more over both of disinfectants are considered bactericidal with average MBC/MIC ratio less than 4. The MBC/MIC ratio < 4 indicates that the agent has bactericidal properties, while a ratio of >4 meaning the agent has bacteriostatic properties [1]. The strain A101 has MBC/MIC ratio as 4, this may lead us to determine the MIC and MBC values at short intervals in order to trace the results.

The fact that the susceptibility of MDR *A. baumannii* evaluation to biocides is important for the successful performance of the disinfection process in the infection control program at hospital.

Antibiotic resistance in *A. baumannii* is a well-studied issue. Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) establish breakpoints for antibiotics. However, no standard and authorized biocide susceptibility testing method has been designed, and MIC breakpoints for biocides have not been established until now [6].

Yet there is not any consensus in definition of biocides resistance of bacteria. Some researchers suggest resistance as a decrease in susceptibility which is determined by an increase in the MIC where as others suggest bacteria surviving at any usable concentration are accepted as resistant [20].

Though MIC is the mostly used value for determining the resistance in practice disinfectants are generally used in higher concentrations than the MIC value [48].

In this study the recommended concentrations were higher than MIC values for both disinfectants. The use of concentrations above the effective level may lead to the emergence and spread of more resistant strains due to selective pressure and becomes a great challenge in nosocomial infection control**.** Use of excessive amounts of disinfectants may cause adverse effects on the environment or human health [20].

Our results displayed no correlation between biocides and antibiotic resistance although the strains were MDR and obtained from ICU patients. Studying environmental isolates give more reliable results than bacterial culture collections in exploring resistance mechanisms [48]. In our study we aimed to determine the susceptibility of the bacteria to disinfectants which are used currently in practice, so we did not investigate the mechanisms.

Despite the fact that some researches have shown that MDR strains carry biocides-resistance genes, some studies proved completely resistance to disinfectants even with recommended concentrations [2]. Similar to our findings, some studies reported there were no relation between antibiotic resistance and biocides and their strains were recorded as susceptible to biocides [1, 55].

In case of Oxivir the MIC values for our strains were 0.09% - 0.045%, and MBCs were 0.18%-0.09%, while the concentration recommended by manufacturer is 0.36%. Similarly, in several other studies, the MIC of the biocides used was less than that of recommended by manufacturers.

Hydrogen peroxide has been routinely employed as a biocide for many years. Hydrogen peroxide resistance is rare according to studies, although Hydrogen peroxide tolerance has been reported in a few bacterial species that produce catalase [44].

For Chlortab all strains were detected as susceptible in our study, with MICs and MBCs ranges less than the manufacturer's recommended concentrations. MIC values were between 12.5% and 6.25%, 156.25–78.12 mg/L. Our results were more susceptible than those from a study conducted in China, where the MIC value for chlorine disinfectants against *A.baumannii* strains were reported as 250 mg/L [2]. In another study MIC values were reported as ranging from 160–640 mg/L in China in 2017 [57].

Iran strains had MICs ranges between 0.08–0.34% for sodium hypochlorite the method of evaluation was different [58], American isolates, had MICs values for bleach ranging between 0.037 - 0.098% for 10 *A. baumannii* patient isolates that were collected during a routine workup [1].

The spread and transmission of bacteria that are resistant to biocides is expected to be influenced by biological, physical, economic, and social factors. These factors change from country to country, region to region, and among different communities [36].

Variations in the results obtained from different countries or even the same country provides further evidence that there are no established testing procedures for determining the MIC breakpoints of biocides. Differences in isolate type, year of isolation and methods used to conduct susceptibility testing all play important roles in determination of susceptibility to disinfectants.

The use of concentrations above the effective level may lead to the emergence and spread of more resistant strains due to selective pressure and becomes a great challenge in nosocomial infection control. It was noted that the high concentrations of biocides are toxic to humans and the environment, in addition to imposing cost to health system .[5]

If biocide susceptibility is not studied on clinical isolates, the importance of decreased susceptibility in control of infection spread will not be clear. By determining the biocide resistance mechanisms in clinical isolates the effective biocide can be used for prevention of outbreak [47].

Till today a unique methodology for susceptibility testing has not been accepted. Also standard MIC breakpoints for disinfectants and various bacteria are also not established yet. The studies which will be performed on this subject will be helpful in developing standard approaches for standardized cut-off MIC values of biocides such as in antibiotics. After evaluating with a standardized MIC values the scientists will be able to choose the best effective biocide to special bacteria resulting with better control in spread of infection [6].

In our opinion the detection of effectiveness of the biocides in every hospital will be beneficial in developing prevention strategy for the breakout of infections. In this policy, periodically evaluation of the strains' susceptibility to biocides obtained from different sources will be the most important goal. As a result the most effective biocide should be chosen for the disinfection [36].

6. CONCLUSION

All strains were susceptible to tested disinfectants. According to the MBC/MIC ratio which were less than 4, both of disinfectants showed bactericidal activity.

The MIC value and MBC value for both disinfectants were very close.

Oxivir excel foam® was more effective than Chlortab at low concentration.

Successful disinfection strategies are principal in preventing disease transmission.

According to our findings there is no correlation between antibiotic resistance and biocides resistance.

Periodic evaluation of biocide susceptibility is necessary to ensure product effectiveness, comply with regulatory requirements, it helps in maintaining the efficacy and safety of biocides and supports effective infection control.

The excessive use or abuse of biocides causes emergence of resistance.

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