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Carbapenem resistance among clinical and environmental Gram-negative isolates recovered from hospitals in Gaza strip, Palestine.

مقاومة الكاربابنيمات بين عزلات سالبة غرام السريرية والبيئية المعزولة من مستشفيات قطاع غزة- فلسطين

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إقـــــــــرار

أنا الموقع أدناه مقدم الرسالة التي تحمل العنوان:

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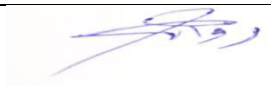
Signature:		التوقيع:
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نتيجة الحكم على أطروحة ماجستير

بناءً على موافقة شئون البحث العلمي والدراسات العليا بالجامعة الإسلامية بغزة على تشكيل لجنة الحكم على أطروحة الباحثة/ **روان حسن عبدالفتاح ريدة** لنيل درجة الماجستير في **كلية *العلوم*** قسم **العلوم الحياتية- أحياء دقيقة** وموضوعها:

مقاومة الكاربابنيمات بين عزلات سالبة غرام السريرية والبيئية المعزولة من مستشفيات قطاع غزة- فلسطين

Carbapenem resistance among clinical and environmental Gram-negative isolates recovered from hospitals in Gaza strip, Palestine

وبعد المناقشة التي تمت اليوم الاثنين 08 ذو القعدة 1438هـ، الموافق 2017/07/31 الساعة الحادية عشر صباحاً في قاعة مؤتمرات مبنى اللحيدان، اجتمعت لجنة الحكم على الأطروحة والمكونة من:

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وبعد المداولة أوصت اللجنة بمنح الباحثة درجة الماجستير في **كلية *العلوم*/** قسم **العلوم الحياتية - أحياء دقيقة.**

واللجنة إذ تمنحها هذه الدرجة فإنها توصيها بتقوى الله ولزوم طاعته وأن يسخر علمها في خدمة دينها ووطنها.

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نائب الرئيس لشئون البحث العلمي والدراسات العليا

أ.د. عبدالرؤوف علي المناعمة

Abstract

Background: The world is threatened by the ongoing emergence of carbapenem resistant organisms, which are contributing to increasing morbidity and mortality rates. The main objective of this study is to highlight carbapenem resistance among clinical and environmental Gram-negative bacteria (GNB) isolates.

Methodology: A total of 140 clinical isolates, 150 environmental swabs, and 110 air samples were collected from Al-Shifa and The European Gaza hospitals. In addition, 70 clinical isolates were obtained from Al-Naser hospital. All isolates/samples were cultured and identified using conventional bacteriological methods. All GNB isolates were tested for their antimicrobial susceptibility using the disc diffusion method. Modified Hodge Test (MHT) was performed to investigate carbapenemsases production.

Results: The overall percentage of carbapenem resistance among GNB was 12.1%. Resistance to Imipenem was 8.1% while resistance to Ertapenem and Meropenem was 3.5% and 0.8% respectively. Al-Naser hospital had the highest resistance rate (17.1%), followed by European Gaza Hospital (12.9%), while that of Al-Shifa hospital was 8.6%. The Intensive Care Units (ICUs) exhibited the highest resistance rate (52.9%), followed by the surgery departments (37.5%). Carbapenem resistance among *Enterobacteriaceae* was 13.2% while in *Pseudomonas* it was 0%. *Klebsiella* spp. was the most resistant to carbapenems (14.4%), followed by *E. coli* (9.8%). Seven isolates were positive (23.3%) for MHT. All *Enterobacteriaceae* isolates had a Multiple Antibiotic Resistance (MAR) index higher than 0.2. GNB was isolated from 17.2% of air samples. The ICUs recorded the highest positivity rate (26.2%). The average levels of bacteria obtained from air samples were (7.8×10^2 CFU/m^3) and of fungi (5.2×10^2 CFU/m^3). Environmental swabs GNB isolates were higher in European Gaza Hospital (22.1%) than Al-Shifa (7.3%). Pediatric Intensive Care Unit showed the highest positivity rate (40.9%), while Neonatal Intensive Care Unit had the lowest positivity rate (8.3%).

Conclusion: The resistance found, after such a recent introduction (10 years) of carbapenems use in Gaza, shows the need for policies to prevent misuse and overuse of carbapenems, the need for infection control procedures and screening policies for carbapenem resistance on a routine basis.

Keywords: Carbapenem Resistant *Enterobacteriaceae* (CRE), Carbapenemsases, Modified Hodge Test (MHT), Multiple Antibiotic Resistance (MAR) index

الملخص

تمهيد: يهدد الانتشار المستمر للميكروبات المقاومة للكاربابنيمات العالم بأسره، وذلك لمساهمتها في رفع معدلات المراضة والوفيات. الهدف الرئيسي من هذه الدراسة هو تسليط الضوء على مقاومة عزلات سالبة غرام السريرية والبيئية للكاربابنيمات.

المنهجية: تم جمع 140 عزلة سريرية , 150 مسحة بيئية و 110 عينة هواء من متشفى الشفاء و الأوروبي. بالإضافة إلى 70 عزلة سريرية من مستشفى النصر. تمت تنمية جميع العزلات / العينات وتشخيصها بالطرق التقليدية. ومن ثم اختبار حساسيتها للمضادات الحيوية. كما وتم التحقق من إنتاج إنزيمات الكاربابنيمازيز عن طريق استخدام فحص هودج المعدل.

النتائج: كان معدل مقاومة البكتيريا سالبة غرام للكاربابنيمات 12.1%. بلغ معدل مقاومتها للاميبنيم 8.1%، بينما كانت المقاومة للارتابينيم و الميروبنيم، 3.5% و 0.8% على التوالي. سجّل مستشفى النصر أعلى نسبة لمقاومة الكاربابنيمات (17.1%) تلاه مستشفى غزة الأوروبي (12.9%)، بينما بلغت نسبة المقاومة في مستشفى الشفاء 8.6%. أظهرت وحدات العناية المركزة اعلى نسب للمقاومة (52.9%)، تلتها وحدات الجراحة (37.5%). كانت مقاومة الكاربابنيمات في البكتيريا المعوية 13.2%، بينما كانت النسبة في الزائفة الزنجارية 0%. كانت عزلات الكلبسيلا الأكثر مقاومة للكاربابنيمات (14.4%)، تلتها عزلات الاشريكية القولونية (9.8%). أظهرت 7 عزلات (23.3%) نتيجة إيجابيه لاختبار هودج المعدل. وقد كان مؤشر المقاومة المتعددة للمضادات الحيوية اعلى من 0.2. لجميع عزلات البكتيريا المعوية. تم عزل بكتيريا سالبة غرام من 17.2% من عينات الهواء. كان المعدل الأعلى من نصيب وحدات العناية المركزة (26.2%). بلغ العد البكتيري الكلي في عينات الهواء 7.8×10^2 وحدة تكوين مستعمرة/م^3, بينما كانت مستويات الفطريات 5.2×10^2 وحدة تكوين مستعمرة/ م^3. بلغت نسبة بكتيريا سالبة غرام التي تم عزلها من المسحات البيئية في مستشفى الأوروبي 22.1%، بينما كانت النسبة في مستشفى الشفاء 7.3%. وأظهرت وحدة العناية المركزة للأطفال أعلى معدل إيجابي (40.9٪)، في حين سجلت وحدة العناية المركزة لحديثي الولادة المعدل الأدنى (8.3٪).

الخلاصة: خلصت الدراسة إلى أن النسبة المرتفعة لمقاومة الكاربابنيمات على الرغم من الفترة القصيرة (10 سنوات) لإدخال هذا النوع من المضادات إلى غزة، تستدعي الحاجة إلى وضع سياسات لمنع إساءة استخدام الكاربابنيمات، وإجراءات مسحية روتينة لمقاومة الكاربابنيمات. كما وهناك حاجة لإتباع إجراءات مكافحة العدوى بشكل مستمر.

كلمات مفتاحية: مقاومة الكاربابنيمات في البكتيريا المعوية، إنزيمات الكاربابنيمازيز، اختبار هودج المعدل، مؤشر المقاومة المتعددة للمضادات الحيوية

“If you always do what you've always done, you'll always get what you've always got.”

-Henry Ford

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List of Abbreviations

CFU	Colony forming unit
CLSI	Clinical and laboratory standards institute
CR	Carbapenem resistance
CRE	Carbapenem resistant *Enterobacteriaceae*
CRGNB	Carbapenem resistant gram negative bacteria
CRKP	Carbapenem resistant *Klebsiella pneumoniae*
ESBL	Extended spectrum beta lactamase
FDA	Food and drug administration
GES	Guiana extended spectrum
GNB	Gram negative bacteria
ICU	Intensive care unit
IMI	Imipenem-hydrolyzing beta lactamase
IMP	Imipenemase metallo beta lactamase
KPC	*Klebsiella pneumoniae* carbapenemase
MALDI-TOF MS	Matrix-assisted laser desorption ionization-time of flight mass spectrometry
MARI	Multiple antibiotic resistance index
MBLs	Metallo beta lactamases
MDR	Multi drug resistant
MHT	Modified Hodge test
MIC	Minimal inhibitory concentration
MRSA	Methicillin resistant *Staphylococcus aureus*
NA	Nutrient agar
NDM	New Delhi metallo beta lactamase
NF-GNB	Non-glucose-fermenting Gram negative bacilli
NICU	Neonatal intensive care unit
NmcA	Non-metallo-carbapenemase-A
OMPs	Outer membrane proteins
OXAs	Oxacillinases
PBPs	Penicillin-binding proteins
PICU	Pediatric intensive care unit
RND	Resistance nodulation division
SDA	Sabouraud dextrose agar
SFC	*Serratia fonticola* carbapenemase
SME	*Serratia marcescens* enzyme
SPSS	Statistical package for the social sciences
VIM	Verona integrin encoded metallo beta lactamase
VRE	Vancomycin resistant *enterococcus*

Chapter I

Introduction

Chapter I
Introduction

1.1 Overview

Carbapenems were developed in the 1980s and are a β -lactam group of drugs that are considered as antibiotics of last resort for treating serious infections with multidrug-resistant (MDR) Gram-negative bacteria (GNB). The broad-spectrum antimicrobial activity of carbapenems included coverage of *Pseudomonas aeruginosa*, which is part of the reasons why they were adequately assigned for the treatment of healthcare-associated infections. Back then, almost all *Enterobacteriaceae* were susceptible to carbapenems (Papp-Wallace, Endimiani, Taracila, & Bonomo, 2011), but this is not the case anymore. The change of the scenario is attributed to the emergence of carbapenem resistance (CR) in non-fermenters GNB (*Acinetobacter baumannii* and *P. aeruginosa*) as well as in fermenters GNB (*Enterobacteriaceae*) over the past few years (Gniadek, Carroll, & Simner, 2016).

Due to the emergence of ESBL-producing *Enterobacteriaceae* and the global spread of *Enterobacteriaceae* and *P. aeruginosa* that produce high levels of AmpC cephalosporinase, GNB, which potentially impact intensive care units (ICUs) patients, are now progressively resistant to antibiotics and particularly to broad-spectrum cephalosporins (Meyer, Schwab, Schroeren-Boersch, & Gastmeier, 2010). This has led to increasing use of carbapenems. The widespread use of carbapenems not only for documented infections, but also for empirical therapy is likely to blame for the emergence of CR, which represents a major concern for ICUs.

Carbapenemases, which are β-lactamases with versatile hydrolytic capacities, have now become a threat to global healthcare because of their linkage to resistance to β-lactam antibiotics and to other classes of antibiotics such as aminoglycosides, fluoroquinolones, and cotrimoxazole (Souli, Galani, & Giamarellou, 2008). Thus, they limit the range of therapeutic options for infections due to MDR strains (Plachouras et al., 2009).

Gaza strip has been witnessing an escalation of antibiotic resistance. The studies conducted concerning this have yielded scary results which are sounding the alarm for urgent action (Al Laham, 2012; Elmanama & Abdelateef, 2013). Carbapenems are one of the few therapeutic agents we have left. But now, with the scarcity in data concerning the prevalence of CR among clinical and environmental GNB isolates in the region, things are getting out of hand and efforts must be targeted to finding urgent solutions.

1.2 Objectives

The main objective of this research is to screen clinical and environmental Gram-negative isolates for CR.

The other specific objectives are:

1. To compare the occurrence of CRGNB among various hospitals.
2. Determine possible alternatives through testing CRGNB to available antimicrobials.
3. Provide microbiological assessment of indoor air quality and the environments of hospitals.

1.3 Significance

There is a notable increase of healthcare-associated infections such as pneumonia and bloodstream infections caused by *Klebsiella pneumoniae* and other Gram-negative organisms (Peleg & Hooper, 2010). Besides increasing incidence, resistance has become an urgent problem. About 10% of hospitalized patients contracts nosocomial infections, and up to 75% of these are caused by organisms resistant to first-line antimicrobial therapy (Lautenbach & Perencevich, 2014). GNB, particularly carbapenem-resistant *K. pneumoniae* (CRKP), has become a major threat to hospitals globally due to its contribution to high morbidity and mortality rates (Munoz-Price & Quinn, 2009). Infections with CRKP and other CRGNB are life threatening, as just few treatment options are available for patients and the outcomes are usually unsatisfying.

WHO's global report issued on April 2014 had some disturbing conclusions. Surveillance of antimicrobial resistance revealed the world is on the verge of entering a post-antibiotic era. At present, the full extent of how this problem is affecting

people, and more expansively in a global scenario, is unclear and needs to be quantified. Dependable data that is predictable and up to date is urgently needed to determine the extent of this potential dilemma (WHO, 2014).

Gaza strip is troubled by wars and has been besieged for a considerable amount of time. This has hindered interaction with the global community. Health and environmental sectors have been adversely affected. The unstable economy continues to cause suffering among the population and malpractices within the medical profession. One example is antimicrobial usage; anyone can have access to antimicrobials without a prescription. Several screening studies have reported a high prevalence of resistant pathogens: both in hospital admitted patients and in the region. MRSA, VRE, MDR *Pseudomonas*, *Acinetobacter* and others are the most commonly reported organisms in pathogen surveys.

There are few reports in Gaza strip and even fewer published data on the prevalence of CRE. This is due to the fact that hospitals have no screening policies for carbapenem resistance and antimicrobial susceptibility testing is not performed for this class of antibiotics on a routine basis. This study is the first inclusive one to provide a baseline for ICUs and surgery departments epidemiology of CRGNB, and the outcomes of this study should guide the local physicians in selecting appropriate empirical therapy and in setting treatment protocols for GNB infections.

Chapter II

Literature Review

Chapter II

Literature Review

2.1 History

Carbapenems are considered one of the most distinctive members of the β-lactam antibacterials family. Olivanic acids produced by *Streptomyces olivaceus* were the first discovered carbapenems (J. Kahan et al., 1979). The discovery of thienamycin in 1976 was found during a soil-screening program purposed to identify peptidoglycan synthesis inhibitors (F. Kahan, Kropp, Sundelof, & Birnbaum, 1983).

At first, a previously unknown *Streptomyces* spp produced thienamycin. This species was then given the name, *"Streptomyces cattleya*", because it has a pigment in its aerial mycelium resembling the color of the cattleya orchid (Bonfiglio, Russo, & Nicoletti, 2002). These compounds were not used clinically due to being chemically unstable. However, the synthesis and approval of a more stable derivative of thienamycin, *N*-formimidoyl thienamycin, which is also known as Imipenem was completed in 1984 (Hellinger & Brewer, 1999). This compound was heralded as a therapeutic agent because of its stability in a solid state and in concentrated solutions. Nevertheless, an extra instability due to the mammalian hydrolase that is known as dehydropeptidase-I (DHP-I), caused a decreasing levels of Imipenem in urine, thereby producing a potentially nephrotoxic metabolite (Birnbaum, Kahan, Kropp, & Macdonald, 1985).

Meropenem was the first carbapenem with both a 1-β-methyl group and 2-thiopyrrolidinyl moiety, which passes on its stability to (DHP-I). Some other carbapenems discovered later such as Biapenem, Panipenem, Ertapenem, Lenapenem, E-1010, S-4661 and BMS-181139 that were used for parenteral administration purposes. Whereas Sanfetrinem, DZ-2640, CS-834 and GV-129606 were Orally-administered (Bonfiglio, et al., 2002)**.**

2.2 Classification of carbapenems

A newly introduced classification system for carbapenems divides them into two groups. Group 1 carbapenems, e.g. Ertapenem, are defined as broad-spectrum agents with limited activity against non-fermentative GNB and fit community-acquired infections, whereas group 2 carbapenems, e.g. Imipenem, Meropenem and Doripenem, are broad-spectrum agents that are active against non-fermentative GNB and particularly suit treating nosocomial infections (Bassetti, Nicolini, Esposito, Righi, & Viscoli, 2009)**.** Another third group which was recently suggested includes carbapenems with activity against MRSA as shown in table 2.1.

Table (2.1): Classification of carbapenems based on their activity/use

Group	**Group 1 carbapenems**	**Group 2 carbapenems**	**Group 3 carbapenems**
Activity	Infections upon hospital admission-limited activity against nonfermentative Gram-negative bacilli	Hospital-acquired infections-Pseudomonas and Acinetobacter.	MRSA
Used carbapenem/s	Ertapenem	Imipenem, Meropenem, Panipenem, Biapenem, Doripenem	CS-023

2.3 Carbapenems activity

Carbapenems, with their broad spectrum of antimicrobial activity, surpass the majority of other antibiotic classes. Their high-affinity binding to most high molecular weight penicillin-binding proteins (PBPs) of Gram-negative and Gram-positive bacteria makes them rapid bactericidal agents (Bassetti, et al., 2009).

Carbapenems (except Ertapenem) have high efficiency against clinically significant non-fermenters GNB such as *P. aeruginosa*, *Burkholderia cepacia* and *Acinetobacter* spp. (Quale, Bratu, Gupta, & Landman, 2006). (See table 2.2)

Table (2.2): Spectrum of activity of four carbapenems

Drug	Strep spp.& MSSA	*Enterobacteriaceae*	Non-fermenters	Anaerobes
Imipenem	+	+	+	+
Meropenem	+	+	+	+
Ertapenem	+	+	Limited activity	+
Doripenem	+	+	+	+

They also retain activity against *streptococci*, methicillin-sensitive *staphylococci*, *Neisseria* and *Haemophilus.* It is worth being emphasized that carbapenems are active against most Gram-positive and Gram-negative anaerobes, including subspecies of *Bacteroides fragilis*, *Bacteroides thetaiotaomicron*, *Prevotella bivia*, *Fusobacterium nucleatum*, *Peptostreptococcus asaccharolyticus* and *Clostridium perfringens*. A feature that most other broad-spectrum antibiotics miss (Tellado & Wilson, 2005).

2.4 Carbapenems' mechanism of action

As a class of β-lactams, carbapenems are not easily diffusible through the bacterial cell wall (Martínez, 2008). Carbapenems use outer membrane proteins (OMPs), which are also known as porins, as a portal of entry to GNB. When carbapenems come across the periplasmic space, they "permanently" acylate the PBPs (Hashizume, Ishino, Nakagawa, Tamaki, & Matsuhashi, 1984)**.**

PBPs are enzymes with a responsibility of peptidoglycan formation in the cell wall of bacteria (i.e., transglycolases, transpeptidases, and carboxypeptidases). Carbapenems inhibit the peptidase domain of PBPs and peptide crosslinking as well as other peptidase reactions. Carbapenems' efficacy is attributed to being able to bind to multiple different PBPs. The inhibition of PBPs leads to a continuing autolysis and eventually a weakening of peptidoglycan. As a result, the cell ends up burst out due to osmotic pressure (Dam, Olrichs, & Breukink, 2009)**.** (Figure 2.1)

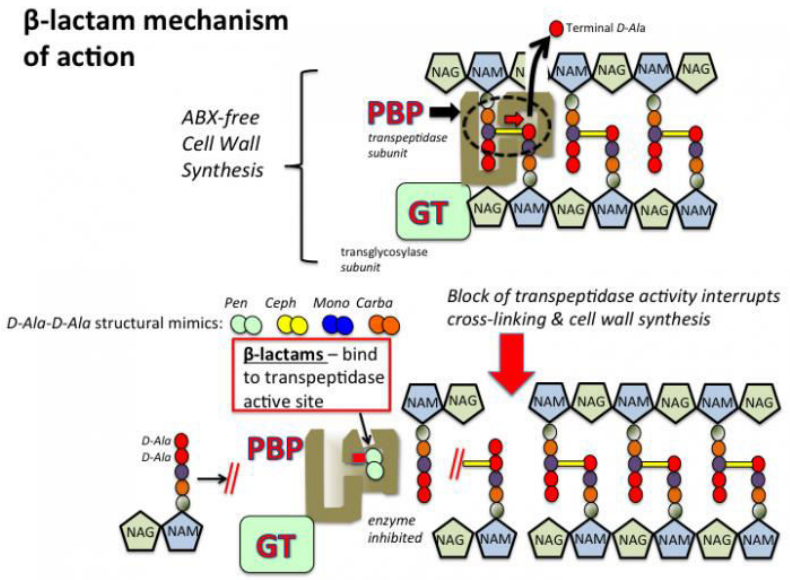

Figure (2.1): Carbapenems' mechanism of action (Tulane.edu, 2017).

2.5 Carbapenems' mechanisms of resistance

Several mechanisms were suggested to explain the resistance of GNB (Figure 2.2).

2.5.1 Carbapenemases

Carbapenemases are specific β-lactamases that are able to hydrolyze carbapenems even at low level. In 1993, the first carbapenemase was identified in *Enterobacter cloacae* clinical isolate (Naas & Nordmann, 1994). These periplasmic enzymes hydrolyze carbapenems, preventing them from reaching their PBP target. β-lactamases are now categorized according to structural similarities into four distinct classes (classes A, B, C and D) (Mammeri, Guillon, Eb, & Nordmann, 2010). Carbapenemases can be detected by disk diffusion and MIC susceptibility testing. Detecting carbapenemases can be confirmed by the Modified Hodge Test (MHT), the carbapenem inactivation method or molecular methods (El-Herte et al., 2012).

2.5.2 Efflux pumps

In this mechanism, the membrane transporter proteins (the efflux pumps) blow out antibiotics from the cell. These MDR pumps are mostly encoded by genes that are considered normal constituents of the bacterial chromosomes. The genes of some of these pumps have high constitutive expression and cause a remarkable resistance to the antibiotics while in other pumps, the expression of their genes comes after the acquisition of regulatory mutations (Lomovskaya et al., 2001). Five major families of

efflux-pump proteins are associated with MDR in Gram-positive and Gram-negative organisms: the ATP-binding cassette superfamily, the major facilitator superfamily, the multidrug and toxic-compound extrusion family, the small MDR family and the resistance nodulation division (RND) family (Piddock, 2006).

2.5.3 Loss of porins

Porins are OMPs that constitute channels for the transportation of molecules across lipid bilayer membranes. Variations in their structure or regulation of porin expression can provide a mechanism to escape from antibacterial pressure. The loss of a membrane protein channel decreases the rate of entry of antibiotics into the periplasm, thus raising the MIC (Vila, Martí, & Sánchez-Céspedes, 2007).

2.5.4 Altered Penicillin binding proteins (PBPs)

This mechanism is not commonly linked to CR in GNB. This is likely due to the high efficiency of the production of carbapenemases and the loss of porins combined, in producing resistance (Spratt, 1988)**.** However, mutations in PBPs and/or decreases in PBPs transcription were found to be contributing to CR phenotypes in *P. aeruginosa, A. baumannii* and *Proteus mirabilis* (Papp-Wallace, et al., 2011).

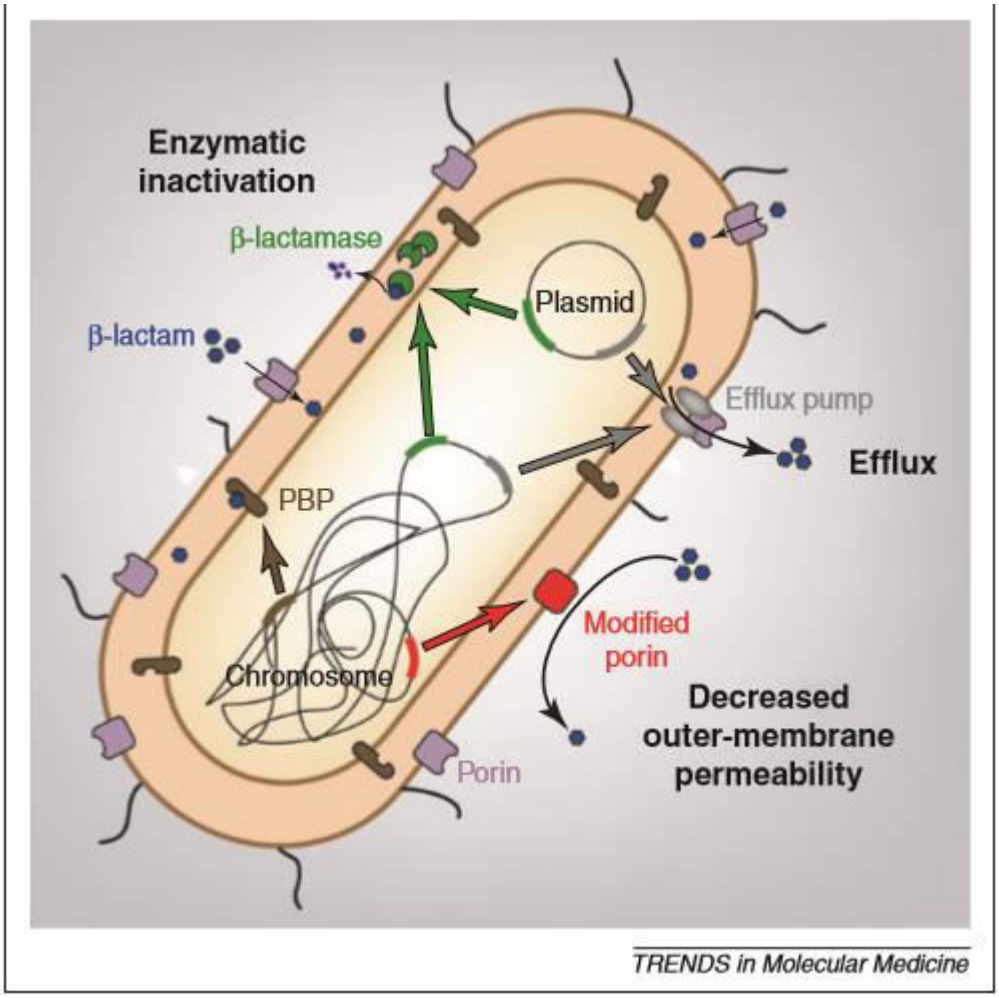

Figure (2.2): Carbapenems' mechanisms of resistance (Cell.com, 2017)

2.6 Ambler classification system of carbapenemases

2.6.1 Class A carbapenemases

Class A carbapenemases is the broadest class. It contains beta-lactamases with an active-site serine at position 70. This class includes chromosomally encoded enzymes Such like: Non-metallo-carbapenemase-A (NmcA), Imipenem-hydrolyzing beta lactamase (IMI-1), *Serratia marcescens* enzyme (SME), and *Serratia fonticola* carbapenemase (SFC-1). This class also has plasmid encoded enzymes such like *K. pneumoniae* carbapenemase (KPC), IMI-2; Guiana extended spectrum (GES) derivatives such as GES-1, GES-2, GES-4, and GES-5. Some of class A carbapenemases hydrolyze a narrow spectrum of β-lactams (e.g. penicillinases) and others hydrolyze a broad spectrum (e.g. ESBL or carbapenemases), but they all have a great potential to hydrolyze carbapenems and they are partially inhibited by

clavulanic acid (Queenan & Bush, 2007). The most frequently detected enzymes in this group are KPCs (Nordmann, Naas, & Poirel, 2011).

2.6.2 Class B carbapenemases

Also called metallo-β-lactamases (MBLs). These enzymes are marked by their resistance to all pencillins, cephalosporins, and carbapenems. Carbapenems hydrolysis varies from one MBL to another. They are mostly of the Verona integron-encoded metallo-*β*-lactamase (VIM) , Imipenemase metallo beta lactamase (IMP) types and more recently, of the New Delhi metallo-*β*-lactamases1 (NDM-1) type (Queenan & Bush, 2007). MBLs can hydrolyze all *β*-lactams except Monobactam (e.g., Aztreonam). MBLs can be plasmid borne or chromosome borne; they require zinc for their activity that can be inhibited by EDTA but not by clavulanic acid, Sulbactam, Tazobactam, or Avibactam (Walsh, Toleman, Poirel, & Nordmann, 2005). However, adding Zn^{2+} ions can reverse the inhibition caused by EDTA.

2.6.3 Class C carbapenemases

This class includes chromosomally encoded enzymes with highly conserved sequences that are known as the cephalosporinases or AmpC enzymes. They hydrolyze β-lactams and β-lactamase inhibitor combinations such as Ceftriaxone or Piperacillin/Tazobactam, but usually not carbapenems. However, CMY-10 is among the first AmpC enzymes that hydrolyze carbapenems (Galleni, Amicosante, & Frère, 1988).

2.6.4 Class D carbapenemases

Enzymes of this class, which are also called OXAs for oxacillinases, have the ability to hydrolyze Oxacillin and Cloxacillin. OXAs include 239 enzymes with the same carbapenemase activity (Nordmann, Poirel, & Dortet, 2012). OXAs were firstly reported from *P. aeruginosa,* but, then have been detected in many other GNB, including *Enterobacteriaceae* (Pfeifer, Cullik, & Witte, 2010). OXA-48 represents the main encountered enzyme around the world. It doesn't have a considerable activity against carbapenems or extended-spectrum cephalosporins (third generation Cephalosporin, Aztreonam), yet it is still strong enough to hydrolyze penicillins. EDTA, Tazobactam, Sulbactam and clavulanic acid cannot inhibit the activity of OXAs (Poirel, Dortet, Bernabeu, & Nordmann, 2011), but NaCl can, *in vitro*

(Nordmann, et al., 2012). Table 2.3 presents general classification of carbapenemases. Table 2.4 shows carbapenemases subgroups of the OXA family.

Table (2.3): General classification of carbapenemases

Class	Active site	Prominent Enzyme	Hydrolysis	Inhibition	Known organisms
A	Serine	GES, SME, NMC, KPC	All β-lactamas antibiotics	Clavulanic acid	Enterobacteriaceae
B	Zn^{2+}	IMP, VIM, SPM, GIM, SIM, NDM	All β-lactamas antibiotics except Azteronam	EDTA	*P. aeruginosa*, Enterobacteriaceae *Acnitobacter* spp.
D	Serine	OXA	Oxacillin and Cloxacillin	Nacl (in vitro)	*Acnitobacter* spp.

Table (2.4): Carbapenemases subgroups of the OXA family

Cluster	Enzyme subfamily	Additional OXA-members
1	OXA-23	OXA-27, OXA-49
2	OXA-24	OXA-25, OXA-26, OXA-40, OXA-72
3	OXA-51	OXA-64 to OXA-71, 75-78, 83, 84, 86-89, 91, 92, 94, 95
4	OXA-58	-
5	OXA-55	OXA-SHE
6	OXA-48	OXA-54, OXA-SAR2
7	OXA-50	OXA-50a- OXA-50d
8	OXA-60	OXA-60a - OXA-60d
9	OXA-62	-

2.7 Carbapenem resistance in *Enterobacteriaceae*

The most common human pathogens fall under the *Enterobacteriaceae* family. They are most likely to cause both community and hospital acquired infections that range from cystitis to pyelonephritis, septicemia, pneumonia, peritonitis, meningitis, and device-associated infections. Many factors come to play when trying to explain the great impact of MDR emergence in *Enterobacteriaceae* on clinical therapy. Those factors include the easily spread of *Enterobacteriaceae* in humans either by hand carriage or by contaminated food and water. In addition to the fact of their ability to acquire genetic material through horizontal gene transfer, with the help of plasmids and transposons (Walsh, Weeks, Livermore, & Toleman, 2011). Carbapenems are considered antibiotics of last resort for serious infections. Thus, the emergence of CRE is a public health concern. The most notable genera that

can develop CR are *E. coli* and *K. pneumonia.* However, CR has been reported also in *Pseudomonas.* CRE bacteria have high levels of resistant to other antibiotics. Infections caused by these bugs are life-threatening. one report cites they can contribute to death in up to 50% of patients who become infected (CDC, 2016).

2.8 Carbapenem resistance in non-glucose-fermenting GNB

There have been a number of studies focusing on CRE which produced guidelines and facilitated infection control efforts. The increase in these studies was primarily due to their becoming more common, their connection with carbapenemases production and ascending reports of nosocomial outbreaks giving rise to high rates of morbidity and mortality (Falagas, Tansarli, Karageorgopoulos, & Vardakas, 2014; Gupta, Limbago, Patel, & Kallen, 2011). In the background, often-neglected non-glucose-fermenting GNB (NF-GNB) are also steadily acquiring resistance to Carbapenems. These organisms can also present a difficult to treat, life threatening clinical conundrum, often in patients who already have significant comorbidities (Gniadek, et al., 2016).
Various mechanisms are involved in CR amidst NF-GNB. These include carbapenemases production, decreased permeability caused by porin mutations, overexpression of efflux pumps and changes in PBPs (Meletis, Exindari, Vavatsi, Sofianou, & Diza, 2012). Production of carbapenemases is the most potent mechanism of concern because of their increasing prevalence, their ability to easily be transmissible as they piggyback on mobile genetic elements, and their alliance with genes which confer resistance to other classes of antimicrobials, creating MDR concerns (Gniadek, et al., 2016).

2.9 Risk factors for acquisition of CRGNB

In an attempt to find out the risk factors for CRGNB acquisition or infection, many studies had revealed that exposure to health care settings and antimicrobials are among the most prominent risks (Marchaim et al., 2010). Poor functional status and intensive care unit (ICU) stay are also strongly linked to the acquisition of CRGNB (Schwaber & Carmeli, 2007). The use of several classes of antimicrobials has been associated with CRKP carriage or infection, including carbapenems (Hidron et al., 2008), cephalosporins (Patel, Huprikar, Factor, Jenkins, & Calfee, 2008),

fluoroquinolones (Hussein et al., 2009), and Vancomycin (Marchaim, et al., 2010). Age, mechanical ventilation, malignancy, heart disease, and ICU stay have been associated with increased mortality among those with CRGNB infections (Daikos et al., 2009).

2.10 Epidemiology of CRGNB

CRGNB has intrigued researchers all over the world. Many countries conducted studies regarding the prevalence of CRGNB including: Germany (Heudorf et al., 2016); (Ehrhard et al., 2014), USA (Lodise, Michael, & Zhao, 2017); (Doll et al., 2017), Spain (Miró et al., 2013), China (Hu et al., 2014), Colombia (Vanegas, Parra, & Jiménez, 2016), Nepal (Karn et al., 2017), Uganda (Okoche, Asiimwe, Katabazi, Kato, & Najjuka, 2015), and India (Nair & Vaz, 2013). Reports on the prevalence of CRGNB in the region are limited to a few countries including: Egypt (Khalifa et al., 2017) and Morocco (El Wartiti, Bahmani, Elouennass, & Benouda, 2012).

Regarding carbapenemases, OXA-48 was reported in Spain (Pitart et al., 2011), USA (Mathers et al., 2013), Greece (Voulgari et al., 2012), France (Cuzon, Ouanich, Gondret, Naas, & Nordmann, 2011), The Netherlands (Kalpoe, Al Naiemi, Poirel, & Nordmann, 2011), Japan (Nagano et al., 2013), Belgium (Glupczynski et al., 2012), Senegal (Moquet et al., 2011), Palestine (Chen et al., 2015), Tunisia (Ktari et al., 2011), Morocco (Hays, Benouda, Poirel, Elouennass, & Nordmann, 2012), Saudi Arabia (Shibl et al., 2013), and Lebanon (El-Herte, et al., 2012). blaOXA23 gene has been reported in Brazil (Carvalho et al., 2009), Algeria (Mesli, Berrazeg, Drissi, Bekkhoucha, & Rolain, 2013), Korea (Jeon et al., 2005), Colombia (Villegas et al., 2007), Turkey (Ciftci et al., 2013; Gur, Korten, Unal, Deshpande, & Castanheira, 2008), Ireland (Boo, Walsh, & Crowley, 2006), Egypt (Fouad, Attia, Tawakkol, & Hashem, 2013), Israel (Higgins, Dammhayn, Hackel, & Seifert, 2010), Tunisia (Mansour et al., 2008), and Bahrain (Mugnier, Bindayna, Poirel, & Nordmann, 2009).

NDM-1 was reported from USA (Li, Munoz-Price, Spychala, DePascale, & Doi, 2016), France (Decousser et al., 2013), New Zealand (Williamson et al., 2012), Germany (Pfeifer et al., 2011), Greece (Voulgari et al., 2014), China (Yang et al., 2012), India (Castanheira et al., 2011), Serbia (Jovcic et al., 2011), Spain (Riazzo et

al., 2017), Kenya (Poirel, Revathi, Bernabeu, & Nordmann, 2011), Oman (Poirel, Al Maskari, Al Rashdi, Bernabeu, & Nordmann, 2010) Kuwait (Jamal et al., 2012), United Arab Emirates (Sonnevend et al., 2013), Lebanon (Baroud et al., 2013), Morocco (Poirel, Benouda, Hays, & Nordmann, 2011), Tunisia (Nasr et al., 2013), Algeria (Mesli, et al., 2013) and in Turkey (Cicek et al., 2014). The isolation of an NDM-1-producing *A. baumannii* in a Czech patient who repatriated in 2011 from Egypt was described (Hrabák et al., 2012). NDM-2 was found in *A. baumannii* isolates in Palestine (Sjölander et al., 2014), and Israel (Espinal et al., 2011).

Reports regarding the detection of different *Enterobacteriaceae* producing KPC were reported in USA (Abdallah et al., 2016), Poland (Baraniak et al., 2017) Israel (Marchaim, Navon-Venezia, Schwaber, & Carmeli, 2008), Greece (Bathoorn et al., 2016), Germany (Wendt et al., 2010), China (Wei et al., 2007), Palestine (Adwan, Rabaya, Adwan, & Al-Sheboul, 2016). There had also been a report from Palestine regarding the emergence of *blaKPC-2* (Liddawi et al., 2012).

Reports of VIM and IMP type producers have been reported in Morocco (Barguigua et al., 2015), Egypt (Poirel, Abdelaziz, Bernabeu, & Nordmann, 2013), Algeria (Meradji et al., 2016), Tunisia (Ktari et al., 2006), Turkey (Malkoçoğlu, Aktaş, Bayraktar, Otlu, & Bulut, 2017), and USA (Castanheira et al., 2016). There had been a report from Saudi Arabia of *P. aeruginosa* harboring the *blaVIM-2* from a Saudi patient hospitalized in France (Guerin et al., 2005), and a report on VIM-producing *P. aeruginosa* from Palestine (Sjölander, et al., 2014), Hungary (Libisch et al., 2008), United Kingdome (Wright, Turton, Livermore, Hopkins, & Woodford, 2014), and Egypt (Zafer, Al-Agamy, El-Mahallawy, Amin, & Ashour, 2015).

2.11 Treatment options for CRE

2.11.1 Polymyxins

Polymyxin E (also known as Colistimethate) and Polymyxin B, are cyclic peptides which with a broad Gram-negative spectrum of activity that covers *Enterobacteriaceae* (except *Proteus* spp. and *Serratia* spp.), *P. aeruginosa*, and *A. baumannii* (Falagas, Kasiakou, & Saravolatz, 2005). Polymyxins cause a leakage of the cellular contents and subsequently, lysing the bacterial cells through an

electrostatic interaction between the cationic polypeptide antimicrobial and the anionic lipopolysaccharides of the bacterial outer membrane (Gales, Jones, & Sader, 2011). One of Polymyxins' success record in treating CRE infection was of a patient with CRKP bloodstream infection (Karabinis et al., 2004).

2.11.2 Tigecycline

The FDA approved antibiotic for treating skin infections, complicated intra-abdominal infections, and community-acquired pneumonia, is bacteriostatic antimicrobial agent that was modified to overcome the resistance mechanisms of tetracycline (Van Duin, Kaye, Neuner, & Bonomo, 2013). The majority of CRE isolates remain sensitive to tigecycline *in vitro*. However, resistance to tigecycline is increasing (Daikos, Markogiannakis, Souli, & Tzouvelekis, 2012; Sader, Farrell, Flamm, & Jones, 2014; Walsh, et al., 2011).

2.11.3 Fosfomycin

Fosfomycin has a broad spectrum bactericidal activity against Gram-positive and Gram-negative organisms. It inactivates the bacterial enzyme pyruvul transferase resulting in an inhibition of bacterial cell wall synthesis (Wisher, 2012). Limited data have demonstrated that fosfomycin has activity against KPC producing *K. pneumoniae* and (NDM)-1 producing *Enterobacteriaceae* (Tuon et al., 2013). fosfomycin is available in many European countries, both in oral and intravenous forms (Wisher, 2012).

2.11.4 Aminoglycosides

The aminoglycosides' mode of action is the inhibition of protein synthesis by binding to the small subunit of the ribosome (30S). Gentamicin is generally the most active aminoglycoside against CRKP *in vitro*. Whereas, Amikacin can be most active against other CRE (Gales, Castanheira, Jones, & Sader, 2012; Kumarasamy et al., 2010). There is a new Sisomicin-derived Aminoglycoside known as Plazomicin (ACHN-490) has also showed *in vitro* activity against CRE (Endimiani, Choudhary, & Bonomo, 2009).

2.11.5 Combination therapy

Since using combination therapy for treating CRE infections may decrease mortality rates compared with monotherapy, it is important to take combination therapy into consideration when a CRE is suspected (Petrosillo, Giannella, Lewis, & Viale, 2013; Qureshi et al., 2012; Tumbarello et al., 2012). Using combination therapy can be a double-edged sword. On one hand, it has many benefits such like reducing the initial inappropriate antimicrobial therapy, potential synergistic effects, and suppression of emerging resistance (Petrosillo, et al., 2013). On the other hand, the risk of developing *Clostridium difficile* infection, colonization or infection with other resistant bacteria increases with combination therapy. Besides some other adverse effects such as nephrotoxicity (Petrosillo, et al., 2013). One of the most recent reviews, with data on 889 patients with CRE infections, had revealed a lower mortality rate when combination therapy with 2 or more agents was used *in vitro* (27.4%) [121 of 441] compared with a single *in vitro* active agent (38.7%) [134 of 346] (Tzouvelekis, Markogiannakis, Piperaki, Souli, & Daikos, 2014).

2.12 Laboratory detection of CRGNB

The Kirby-Bauer disk susceptibility test can be used for screening CRGNB. According to CLSI recommendations, very low carbapenems concentrations should be used for CRE screening (CLSI, 2016). That is because the low level of resistance *in vitro* could make some carbapenemases- producing organisms test as susceptible to carbapenems. This mainly happens with Imipenem and Meropenem where CRE may be found to exhibit elevation but not resistance for minimum inhibitory concentrations (MICs) and/or zones of inhibition. MHT is a reliable confirmatory test for carbapenemases production (Anderson et al., 2007) however, NDMs may show negative or weakly positive MHT results making detection more complex (Castanheira, et al., 2011). Three different tests can be used to screen MBLS: the double-disk synergy test, the combination disk test, and the bioMérieux MBL E-test (Galani et al., 2008). PCR play a major role in the identification of the genes that code for all carbapenemases classes. Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS); which relies on changes in mass to charge ratios, can also be used to determine resistance patterns in bacteria. Since the

production of carbapenemases is the most common mechanism of resistance in cabapenem-resistant bacteria, disrupting the structure of β-lactam antibiotics results in a change of the antibiotic mass. Therefore CRGNB can be detectable by MALDI-TOF MS. Interestingly the results can be available in 4-5 hours (Bilecen, Yaman, Ciftci, & Laleli, 2015).

2.13 Prevention the spread of CRGNB

The two essential tasks needed to halt the spread of CRE remain discovering which patients are at highest risk, as well as robust transmission prevention. Even though organism acquisition is most commonly happen by nosocomial spread, there are additional worrisome amplifications. Community acquisition, probably involving publically-available contaminated water sources, can fan the flames of CRE transmission (Walsh, 2010; Walsh, et al., 2011). There is an arsenal of documented methods which can help slow the global spread of these harmful organisms. An integrated management approach is necessary. Complicated infection control strategies require a global effort in order to be effective against CRE. These strategies must include proper hand hygiene and integrated prevention strategies in high-risk environments, like long-term care facilities. Adequate cohort nursing care along with surveys pinpointing outbreaks and active surveillance add to the arrayed, available arsenal necessary to make significant gains fighting the spread of CRE (Lledo et al., 2009; Munoz-Price et al., 2010).

Chapter III

Materials & Methodology

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Materials and Methodology

3.1 Materials

3.1.1 Media

All media used in this research (table 3.1) were purchased from HiMedia, India and were prepared according to manufacturer recommendations.

Table (3.1): Commercial media used in isolation, identification and antimicrobial susceptibility testing

Media	Manufacturer
MacConkey agar	HiMedia-India
Blood agar	HiMedia-India
Brain heart infusion broth	HiMedia-India
Nutrient agar	HiMedia-India
Muller Hinton agar	HiMedia-India
Sabouraud dextrose agar	HiMedia-India
Triple sugar iron agar	HiMedia-India

3.1.2 Reagents and Disposables

Table (3.2): A list of the most common reagents and disposables used

Reagent	Disposable
Indole	Petri dishes (90 mm)
Urease	Plastic sterile loops (10 ul)
Citrate	Sterile cotton swabs
Methyl Red	Sterile cups
Voges-Proskauer	
Oxidase test	
Normal saline	

3.1.3 Equipment

Equipment and tools used to collect air samples, carry out microbiological investigations and to store isolates are listed in table 3.3

Table (3.3): List of equipment utilized in this research

Equipment	Manufacturer
Incubator 37 °C	Heraeus (Germany)
Refrigerator	Sanyo (Italy)
Autoclave	Sturdy (Germany)
Air sampler	FSC- IV (China)

3.2 Methods

3.2.1 Sampling

3.2.1.1 Sample size

A total of 140 clinical isolates, 150 environmental swabs, and 110 air samples were collected from Al-Shifa and The European Gaza hospitals. In addition, 70 clinical isolates were collected from Al-Naser Hospital (Table 3.4). Air samples and environmental swabs were obtained from the following departments: ICUs, Pediatric Intensive Care Unit (PICU), Neonatal Intensive Care Unit (NICU), and surgery departments.

Table (3.4): Number and distribution of collected samples

Hospital	Air samples	Environmental swabs	Clinical isolates
Al-Shifa Hospital	54	82	108
European Gaza Hospital	56	68	32
Al-Naser Hospital	-	-	70
Total	110	150	210

3.2.1.2 Sampling duration

The sampling period started from September 2016 and continued for seven months.

3.2.1.3 Sampling and transportation

Sterile cotton tipped swabs were used to collect the clinical isolates from the hospitals' microbiology labs (Adler et al., 2011). Environmental surfaces including bed rails, bed sheets, tables, door handles, sinks, soaps and floors were sampled using pre-moistened sterile swabs. These swabs were used to swab an area of 3 X 3 cm (Figure 3.1) with the aid of sterile plastic windows (Thom et al., 2012).

The swabs were immediately placed in transport media and along with other samples were sent to the microbiology laboratory of the Islamic University of Gaza, where they were streaked on both MacConkey and Blood Agar plates and incubated for 48 hours at 37°C (Shimose et al., 2016).

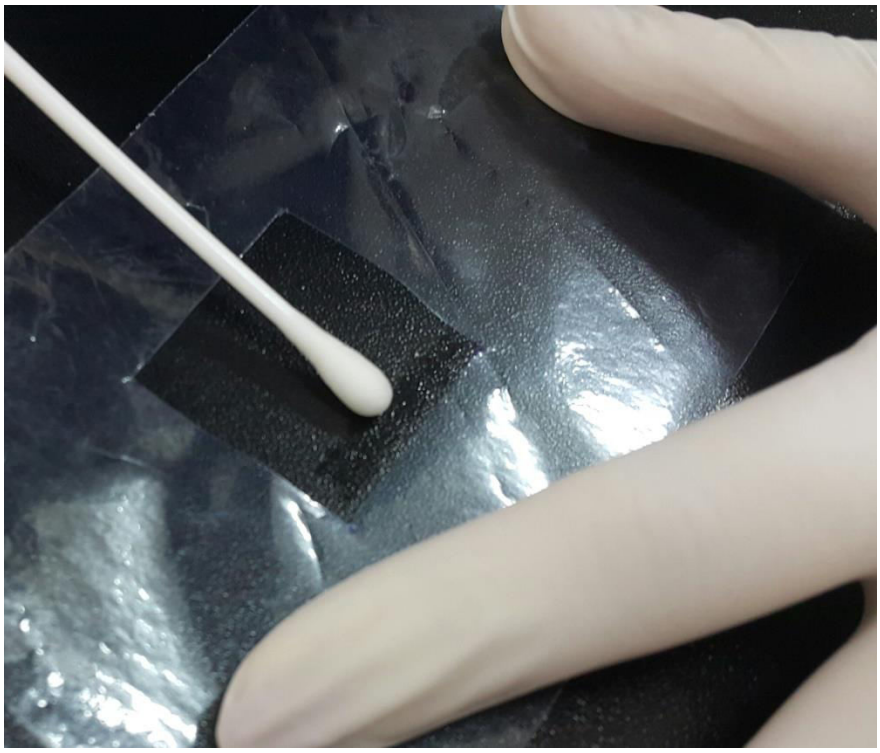

Figure (3.1): Sterile 3x3 plastic window used for sampling surfaces

For air samples collection, 150 liters of air were aspirated for each sample from different sites of the investigated departments using an air sampler (Figure 3.2). The 150 liters were distributed as 50 liters for each culture media that was used.

MacConkey agar plates were used to grow GNB, whereas Nutrient Agar (NA) and Sabouraud Dextrose Agar (SDA) plates were used for total plate count and yeast and molds (fungi) count, respectively. The plates were then transported to the laboratory and incubated for 48 hours at 37°C for bacteria and at 25 ^{o}C for fungi for 7 days (Park, Yeom, Lee, & Lee, 2013).

Figure (3.2): FSC- IV air biological sampler used to sample 50 liters of air

3.2.2 Microbiological investigation

3.2.2.1 Swab cultures

After the incubation period, positive cultures were subcultured onto Blood agar and isolates were identified based on colony color and morphology in addition to conventional biochemical tests (e.g., oxidase, indole, methyl red, Voges-Proskauer, citrate, and urease tests). Ambiguous results were confirmed using API 20 E (bioMérieux).

3.2.2.2 Air samples

After incubation**,** microbial counts were expressed in terms of colony forming units (CFU) per m^3. Gram negative isolates were identified as described in section 3.2.2.1.

3.2.3 Antimicrobial susceptibility testing

The susceptibilities of the isolates to carbapenems and other antibiotics (Table 3.5) were determined using the disc diffusion (modified Kirby-Bauer) method according to the methods and interpretive criteria of the Clinical and Laboratory Standards Institute (CLSI, 2016).

Table(3.5): List of antimicrobial agents used for the tested isolates

Antimicrobials	Abbreviation	Concentration	*Enterobacteriaceae*	*Pseudomonas* spp
Amikacin	AK	30 mcg	X	X
Amoxicillin	AMC	30 mcg	X	
Ampicillin	AMP	10 mcg	X	
Aztreonam	AT	30 mcg	X	X
Ceftazidime	CAZ	30 mcg	X	
Ceftriaxone	CTR	30 mcg		X
Cefuroxime	CXM	30 mcg	X	
Chloramphenicol	C	30 mcg	X	
Ciprofloxacin	CIP	5 mcg	X	X
Etrapenem	ETP	10 mcg	X	
Imipenem	IMI	10 mcg	X	X
Meropenem	MEM	10 mcg	X	X
Gentamicin	GEN	10 mcg	X	X
Piperacillin	PIP	100 mcg	X	X
Tetracycline	TE	30 mcg	X	
Trimethoprim/Sulfonamides	COT	25 mcg	X	

3.2.4 The Multiple Antibiotic Resistance (MAR) index and MDR

The MAR index was calculated for each isolate by dividing the number of antibiotics for which each isolate was resistant by the number of antibiotics for which each isolate was tested. Isolates were determined MDR by their resistance to one or more antibiotics from each of at least three different families.

3.2.5 Modified Hodge Test

Isolates that showed resistance to at least one of the tested carbapenems (Etrapenem, Imipenem, Meropenem) were further investigated for carbapenemases production by MHT according to CLSI guidelines (CLSI, 2016).

A lawn of pre-tested carbapenem-sensitive *E. coli* was streaked to Mueller Hinton agar plates and left for a while to dry. Then a 10 μg carbapenem disc was placed in the center of the test area; Ertapenem disc was used for the isolates that were resistant to Ertapenem, and Imipenem disc was used for the isolates that showed resistance to Imipenem, while Meropenem disc was used for the isolates that were resistant to Meropenem. Test organisms were then streaked from the edge of the disc to the edge of the plate. After incubation, the plates were examined for an inward distortion of zone of inhibition (Clover leaves appearance).

3.3 Permissions and ethical considerations

Prior the initiation of the research work, approval was obtained from the Helsinki Committee (Appendix 1). In addition, formal applications for permissions were submitted to the relevant authorities for collection of samples and access to patients' records (Appendix 2).

3.4 Data analysis

Collected data were summarized, tabulated and analyzed using Statistical Package for Social Sciences (SPSS) software. Chi square test was used to detect significant differences among hospitals and or samples. The results are presented as tables.

Chapter IV

RESULTS

Chapter IV
RESULTS

A total of 140 clinical isolates, 150 environmental swabs, and 110 air samples were collected from Al-Shifa and The European Gaza hospitals. In addition, 70 clinical isolates were collected from Al-Naser hospital. In the following sections, a description of the results is provided.

4.1 Air samples

Out of 110 air samples, only 19 exhibited growth for GNB (17.3%) (Table 4.1). Although the European Gaza Hospital showed the highest positive rate, no statistically significant difference was found.

Table (4.1): The results of air samples by hospital

Hospital	Gram negative bacteria		Total
	Positive	Negative	
Al-Shifa hospital	8	46	54
	14.8%	85.2%	100.0%
European Gaza hospital	11	45	56
	19.6%	80.4	100.0%
Total	19	91	110
	17.3%	82.7%	100.0%
P value	0.33		

An attempt to correlate the presence of GNB and the sampled department is shown in table 4.2. The highest incidence of GNB was in the ICUs, while the surgery departments' air samples showed the least (No statistically significant differences).

Table (4.2): The results of air samples by department

Department	Gram negative bacteria		Total
	Positive	Negative	
ICUs	11	31	42
	26.2%	73.8%	100.0%
NICU	2	12	14
	14.3%	85.7%	100.0%
Surgery	3	35	38
	7.9%	92.1%	100.0%
PICU	3	13	16
	18.8%	81.3%	100.0%
Total	19	91	110
	17.3%	82.7%	100.0%
P value	0.19		

The frequencies of isolation of each identified genus are listed in table 4.3. *Citrobacte*r and *Enterobacter* followed by *Escherichia and Providencia* were the most frequently isolated Gram negative. All isolates belonged to the *Enterobacteriaceae* family except two isolates were identified as *Pseudomonas*.

Table (4.3): Gram negative bacteria isolated from air samples from various departments

Isolate	Frequency	Out of positive samples (19%)	Out of total samples (110%)
Citrobacter spp.	4	21.1	3.6
Enterobacter spp.	4	21.1	3.6
Escherichia spp.	3	15.8	2.7
Providencia spp.	3	15.8	2.7
Pseudomonas spp.	2	10.5	1.8
Serratia spp.	1	5.3	0.9
*Lost isolates	2	10.5	1.8
Total	19	100.0	17.3

*Two isolates failed to grow upon revival from cold storage and therefore, were not identified properly

Air samples were tested for their total bacterial count and fungal count. The results according to the different departments are depicted in table 4.4 and according to

hospitals are presented in table 4.5. The results were categorized as counts lower than 1000 CFU/m^3 and counts higher that 1000 CFU/m^3. Statistically significant differences were found in both categories among the various departments. NICU demonstrated the lowest frequency in both parameters. As shown below, no single sample contained counts higher than 1000 CFU/ m^3.

The surgery departments recorded the highest percentage of samples containing fungi counts higher than 1000. Similar results were found for both ICUs and surgery department with regard to total plate counts. In general, bacterial counts higher than 1000 were more frequent (25.5%) than those of fungi (12.7%). Of the two hospitals, statistically significant differences were found with regard to fungal count, but no statistically significant differences were found regarding total bacterial count.

Table (4.4): Total plate count and fungi count by department

Department	Total plate count CFU/1000 L		Fungi count CFU/1000 L	
	Lower than 1000	Higher than1000	Lower than1000	Higher than1000
ICU	28	14	41	1
	66.7%	33.3%	97.6%	2.4%
NICU	14	0	14	0
	100%	0.0%	100%	0.0%
Surgery	26	12	28	10
	68.4%	31.6%	73.7%	26.3%
PICU	14	2	13	3
	87.5%	12.5%	81.3	18.8%
Total	82	28	96	14
	74.5%	25.5%	87.2%	12.7%
P value	0.04		0.005	

Table (4.5): Total plate count and fungi count by hospital

Hospital	Total plate count		Fungi count		P value
	Lower than 1000	Higher than1000	Lower than1000	Higher than1000	
European	42	14	43	13	0.08
	75%	25%	76.8%	23.2%	
Al-Shifa	40	14	53	1	
	74.1%	25.9%	98.1%	1.9%	
Total	82	28	96	14	
	74.5%	25.5%	87.3%	12.7%	
P value	0.5		0.001		

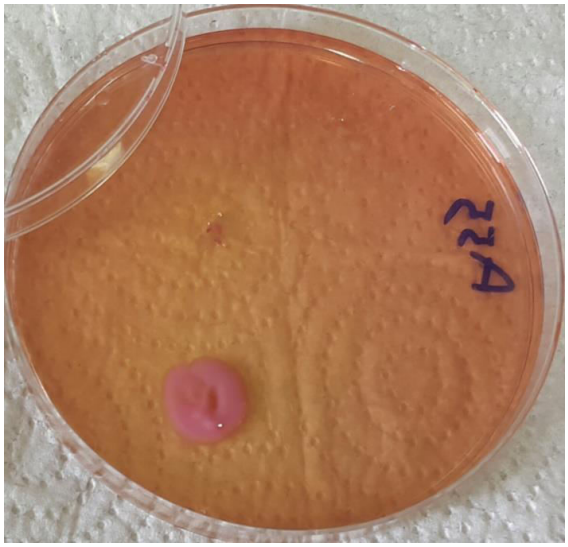

Figure (4.1): Gram-negative bacterial growth on MacConkey from an air sample

In trying to find a correlation between total plate count and isolated Gram-negative positive air samples, no statistically significant differences were found in spite of the high averages of total bacterial counts (Table 4.6). Two other correlations were attempted to be found. One of which was between Gram-negative positive air samples and the season in which the sampling was carried out (Table 4.7), While the other one was between the counts of fungi and season (Table 4.8). Statistically significant differences were found between the two sets in both cases. The greatest percentage (84.2%) of GNB was isolated in fall while the lowest (5.3%) was in winter. Regarding the counts of fungi, levels of fungi were the highest in winter (92.9%) and the lowest in spring (0.0%).

Table (4.6): Relationship between total plate count and Gram negative bacteria

Total plate count	Gram negative bacteria		Total
	Positive	Negative	
Lower than 1000	14 17.1%	68 82.9%	82 100.0%
Higher than 1000	5 17.9%	23 82.1%	28 100.0%
Total	19 17.3%	91 82.7%	110 100.0%
P value	0.5		

Table (4.7): The results of air samples by season

Gram negative bacteria	Season			Total
	Fall	Winter	Spring	
Positive	16 84.2%	1 5.3%	2 10.5%	19 100.0%
Negative	43 47.3%	36 39.6%	12 13.2%	91 100.0%
Total	59 53.6%	37 33.6%	14 12.7%	110 100.0%
P value	0.008			

Table (4.8): Fungi count by season

Fungi count	Season			Total
	Fall	Winter	Spring	
Lower than 1000	58 60.4%	24 25.0%	14 14.6%	96 100.0%
Higher than 1000	1 7.1%	13 92.9%	0 0.0%	14 100.0%
Total	59 53.6%	37 33.6%	14 12.7%	110 100.0%
P value	<0.001			

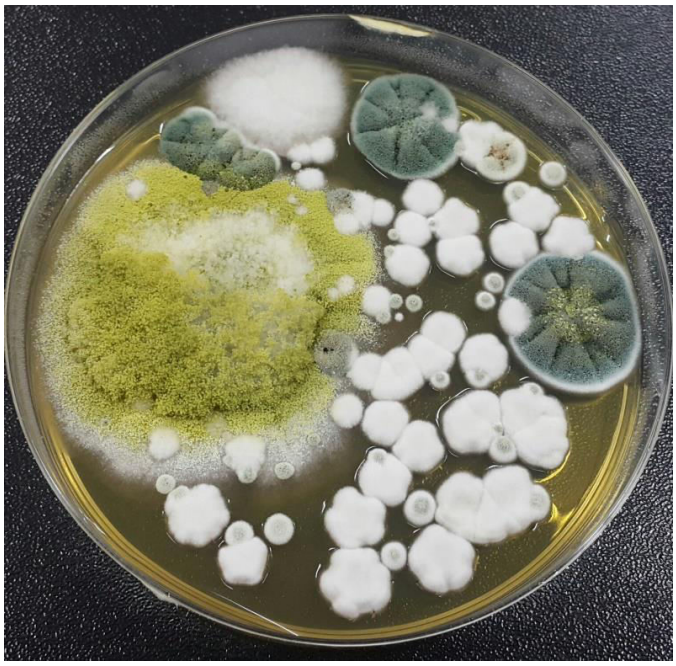

Figure (4.2): Fungal growth from an air sample

4.2 Environmental swabs

A total of 21 out of 150 (14%) environmental swabs were positive for GNB with a significant difference between the two hospitals (Table 4.9) as well as between the departments (Table 4.10). With respect to hospitals, the European hospital had the highest positivity rate (22.1%). Regarding departments, PICU exhibited the highest positivity rate (40.9%).

Table (4.9): The results of environmental swabs according to hospital.

Hospital	Gram negative bacteria		Total
	Positive	Negative	
Al-Shifa	6	76	82
	7.3%	92.7%	100.0%
European Gaza	15	53	68
	22.1%	77.9%	100.0%
Total	21	129	150
	14.0%	86.0%	100.0%
P value	0.009		

Table (4.10): The results of environmental swabs according to department

Department	Gram negative bacteria		Total
	Positive	Negative	
ICUs	6	52	58
	10.3%	89.7%	100.0%
Surgery	5	53	58
	8.6%	91.4%	100.0%
NICU	1	11	12
	8.3%	91.7%	100.0%
PICU	9	13	22
	40.9%	59.1%	100.0%
Total	21	129	150
	14.0%	86.0%	100.0%
P value	0.001		

The frequencies of isolation of each identified genus are listed in table 4.11. *Klebsiella* spp. Followed by *E. coli* were the most isolated bacteria. All isolates belonged to the *Enterobacteriaceae* family.

Table (4.11): Gram negative bacteria isolated from environmental swabs from various departments

Isolate	Frequency	Out of positive samples (21%)	Out of total samples (150%)
Klebsiella spp.	11	52.4	7.3
Escherichia spp.	7	33.3	4.7
Providencia spp.	1	4.8	.7
Citrobacter spp.	1	4.8	.7
Lost Isolates	1	4.8	.7
Total	21	100	14.1

*One isolate failed to grow upon revival from cold storage and therefore, was not identified properly

In trying to correlate between presence of GNB and the sampling time as shown in table 4.12, the highest incidence of GNB was in the morning, while the lowest was at noontime. Although the morning collections yielded higher total GNB isolates counts, the differences were not significant.

Table (4.12): The results of environmental swab according to sampling time.

Gram negative bacteria	Sampling time		Total
	Morning	Noontime	
Positive	15 18.5%	6 8.7%	21 14%
Negative	66 81.5%	63 91.3%	129 86%
Total	81 100%	69 100%	150 100.0%
P value	0.06		

The distribution of the different types of bacteria obtained from air samples, environmental swabs and clinical isolates is presented below in table 4.13.

Table (4.13): Different types of Gram negative bacteria isolated from air samples, environmental swabs and clinical isolates

Isolate	Type of isolate			Total
	Air isolates	Environmental swabs isolates	Clinical isolates	
Escherichia spp.	3	7	81	91
	17.6%	35.0%	38.6%	36.8%
Klebsiella spp.	0	11	79	90
	0.0%	55.0%	37.6%	36.4%
Enterobacter spp.	4	0	12	16
	23.5%	0.0%	5.7%	6.5%
Citrobacter spp.	4	1	13	18
	23.5%	5.0%	6.2%	7.3%
Serratia spp.	1	0	0	1
	5.9%	0.0%	0.0%	0.4%
Providencia spp.	3	1	0	4
	17.6%	5.0%	0.0%	1.6%
Proteus spp.	0	0	5	5
	0.0%	0.0%	2.4%	2.0%
Shigella spp.	0	0	1	1
	0.0%	0.0%	0.5%	0.4%
Pseudomonas spp.	2	0	19	21
	11.8%	0.0%	9.0%	8.5%
Total	17	20	210	247 100%

4.3 Antimicrobial susceptibility testing

The isolates showed 100% resistance to Ampicillin, Amoxicillin, and Aztreonam. Resistance to Chloramphenicol was 42%, Trimethoprim/Sulfonamides, 88.5%, Cefuroxime, 96.9%, Gentamicin, 0.8%, Amikacin, 1.3%, Tetracycline, 62.4%, Piperacillin, 99.1%, Ceftriaxone, 47.6%, Ciprofloxacin, 15.5%, and Ceftazidime, 42.5%. Antibiotic resistance profiles of the isolated organisms are presented in table 4.17.

The overall percentage of CR among GNB was (30/247) 12.1%. CR among *Enterobacteriaceae* was (30/226) 13.2% and 0% in *Pseudomonas*. With respect to CR among hospitals, Al-Naser Hospital had the highest resistance rate (17.1%), followed

by European Gaza Hospital (12.9%), while that of Al-Shifa Hospital was 8.6% (Table 4.14). Resistance to Imipenem, Ertapenem and Meropenem is presented in table 4.15. CR among air samples, environmental swabs and clinical isolate is illustrated in table 4.16. CR in *Klebsiella* spp. was (13/90) 14.4% and in *E. coli* (9/91) 9.8%, while in other *Enterobacteriaceae,* it was (8/28) 28.5%. The ICUs exhibited the highest CR rate, 9/17 (52.9%), followed by surgery departments, 3/8 (37.5%), and PICU, 4/12 (33.3%). Outpatient clinics had a rate of 3/49 (6.1%), while other departments had a CR percentage of 11/19 (57.8%).

Table (4.14): Carbapenem resistance among hospitals

Hospital	Frequency	Percent
Al-Naser (N=70)	12	17.1%
European Gaza (N=62)	8	12.9%
Al-Shifa (N=115)	10	8.6%

Table (4.15): Antimicrobial Susceptibility Testing of carbapenems

	Imipenem N=247		Ertapenem N=226		Meropenem N=247	
	Frequency	Percent	Frequency	Percent	Frequency	Percent
Sensitive	153	61.7	165	66.5	218	87.9
Intermediate	74	29.8	53	21.4	27	10.9
Resistant	20	**8.1%**	8	**3.5%**	2	**.8%**

Table (4.16): Resistance to carbapenems by source of isolate

Carbapenems		Air N= 17			Environmental swabs N= 20			Clinical N=210			P value
		S	I	R	S	I	R	S	I	R	
Ertapenem	N	6	7	2	7	12	1	152	34	5	<0.001
	%	40.0	46.7	13.3	35.0	60.0	5.0	79.6	17.8	2.6	
Imipenem	N	4	8	5	4	12	4	145	54	11	<0.001
	%	23.5	47.1	29.4	20.0	60.0	20.0	69.0	25.7	5.2	
Meropenem	N	9	7	1	17	3	0	192	17	1	<0.001
	%	52.9	41.2	5.9	85.0	15.0	0.0	91.4	8.1	0.5	

4.4 The Multiple Antibiotic Resistance (MAR) index and MDR

MAR index was calculated for each isolate by dividing the number of antibiotics for which each isolate was resistant by the number of antibiotics for which each isolate was tested. All *Enterobacteriaceae* isolates had a MAR index higher than 0.2, while those of *Pseudomonas* had an average of 02. All *Enterobacteriaceae* isolates were 100% MDR, while those of *Pseudomonas* were 47.6%. (Table 4.17).

Table (4.17): Antibiotic resistance profiles of the isolated organisms

Antimicrobial	*Escherichia* spp.	*Klebsiella* spp.	*Enterobacter* spp.	*Citrobacter* spp.	Other E*	*Pseudomonas* spp.
N	91	90	16	18	11	21
	Resistance %					
Ceftazidime	44	40	43.8	33.3	64	NT
Ciprofloxacin	19.8	14.4	6.3	0	45.4	0
Piperacillin	100	100	93.8	100	9	100
Tetracycline	67	62.2	56.3	50	36.3	NT
Ampicillin	100	100	100	100	100	NT
Amoxicillin	100	100	100	100	100	NT
Trimethoprim/Sulfonamides	92.3	90	87.5	72.2	27.2	NT
Azteronam	100	100	100	100	100	100
Cefuroxime	93.4	98.9	100	100	100	NT
Gentamycin	0	2.2	0	0	0	0
Amikacin	1.1	2.2	0	0	0	0
Ceftriaxone	NT	NT	NT	NT	NT	47.6
	MAR index and MDR %					
MAR index	0.6	0.5	0.5	0.5	0.6	0.2
MDR %	100	100	100	100	100	47.6

E*= *Enterobacteriacae, NT=Not tested.*

4.5 Modified Hodge Test

Among 30 isolates that were resistant to at least one of the tested carbapenems, seven were positive (23.3%) for MHT. Inward distortion of zone of inhibition was an indicator of carbapenamases production. (Figure 4.3). Out of 7 MHT positive isolates, the frequency of *Klebsiella* spp. was 57.1% and of *Citrobacter* spp. 42.8%.

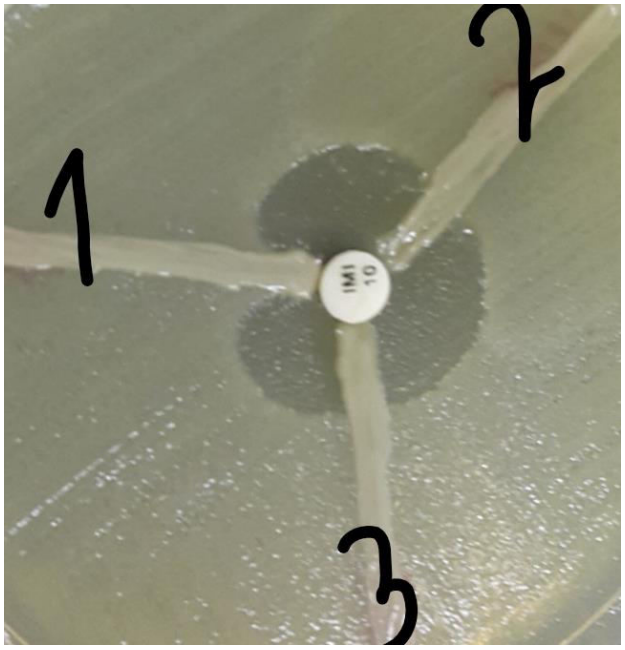

Figure (4.3): Modified Hodge Test. Isolate number 1 is positive whereas isolates number 2 and 3 are negative.

Chapter V

Discussion

Chapter V
Discussion

The overall percentage of CR among GNB was 12.1%. This is comparable to the prevalence rate (13.8%) obtained from Germany (Heudorf, et al., 2016), a little higher than that of Jordan, 5.6% (Wadi, Haloub, Al Ahmad, Samara, & Romman, 2011), Nepal 7.4% (Karn, et al., 2017) and Colombia 8.8% Colombia (Vanegas, et al., 2016), but much lower than that obtained from Egypt, 50.8% (Khalifa, et al., 2017). Our study found a CRE rate of 13.2% which is close to that of India, 12.26% (Nair & Vaz, 2013) and a little lower than that of Uganda, 22.4% (Okoche, et al., 2015). However, other studies have reported lower rates compared to Gaza. A rate of 1.2% was observed in Germany (Ehrhard, et al., 2014), 2.8% in Morocco (El Wartiti, et al., 2012) , 1% in China (Hu, et al., 2014), 2.3% (Lodise, et al., 2017) and 0.3% (Doll, et al., 2017) in USA, and 0.04% in Spain (Miró, et al., 2013).

These proportional variances could be attributed to the restrictions imposed on antibiotic use and the time each country started using carbapenems. Antimicrobial therapeutic protocols and practices vary from one hospital/city/country to another making comparisons and interpretations of prevalence of CRE variations a difficult task. Sample size, sample sources, time the study took place and other factors may contribute to variable prevalence rates.

Our finding that ICUs exhibited the highest CR rate, 9/17 (52.9%), comes in accordance with the outcome of a Chinese study done by (Xu et al., 2016) and an American study conducted by (Brennan, Balke, Coyle, & Mollon, 2017). This might be due to the weak health conditions of hospitalized patients in ICUs and their need for intensive use of antibiotics. However, outpatient clinics also showed a considerable CR rate, 3/49 (6.1%) which indicates that CR is not limited to hospitals but can also be acquired from the community. Thus, infection control measures should be established not only for hospitals but should be promoted also in communities.

Klebsiella spp. was found to be the most resistant to Carbapenems (14.4%), followed by *E. coli* (9.8%). This is similar to the finding of a Chinese study done by (Zhang, Chan, Zhou, & Chen, 2017), but divergent from the outcome of a different Chinese study (Xu, et al., 2016) where *P. aeruginosa* and *A. baumannii* had the highest CR rates. This could be attributed to different antimicrobial treatment protocols for the aforementioned bacteria.

In this study, seven isolates (23.3%) were positive for MHT. A higher rate (47.4%) was documented in Colombia Colombia (Vanegas, et al., 2016). In addition, very much higher rates were documented in Pakistan 69% (Amjad et al., 2011), USA 76% (Doll, et al., 2017), and India 62.5% (Soni, Bansal, Subhedar, Nayak, & Sharma, 2016). However, a Moroccan study conducted by (El Wartiti, et al., 2012) revealed a lower rate of 2.8% for carbapenemases producing *Enterobacteriaceae*. This could mean that the MHT-negative isolates harbor different mechanisms for CR other than the production of carbapenemases (e.g., efflux pump or altered porins).

In the present study, the average levels of bacteria obtained from air samples were (7.8×10^2 CFU/m^3) and of fungi (5.2×10^2 CFU/m^3). Our investigation showed a total bacterial load exceeding 7.5×10^2 CFU/m^3 which, according to a work conducted by (de Aquino Neto & de Góes Siqueira, 2000) is considered contaminated. The study also exhibited a total fungal load exceeding 3×10^2 CFU/m^3 which, according to (Cappitelli et al., 2009) is considered contaminated. This finding emphasizes the need for regular indoor air quality assessment. A study done in Thailand by (Apisarnthanarak, Tantajina, Laovachirasuwan, Weber, & Singh, 2016) revealed similar average levels of bacteria (7.8×10^2 CFU/m^3). A Korean study conducted by (Park, et al., 2013) reported comparable averages of bacteria (7.2×10^2 CFU/m^3) and fungi (5.5×10^2 CFU/ m^3). A study from Poland found lower averages of 2.5×10^2 – 4.4×10^2 CFU/m^3 for airborne bacteria (Augustowska & Dutkiewicz, 2006). While higher averages of (2.4×10^3 CFU/m^3) for airborne bacteria were reported in Iran (Shamsizadeh et al., 2017). A study conducted in Canada (Gilbert, Veillette, & Duchaine, 2010) showed a range of 1.4×10^1 - 7.4×10^1 CFU/m^3 for airborne bacteria and 5×10^1 - 6×10^2 CFU/m^3 for fungi.

Our study revealed that levels of fungi were the highest in winter (P<0.001) which is in accordance with a Turkish study (Suerdem & Yildirim, 2009) and an Indian study (Reddy, Sarita, & Srinivas, 2015). This is possibly due to the effects of temperature and humidity.

GNB was isolated from 17.2% of air samples. A lower rate (3.05%) was documented by (Okten & Asan, 2012) in a Turkish study concerning indoor and outdoor airborne fungi and bacteria. A higher rate (56.9%) was found in an Ethiopian study regarding identification, characterization and Antibiotic Susceptibility of indoor airborne bacteria (Leta, Aragaw, & Merid, 2014). In our study, ICUs exhibited the highest positivity rate. Crowded conditions, lack of ultraviolet light, and insufficient ventilation may be contributing factors. The greatest percentage (84.2%) of GNB was isolated in fall (P=0.008) while the lowest was in winter. Since most diseases peak and are likely to spread in summer and fall, seasons might have contributed to this variation.

Citrobacter and Enterobacter spp. were the most isolated bacteria. This is in contrast to the finding of a Sudanese study conducted by (Yagoub & Agbash, 2010) and an Indian study done by (Tambekar, Gulhane, & Bhokare, 2007) where *P. aeruginosa* was the most frequently isolated bacteria. No significant differences were found between the average of airborne bacteria and fungi (P=0.08), nor between airborne bacteria and GNB (P=0.5). CR among GNB isolated from air samples was high. A very alarming finding that should be taken seriously. These CRE pathogens would likely be the cause of future infections.

With respect to bacteria isolated from environmental swabs, 47.3% (71/150) were culture positive. That is lower than the finding (57.4 %) of an Iranian study (Ekrami, Kayedani, Jahangir, & Kalantar, 2011), and (96.2%) of a Moroccan study (Lalami et al., 2016). GNB accounted for 29.6% of the total positive cultures. In a Moroccan study regarding microbiological monitoring of environment surfaces in a hospital (Lalami, et al., 2016), the level of isolated GNB was 73.33%. A much lower rate of (4.9%) was documented in a German study conducted by (Lemmen, Häfner, Zolldann, Stanzel, & Lütticken, 2004). In the present study, 18.5% of GNB were recovered from samples collected in the morning, whereas 8.7% were from samples

collected at noontime. This finding is supported by that of (Lerner et al., 2013), giving an assumption that time period amongst cleaning and testing is a contributing factor, hence emphasizes the importance of regular cleaning.

In our study, *Klebsiella* spp. was the most isolated bacteria in contrast to the finding of (Lalami, et al., 2016) where *Aeromonas salmonicida* was the most predominant isolated bacteria. In our study, PICU had the highest positivity rate. A study conducted in Mexico by (Garcia-Cruz, Najera-Aguilar, & Arroyo-Helguera, 2012) revealed that the emergency department had the highest positivity rate. CR among GNB isolated from environmental swabs was 25%. This is comparable to the finding (24%) of (Lerner, et al., 2013). These findings about contaminated surfaces with CR bacteria rendering increasingly difficult-to-treat nosocomial infections. Since these surfaces serve as cross-transmission reservoirs of infections, disinfectants, such as bleach, should be checked for quality and strength.

Chapter VI

Conclusions & Recommendations

Chapter VI
Conclusions and Recommendations

6.1 Conclusions

This study provided some insight into CR among clinical and environmental GNB isolates that were obtained from three of Gaza strip hospitals as well as microbial assessment of indoor air quality and the environments of hospitals. The following conclusions were drawn from the results of this study:

1. The overall percentage of CR among GNB was 12.1%. CR among *Enterobacteriaceae* was 13.2% while in *Pseudomonas* it was 0%.
2. Al-Naser Hospital had the highest resistance rate (17.1%), followed by European Hospital (12.9%), while that of Al-Shifa Hospital was 8.6%. The ICUs exhibited the highest resistance rate (52.9%).
3. *Klebsiella* spp. was the most resistant to Carbapenems (14.4%), followed by *E. coli* (9.8%).
4. Seven CR isolates (23.3%) exhibited positive results for MHT (Carbapenemase producers).
5. All isolates had a MAR index higher than 0.2.
6. The average level of bacteria obtained from air samples was (7.8×10^2 CFU/m^3) and of fungi (5.2×10^2 CFU/m^3). Levels of fungi were the highest during winter.
7. GNB was isolated from 17.2% of air samples; 19.6% from the European Hospital and in 14.8% from Al-Shifa Hospital. ICUs exhibited the highest positivity rate (26.2%).
8. The greatest percentage (84.2%) of GNB was isolated in fall while the lowest was in winter. *Citrobacter and Enterobacter* spp. were the most frequently isolated bacteria
9. About 47% of the environmental swabs were culture positive.
10. GNB accounted for 29.6% of the total positive cultures; 22.1% from the European Hospital and 7.3% from Al-Shifa Hospital. PICU showed the highest positivity rate (40.9%), *Klebsiella* spp. was the most commonly isolated bacteria.

11. Higher percentage (18.5%) of GNB was recovered from samples collected in the morning than samples collected at noontime (8.7%).

6.2 Recommendations

In consideration of the aforementioned conclusions, the following recommendations are suggested:

1. Further studies concerning CR need to be conducted in Gaza to include larger number of samples and wider study area.
2. Setting local standards for the air quality in hospitals and even for specific departments/wards.
3. Screening for resistance and antimicrobial susceptibility testing need to be performed for this class of antibiotics on a routine basis.
4. Urgent policies are required to prevent misuse and overuse of Carbapenems.
5. In light of the excellent results of Gentamicin and Amikacin, we recommend reconsidering the current therapeutic protocols.
6. Infection control measures should be established not only in hospitals but should be promoted also in communities.
7. Standard precautions and transmission-based precautions should be considered to prevent the transmission of antibiotic resistant infections.
8. Regular indoor air quality assessment and environmental screening of hospitals are highly needed.
9. Ultraviolet light and sufficient ventilation need to be checked in closed departments like ICUs.
10. The effectiveness of routinely used disinfectants needs to be evaluated regularly.
11. Hydrogen peroxide vapor (HPV) should be considered as a cleaning routine for rooms after discharging patients.
12. Appropriate air filtration systems should be used and maintained within hospitals.

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Appendix 1

المجلس الفلسطيني للبحث الصحي

Palestinian Health Research Council

تعزيز النظام الصحي الفلسطيني من خلال مأسسة استخدام المعلومات البحثية في صنع القرار

Developing the Palestinian health system through institutionalizing the use of information in decision making

Helsinki Committee

For Ethical Approval

Date: 01/08/2016

Number: PHRC/HC/138/16

Name: RAWAN H. RAIDAH

الاسم: روان ريدة

We would like to inform you that the committee had discussed the proposal of your study about:

نفيدكم علماً بأن اللجنة قد ناقشت مقترح دراستكم حول:

Carbapenems Resistance among Gram Negative Isolates from the ICUs of Gaza Strip Hospitals

The committee has decided to approve the above mentioned research. Approval number PHRC/HC/138/16 in its meeting on 01/08/2016

و قد قررت الموافقة على البحث المذكور عاليه بالرقم والتاريخ المذكوران عاليه

Signature

Member

Member

Chairman

Genral Conditions:-

1. Valid for 2 years from the date of approval.
2. It is necessary to notify the committee of any change in the approved study protocol.
3. The committee appreciates receiving a copy of your final research when completed.

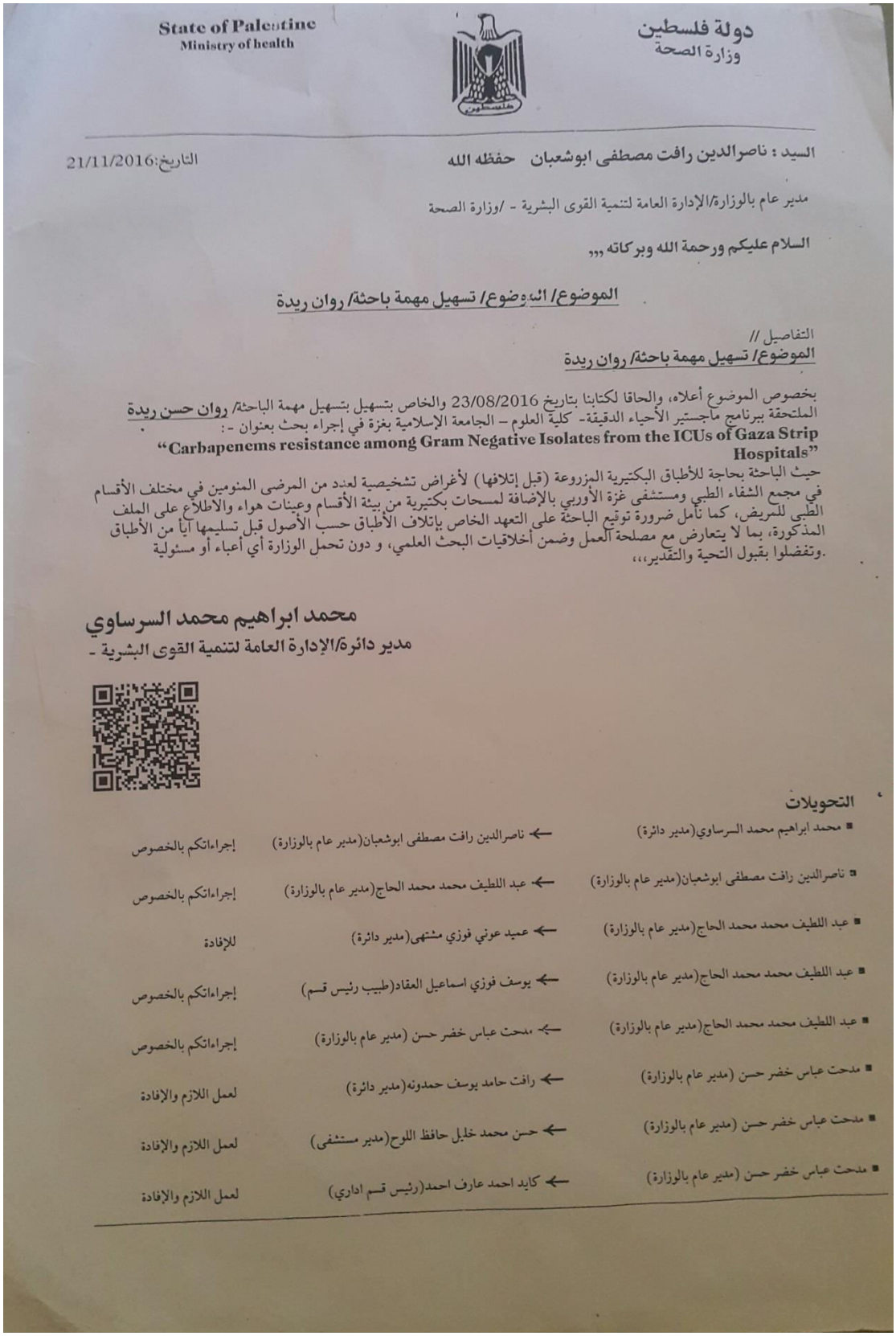
Specific Conditions:-

E-Mail:pal.phrc@gmail.com

Gaza - Palestine غزة - فلسطين

شارع النصر - مفترق العيون

Appendix 2



State of Palestine
Ministry of health

دولة فلسطين
وزارة الصحة

السيد : ناصرالدين رافت مصطفى ابوشعبان حفظه الله

التاريخ:21/11/2016

مدير عام بالوزارة/الإدارة العامة لتنمية القوى البشرية - /وزارة الصحة

السلام عليكم ورحمة الله وبركاته ,,,

الموضوع/ الموضوع/ تسهيل مهمة باحثة/ روان ريدة

التفاصيل //
الموضوع/ تسهيل مهمة باحثة/ روان ريدة

بخصوص الموضوع أعلاه، والحاقا لكتابنا بتاريخ 23/08/2016 والخاص بتسهيل بتسهيل مهمة الباحثة/ روان حسن ريدة الملتحقة ببرنامج ماجستير الأحياء الدقيقة- كلية العلوم – الجامعة الإسلامية بغزة في إجراء بحث بعنوان -:
"Carbapenems resistance among Gram Negative Isolates from the ICUs of Gaza Strip Hospitals"
حيث الباحثة بحاجة للأطباق البكتيرية المزروعة (قبل إتلافها) لأغراض تشخيصية لعدد من المرضى المنومين في مختلف الأقسام في مجمع الشفاء الطبي ومستشفى غزة الأوربي بالإضافة لمسحات بكتيرية من بيئة الأقسام وعينات هواء والاطلاع على الملف الطبي للمريض، كما نأمل ضرورة توقيع الباحثة على التعهد الخاص بإتلاف الأطباق حسب الأصول قبل تسليمها أيا من الأطباق المذكورة، بما لا يتعارض مع مصلحة العمل وضمن أخلاقيات البحث العلمي، و دون تحمل الوزارة أي أعباء أو مسئولية .وتفضلوا بقبول التحية والتقدير،،،

محمد ابراهيم محمد السرساوي
مدير دائرة/الإدارة العامة لتنمية القوى البشرية -

التحويلات

■ محمد ابراهيم محمد السرساوي(مدير دائرة)	← ناصرالدين رافت مصطفى ابوشعبان(مدير عام بالوزارة)	إجراءاتكم بالخصوص
■ ناصرالدين رافت مصطفى ابوشعبان(مدير عام بالوزارة)	← عبد اللطيف محمد محمد الحاج(مدير عام بالوزارة)	إجراءاتكم بالخصوص
■ عبد اللطيف محمد محمد الحاج(مدير عام بالوزارة)	← عميد عوني فوزي مشتهى(مدير دائرة)	للإفادة
■ عبد اللطيف محمد محمد الحاج(مدير عام بالوزارة)	← يوسف فوزي اسماعيل العقاد(طبيب رئيس قسم)	إجراءاتكم بالخصوص
■ عبد اللطيف محمد محمد الحاج(مدير عام بالوزارة)	← مدحت عباس خضر حسن (مدير عام بالوزارة)	إجراءاتكم بالخصوص
■ مدحت عباس خضر حسن (مدير عام بالوزارة)	← رافت حامد يوسف حمدونه(مدير دائرة)	لعمل اللازم والإفادة
■ مدحت عباس خضر حسن (مدير عام بالوزارة)	← حسن محمد خليل حافظ اللوح(مدير مستشفى)	لعمل اللازم والإفادة
■ مدحت عباس خضر حسن (مدير عام بالوزارة)	← كايد احمد عارف احمد(رئيس قسم اداري)	لعمل اللازم والإفادة