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Arab Academy for Science, Technology & Maritime Transport

AASTMT RESEARCH 2020 CALL FOR COLLABORATION RESEARCH AND INNOVATION PROJECT

Co-existence of Antibiotic Resistance and Virulence Factors in Carbapenem Resistant Gram Negative Clinical Isolates From Alexandria, Egypt.

Proposal's Theme: Medical (Life) Sciences.

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Abu-Qir – El Alamein

Table of content:

- **The actual proposal starts at page "3" and ends at page "32".**

Proposal Details

Title: Co-existence of Antibiotic Resistance and Virulence Factors in Carbapenem Resistant Gram Negative Clinical Isolates From Alexandria, Egypt.

Short Title or Acronym: MDR-GNB

Keywords: Gram negative bacteria, Carbapenem resistance, Carbapenemases, Virulence factors, Phenotypic detection, Molecular detection, Genotyping.

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Proposal Summary: English and Arabic

The emergence of multidrug resistance (MDR) Gram negative pathogens such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumoniae*, represents a global serious problem in health care settings and plays a major role in hospital-acquired infections. These pathogens showed low susceptibility to different antimicrobial agents and they are among the most difficult pathogens to treat. Therefore, infections with such pathogens are frequently associated with high morbidity and mortality rates.

The pathogenicity of bacteria is multifactorial and it depends partially on the nature of the bacterial species or strain i.e. each pathogen has its own virulence factors. For example, the virulence factors of *Klebsiella pneumoniae* include LPS, capsule, urease, adhesins and outer-membrane proteins while the virulence factors of *Acinetobacter baumannii* includes surface motility, hemolysis on blood agars, siderophore production, efflux pumps, porins and exoprotease activity. In addition to biofilm formation, which is one of the most important virulence factors associated with different clinical isolated, the pathogenicity of *Pseudomonas aeruginosa* is mediated by many virulence factors e.g. hemolysin, phospholipase, gelatinase, DNAse, exotoxin A, protease and siderophore.

Carbapenems represent the main therapy for treatment of infections caused by such pathogens. Unfortunately, a drastic increase of carbapenem resistant Gram negative isolates has been recorded in the recent years worldwide.

The relationship between the carbapenem resistance among different MDR Gram negative pathogens and the production of different virulence factors is still not well understood and the published data are relatively low especially from Egypt.

The aim of the present study is to investigate the co-existence and interplay between carbapenem resistance and virulence factors in Gram negative clinical isolates especially *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumoniae*, collected from Alexandria.

In the current study, multidrug-resistant *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Klebsiella pneumoniae* isolates will be collected from different clinical specimens. The isolates will be identified by conventional and non-conventional microbiological methods. The carbapenem resistance and virulence factors of tested isolates will be characterized phenotypically and genetically. Moreover, the correlation between the resistance to carbapenem and virulence factors production among tested clinical isolates will be assessed.

ملخص الاقتراح: باللغتين الإنجليزية والعربية

يمثل ظهور البكتيريا المرضية سالبة الجرام المتعددة المقاومة للمضادات الحيوية (مثل السودومونس ايروجينوزا والاسينيتوباكتربومنياى والكليبسيلا نيومونيا) مشكلة عالمية خطيرة في مراكز الرعاية الصحية وتلعب دورًا رئيسيًا في العدوى المكتسبة من المستشفيات. وقد أظهرت هذه البكتيريا حساسية منخفضة لمضادات الميكروبات المختلفة وتعتبر من بين أكثر البكتيريا صعوبة في العلاج. ولذلك فان العدوى بهذه البكتيريا تكون مرتبطة غالبا بارتفاع معدلات المراضة (الاعتلال) والوفيات.

ان قدرة البكتيريا على احداث المرض تتأثر بالعديد من العوامل وتعتمد جزئيًا على نوع البكتيريا المسببة للعدوى ، أي أن لكل بكتيريا مرضية عوامل الضراوة الخاصة بها. فعلى سبيل المثال تشتمل عوامل الفوعة للكليبسيلا نيومونيا على متعدد السكريات الدهني والكبسولة وانزيم اليوريازوالمواد اللاصقة وبروتينات الغشاء الخارجي بينما تشمل عوامل الضراوة للاسينيتوباكتربومنياي على الحركة السطحية وانحلال الدم على أجار الدم وإنتاج حاملات الحديد ومضخات التدفق والبورينات ونشاط البروتياز الخارجى بالإضافة إلى تكوين الأغشية الحيوية والذي هو أحد أهم عوامل الضراوة المرتبطة بالعزلات السريرية المختلفة. بالاضافة الى ذلك فإن قدرة السودومونس ايروجينوزا على احداث المرض تعتمد على انتاج كل من الهيموليسين والفوسفوليباز والجيلاتيناز والانزيم المحلل للحمض النووي والسموم الخارجية أ وانزيم البروتياز وحاملات الحديد.

تمثل مجموعة الكاربابينيم العلاج الرئيسي للالتهابات التي تسببها البكتيريا المرضية. ولكن لسوء الحظ فقد تم في الاونة الأخيرة تسجيل زيادة كبيرة في العزلات السالبة الجرام المقاومة للكاربابينيم في جميع أنحاء العالم. ان العلاقة بين مقاومة الكاربابينيم وإنتاج عوامل الضراوة المختلفة في البكتيريا سالبة الجرام متعددة المقاومة للمضادات الحيوية لازالت غير مفهومة جيدًا والبيانات المنشورة منخفضة نسبيًا خاصة من مصر.

ان الهدف من هذه الدراسة هو دراسة التعايش والتفاعل بين مقاومة الكاربابينيم وعوامل الضراوة في العزلات السريرية سالبة الجرام وخاصة السودوموناس ايروجينوزا والاسينيتوباكتربومنياي والكليبسيلا نيومونيا والتي سوف تجمع من الإسكندرية. سيتم في هذه الدراسة جمع عزلات سريرية متعددة المقاومة للمضادات الحيوية لكل من السودوموناس ايروجينوزا والاسينيتوباكتربومنياي والكليبسيلا نيومونيا وسيتم التعرف على هذه العزلات بالطرق الميكروبيولوجية التقليدية وغير التقليدية. كما سيتم توصيف مقاومة الكاربابينيم وعوامل الضراوة في العزلات المختبرة من الناحية المظهرية والوراثية. علاوة على ذلك سيتم تقييم العلاقة بين مقاومة الكاربابينيم وإنتاج عوامل الضراوة بين العزلات السريرية المختبرة.

Introduction/Background

Antibiotics play a vital role in controlling and treating different bacterial infections through using different mechanisms against various targets in bacteria to prevent their pathogenesis. Consequently, bacteria are endlessly using methods to overcome the activity of the antibiotics by using distinct types of mechanisms and hence the challenge of antibiotic resistance commenced.(1)

As the global antibiotic consumption increased by 65% between 2000 and 2015 world-wide, bacterial resistance towards antibiotic is considered a major public health problem. Widespread excessive dispensing and irresponsible use of antibiotics has resulted in the development of resistant strains with reduced available therapeutic options, causing profound consequences leading to high rates of morbidity and mortality, or clinical complications.(2-4)

Unfortunately, most antibiotics are available over the counter in the developing countries and can be dispensed without prescription; therefore, patients and general public education are crucially needed.(5) Antibiotic-resistant bacteria are estimated to cause 33,000 deaths in Europe and 700,000 in the world each year. In the worst-case scenario, by 2050, 10 million people in the world will die every year from bacterial infections, exceeding 1.8 million deaths from cancer.(6)

In 2017, the World Health Organization (WHO) has published a list of antibiotic-resistant priority pathogens, which present a great threat to humans and to which new antibiotics are urgently needed. The list is classified according to the urgency of the need for new antibiotics as critical, high, and medium priority. The majority of the WHO list is Gram-negative bacteria due to their distinctive structure. Gram-negative bacteria are more resistant than Gram-positive bacteria, and can cause significant morbidity and mortality worldwide. Bacteria have demonstrated a diverse set of mechanisms for degrading antibiotics, modifying the antibiotic target site, or modulating the influx/efflux of antibiotic into or out of the bacterial cell.(5)

One of the most important resistance issues through the past 20 years is related to the rapidity of emergence of extended-spectrum beta-lactamases (ESBLs), first noted in the late 1990s. The increased rate of ESBL-producing bacteria leads to the wide use of carbapenem antibiotics, a drug group of last resort. The escalation of carbapenem usage has rapidly led to resistance through the emergence and spread of carbapenemases, threatening their effectiveness against this group of organisms.(7,8)

Carbapenemases are members of the molecular class A, B, and D β-lactamases. Class A and D enzymes have a serine-based hydrolytic mechanism, while class B enzymes are metallo-β-lactamases that contain zinc in the active site. The most important ones in terms of clinical setting include: (i) *Klebsiella pneumonia* carbapenemase (KPC), class A serine based β-lactamases, (ii) class B, New Delhi Metallo-β-lactamases (NDM), (iii) Verona integrin encoded Metallo-β-lactamase (VIM), (iv) class D, OXA or OXA-48-like carbapenemases and (v) IMP, active on imipenem.(9)

Among Gram negative bacteria, carbapenem resistance rates for *Klebsiella pneumonia* (*K. pneumonia)* are above 25% while 20 to 40% is for *Pseudomonas aeruginosa (P. aeruginosa)* and 40 to 70% ICU acquired infections being carbapenem-resistant for

Acinetobacter baumannii (A. baumannii). (10) A carbapenem resistant *Enterobacteriaceae* (CRE) surveillance study in Egypt during the 2011-2017 periods revealed approximately 50% of *K. pneumoniae* isolates were carbapenem resistant.(11)

The emergence of carbapenem-resistant *P. aeruginosa* and MDR *P. aeruginosa* strains has been documented worldwide. Acquired carbapenem resistance in *P. aeruginosa* is related to a plasmid or integron-mediated MBL carbapenemases, mainly IMP and VIM enzymes that are widely spread and involved in the development of different nosocomial outbreaks where *P. aeruginosa* is the causative agent.(12) The presence of NDM-1 and other carbapenemases, such as KPC in *P. aeruginosa,* has been reported since 2011.(13) OXA-40 and OXA-198 have been reported in *P. aeruginosa* resistant to carbapenems, whose genes encoding this carbapenemase have been found on plasmids.(14)

Besides antibiotic resistance, Gram negative bacteria are able to produce potential virulence factors contributing in their pathogenicity. Although virulence and resistance are developed at different times (virulence from the beginning of host colonization and resistance from the appearance of antibiotics), they are not independent characteristics. There is a relationship between them depending on the bacterial species, the specific mechanisms of resistance and virulence, the immune system of the host, the ecological niche and environmental conditions. Also, other factors like diet, age, and stress determine the host susceptibility to infection.(15)

Certain multidrug resistant opportunistic species, such as *P. aeruginosa* and *A. baumannii*, can colonize niches where many other species cannot survive (environments with high antibiotic pressure) and can even displace the commensal flora. This is one example of how antimicrobial resistance can increase the virulence or fitness of certain species in some environments, often helping these species to colonize new niches. Therefore, although antibiotic resistance is not in itself a virulence factor, in certain situations it is a key factor in development of infection, and it may be considered as virulence like factor in specific ecological niches which antibiotic-resistant bacteria are able to colonize. This is especially true in the hospital environment (intensive care units, burn units, etc.), in which if an opportunistic pathogen is drug resistant; it can cause disease more readily.(16) Also, Extended-spectrum β-lactamase (ESBL)-producing *K. pneumoniae* strains have been suggested to possess higher pathogenic potential than nonproducers.(17)

Regarding the relation between virulence and resistance, three scenarios have been reported: (i) an increase of resistance accompanied by an increase of virulence; (ii) an increase of resistance accompanied by a decrease of virulence; and (iii) an increase of resistance that does not cause effects on virulence.(18)

Bacterial genotyping methods have been used in several areas of microbiology and have facilitated the identification of bacterial strains, as well as the study of virulence and resistance factors. Constituting, in this way, as complementary or alternative to phenotypic methods.(19) The adoption of genotyping and whole genome sequencing of large sets of clinical bacterial isolates has greatly expanded our knowledge of how antibiotic resistance emerges.(20) So, Rapid, reliable and accurate molecular typing methods are essential for outbreaks detection and infectious diseases control, for monitoring the evolution and dynamics of microbial populations, and for effective epidemiological surveillance.(21)

The development of new strategies involving new antimicrobials or non-antimicrobial compounds and of novel diagnostic methods that focus on high-risk clones and rapid tests

to detect virulence markers may help to resolve the increasing problem of the association between virulence and resistance, which is becoming more beneficial for pathogenic bacteria.(15)

Early recognition of strains belonging to clones with high transmissibility potential would be beneficial by leading to the implementation of infection control measures in order to prevent further spread in hospitals. Study of the biological properties of widespread clones will be important to understand their evolutionary success and to determine whether they share special properties such as enhanced virulence or an increased capacity to survive in the hospital environment, or if their success is merely due to the fact that they acquired and express antimicrobial resistance genes.

Questions and Objectives

1- What is the prevalence of carbapenem resistance in clinical isolates of the most frequent Gram negative pathogens in Alexandria?
2- What are the virulence factors contributing in the pathogenicity of the resistant isolates?
3- What are the dominant carbapenemases present in the resistant isolates?
4- What is the clonal relatedness of the multi-drug resistant isolates studied with respect to each other and the international clones?
5- What are the solutions suggested to solve the problem of carbapenem resistance spread?

Antimicrobial agents are considered as one of the most useful and successful forms of chemotherapeutics in the history of medicine. Unfortunately, resistance to such antimicrobials is widely spread sooner or later after their usage which represents one of the major problems facing the health authorities. The increased dissemination of microbial resistant to antibiotics is caused by various mechanisms which may include: misuse and overuse of antibiotics, selective pressure, gene transfer and non-human use of antimicrobial agents.

The emergence of antimicrobial resistance affects many sectors in the healthcare settings which will be negatively reflected on the whole community and can lead to many consequences such as: high morbidity and mortality rate and loss of protection for patients, increased healthcare costs. In the last decade, many multi-drug-resistant Gram negative pathogens have been isolated globally, regionally and locally. Nowadays, these pathogens are among the most difficult pathogens to treat. Carbapenems represent the main therapy for serious infections caused by such pathogens. Unfortunately, a dramatic increase in carbapenem-resistant Gram negative isolates has been recorded in recent years. The recent worldwide emergence of carbapenem resistance in Gram-negative bacteria has mainly been manifested by the infiltration of different types of carbapenemases.

Pathogenicity is defined as the ability of microorganisms to cause disease. This ability is mediated by a group of virulence factors which depend on the genetic components of the pathogen. It was originally believed that the development of antibiotic resistance reduces the survival rates of the resistant clinical isolates. However, the persistence of the resistance phenotype indicates that resistant isolates being more pathogenic. The relation between the resistance mechanisms among Gram negative pathogens (e.g. *P. aruginosa*, *A. baumannii* and *K. pneumoniae*) to carbapenems and the production of different virulence factors is still not well understood and the published data are controversial.

The aim of the present study is:
1- Study the level of resistance to antibiotics in clinical isolates of the most frequent Gram negative pathogens in Alexandria such as *K. pneumoniae, P. aeruginosa* and *A. baumannii*.
2- Study some virulence factors of the selected isolates and their relevance to the level of resistance to antibiotics.
3- Characterisation of carbapenem resistant clinical isolates through the detection and identification of the most important carbapenemases such as OXA, KPC and NDM types and the presence of mobile structures as its genetic support.

4- Epidemiological study of the clonal relatedness of the resistant isolates by genotyping techniques such as Multi Locus Sequence Typing, determination of plasmid profiles and comparison with international clones.
5- Cell line model will be established in case of identification of interesting clone or hypervirulent strains to analyse the pathogenicity and explore combinations of antibiotics to overcome the problem of resistance

The results of the current study will determine the exact prevalence rate of different carbapenemases in clinical isolates collected from Alexandria and understand the molecular bases of carbapenem resistance mechanisms of the tested isolates. In addition, the interplay between carbapenem resistance and production of different virulence factors will be well understood. These results will also help the healthcare professionals to select the proper antimicrobial agents to treat different microbial infections caused by Gram negative pathogens and to implement a proper stewardship antibiotic program to combat the spread of deadly pathogens. The consequences of resistance to antibiotics are critical as it costs money, livelihoods and lives and threatens to undermine the effectiveness of health programmes worldwide. Moreover, it has recently been described as a threat to global stability and national security and a factor to consider driving countries to poverty. Many studies have demonstrated that resistant clones can be replaced by susceptible ones what is threatening as resistance is slow to reverse or is irreversible.

The United Nations Organization launched the Sustainable Development Goals (SDGs) as an agenda with to end poverty, protect the planet and ensure prosperity for everyone by 2030. Resistance to antibiotics has put into danger many of them:

SDG 3, Good Health and Well-being: Resistance is responsible of high rates of mortality and morbidity, increased costs and resources and changes in control protocols. The number of deaths per year attributable to AMR by 2050 if current resistance rates increased by 40% will be more that the ones caused by cancer.
SDG 5, Gender Equity: there are increasing evidence that infections affect differently to men and women. There are some publications indicating that there are worse outcomes and a higher rate of mortality in women with infections caused by resistant isolates such as blood infections by *Staphyloccus aureus*.
SDG 1, No Poverty; SDG 8, Decent Work and Economic Growth and SDG10, Reduced Inequalities: the increasing economic cost of resistance leads countries to poverty. The World Bank published a report in 2017 on drug-resistant infections as a threat to our economic future.
SDG 6, Clean Water and Sanitation; SDG 14, Life Below Water; SDG 15, Life on Land: world's rivers awash with dangerous levels of antibiotics and resistant bacteria from the hospital and community activities and pollution driven by pharmaceutical companies due to the manufacturing of molecules in countries where the regulations are not strict enough.
SDG 11Sustainable Cities and Communities, SDG 12 Responsible consumption and Production: the overuse of antibiotics is increasing all over the world and it's the main reason why resistance rates are increasing dramatically. Plans for appropriate use of antibiotics are mandatory to reduce resistance.
SDG 17, Partnership for the Goals: All approaches need to focus on an Integrative One Health Risk Management taking in account not only human health but also animal and environmental health. Multidisciplinary teams coordinating, collaborating and communicating to achieve the best health outcomes for people, animals, plants and our environment.

Project Description

Antimicrobial resistance represents a global health problem and has reached crisis points in many areas around the world. In fact, it is considered by the World Health Organization one of the top ten global threats for human development. Recently, many multi drug resistant (MDR) Gram negative pathogens were isolated globally. These pathogens showed resistance to most available antimicrobial agents. Infections with such pathogens are associated with high rate of morbidity and mortality. A bacterial isolate is considered as MDR when it shows resistance to at least one antimicrobial agent in three or more antimicrobial categories (aminoglycosides, carbapenems, cephalosporins, fluoroquinolones, penicillins plus β-lactamase inhibitors, polymyxins, etc). In addition, these pathogens play a major role in nosocomial infections especially in the intensive care units.

Carbapenems represent the main therapy for the serious infections caused by such pathogens. Unfortunately, a dramatic increase of carbapenem resistant Gram negative isolates has been recorded in the recent years.

Resistance to carbapenems is mainly mediated by: (i) changes in porin proteins, (ii) development of efflux pumps, (iii) modification of penicillin-binding proteins, and production of different types of β-lactamases. According to the Ambler classification (Ambler 1980), there are four classes of β-lactamases (A, B, C and D). According to the Ambler classification (Ambler 1980), there are four classes of β-lactamases (A, B, C and D). Classes A, C and D are serine type enzymes while class B is metallo-type enzymes (metallo-β-lactamases, MBLs) which require zinc for their catalytic activity.

In Egypt, the data regarding the molecular mechanism of carbapenem resistance in Gram negative pathogens relatively is limited.

The virulence mechanisms in bacteria have been modified over millions of years to evade host defense systems. Many virulence factors are produced by microorganisms to evoke disease (e.g. toxins, capsule, porins, enzymes, etc.). The coexistence of antibiotic resistance genes and virulence factors was observed in different clinical isolates. The data about the interplay between antibiotic resistance and virulence factors in different Gram negative clinical isolates is limited.

Comprehensive analysis of the coexistence profiles of antibiotic resistance genes and virulence factors in bacterial genomes will improve the understanding of bacterial pathogenesis and development and widespread of microbial resistance.

The aim of the present study is to investigate the prevalence of the genes encoding carbapenem resistance and different virulence factors in MDR Gram negative clinical isolates collected from Alexandria. Moreover, the relation between antibiotic resistance and virulence will be also explored.

 # The main points to be addressed:

1- Knowledge about the prevalence of the resistance to carbapenems among nosocomial isolates from hospitals of Alexandria. Not many reports until now shows the situation in Egypt in comparison with other countries worldwide. The data obtained will allow developing more effective control measures to avoid the fatal consequences of this type of infections in terms of mortality and morbidity.
2- Development of molecular biology techniques such as MLST and sequencing for rapid detection and identification of resistance to carbapenems and analysis of its usefulness, advantages and disadvantages as an infection control tool.
3- Identification of plasmids and integrons as mobile genetic elements with the ability to spread the resistance among nosocomial isolates.
4- To implement a multidisciplinary approach with groups from different areas of research under a One Health concept, human health linked to animal, plants and environmental health.

Our project aims to get a better insight into antibiotic resistance dynamics under the selection pressure of antibiotics, select antibiotic resistant bacteria in humans, animals and the environment. We propose a holistic approach combining and integrating different detection tools (microbiological, pharmaceutical and genomics) for monitoring and control antibiotic resistance in Gram negative bacteria causing nosocomial infections in Alexandria.

We also propose to evaluate the extent of antibiotic resistance dissemination by screening of plasmids and integrons leading to facilitate antibiotic resistance spread. The analysis of correlations among factors affecting antibiotic resistance spread (antibiotic resistance genes, mobile genetic elements, antibiotic resistant bacteria and transfer capacity of antibiotic resistance among bacteria) aimed by our multidisciplinary/multi-sites approach will certainly bring a loop forward into the understanding of the antibiotic resistance dynamics from a One Health approach.

This project will be accomplished by an international interdisciplinary consortium with the Faculty of Medicine & Nursing of the University of the Basque Country (UPV/EHU) that will work in a collaborative manner, tackling from complementary views through their respective areas of expertise, this challenging public health threat. The group of the University of the Basque Country belongs to a Joint Research Laboratory on Environmental Antibiotic Resistance including several high performance research groups. The group is focussed on different aspects of antibiotic resistance spread: (a) On the clinical level, they focus on new targets to control resistance and persistence mechanisms of *A. baumanni*; b) On the molecular level, on one of the key conjugative proteins, the coupling protein aiming to inhibit conjugation to stop the spread of ARGs among bacteria; (c) On the environmental level, they reported high prevalence of transfer genes in different hotspots of antibiotic resistance spread) and; (d) They develop high throughput methods for ecotoxicological assessment, effect-directed analysis and metabolomics to monitor antibiotic resistance.

To undertake this project, we have a multidisciplinary team that works in complementary research areas. This collaboration will allow us to undertake more ambitious and innovative projects than those that we could address individually.

Research Design and Methods

1- Patient and bacterial isolates data, species identification and antimicrobial susceptibility testing

- Isolates from various clinical specimens will be collected from different patients admitted to multiple medical facilities.
- Identification of all clinical isolates will be performed the species level by means of conventional identification methods (Gram staining and culturing on specified media), and the VITEK 2 ID automated system (using VITEK®2 GN ID card, bioMérieux, Marcy L'Etoile, France).
- Antimicrobial susceptibility testing of all isolates will be performed using the VITEK 2 automated system (using the specified VITEK® 2 AST cards, bioMérieux, Marcy L'Etoile, France) and/or the agar/broth dilution. MICs will be interpreted using the resistance breakpoints for the selected organisms from EUCAST (Version 10.0, January 2020, http://www.eucast.org/clinical_breakpoints/).

2- Detection of virulence factors.

Phenotypic and/or genotypic characterization of different virulence factors will be studied (according to the nature of each organism) including enzymes, toxins and biofilm production:

a- Biofilm formation will be assessed as described by O'Toole (22) with some modifications. Biofilms are developed in 24 well plates. Overnight cultures of bacteria are adjusted to an optical density of 0.2 at 600 nm; one hundred microliters are placed in each well containing 900 µl of M63 medium (minimal salts medium) supplemented with casamino acids (0.5% w/v) and incubated 24 h at 37°C. Planktonic cells are removed and the wells containing biofilms are rinsed three times with distilled water and air dried for approximately 20 minutes. The remaining adherent bacteria are stained with 1 ml/well of 0.7% crystal violet (wt/vol) solution for 12 min. Excess stain is removed by three washes with distilled water. Crystal violet-stained biofilm is solubilized in 1 ml of 33% acetic acid (vol/vol), and the plates incubated at RT in an orbital shaker for 1 min at 400 rpm and the amount of dye (proportional to the density of adherent cells) is determined at 620 nm using a microplate reader. Results are corrected for background staining by subtracting the value for crystal violet bound to uninoculated control wells. The biofilm assay is performed four independent times.

b- For motility, we will inoculate a motility agar plate with 1 microliter of an overnight culture at 0.5 McFarland and incubate at 37ºC. Observation of the shape of the colonies and the characteristics of the growth will be analyzed at 24, 48 and 72 hours.

3- Phenotypic detection of carbapenemases:

a. Modified Hodge Test (MHT (23):
A sterile Müller-Hinton agar plate was aseptically inoculated with a 1:10 dilution of 0.5 McFarland suspension of the *E. coli* ATCC 8739 by using a sterile swab and allowed to dry for 3-5 min. Meropenem disc (10 µg) was aseptically placed in the centre of the test area and each tested isolate was streaked, in a straight line, from the disc to the edge of the plate using sterile swabs. *K. pneumoniae* ATCC 10031 was used as a negative control.

The plates were then incubated for 18-24 h at 37°C. Development of a clover leaf-like indentation of the carbapenem susceptible *E. coli* strain growing along the test organism growth streak within the disc diffusion zone indicates that this isolate is a carbapenemase producer.

b. Carbapenem Inactivation Method (23):
Meropenem disc (10 µg) was incubated for 4 h in an overnight culture of each tested isolate. A 0.5 McFarland suspension of *E. coli* ATCC 8739 was used to inoculate a Müller-Hinton agar by using a sterile swab. After incubation, the meropenem disc was transferred onto the inoculated Müller-Hinton agar plate and incubated for 18-24 h at 37ºC. The absence of an inhibition zone was interpreted as the presence of carbapenemase activity due to enzymatic hydrolysis of meropenem, whereas a clear inhibition zone (≥ 20 mm) indicated the absence of carbapenemase activity.

c. Combined Disc Test (23):
Combined disc test was used for the detection of MBLs. On a Müller-Hinton agar plate inoculated with the tested isolates, imipenem (10 µg) and imipenem/EDTA discs (10/930 µg) were placed with not less than 20 mm distance from Centre to center of the discs. The inhibition zones of the imipenem and imipenem/EDTA discs were compared after 16-18 h of incubation at 37°C. An increased inhibition zone of ≥ 7 mm with the imipenem/EDTA disc compared to the imipenem disc alone was considered as MBL positive production.

d. Boronic acid disc test (23):
Ten microliters of 3-aminophenylboronic acid (PBA) dissolved in dimethyl sulfoxide solution were aseptically dropped onto imipenem and meropenem discs. Treated and untreated imipenem and meropenem discs were also transferred onto Müller-Hinton agar plate inoculated with the tested isolate. In addition, 400 µg PBA disc lacking either antibiotic was used on the same plate as a control. After incubation for 18-24 h at 37°C, the inhibition zone diameters around the treated imipenem and meropenem discs were compared with the diameters around the plain antibiotic discs. A ≥ 5 mm difference in zone diameter was considered as a positive result.

4- Detection of carbapenemase-encoding genes

The presence of the carbapenemase-encoding genes $bla_{\text{OXA-51-like, -23-like, -58-like, -40-like,-48, -143-like and -235-like}}$ will be investigated by PCR (24-26). MBLs genes will be investigated by in-house PCRs targeting the genes: bla_{VIM}, bla_{IMP}, and bla_{NDM}. Positive PCR products will be purified by PCR purification kits and allelic variants determined by Sanger sequencing followed by NCBI BLAST analysis.

5- Molecular typing

- Concerning, *A. baumanii* clinical isolates, multi-locus sequence typing (MLST) will be performed using the Oxford and Pasteur typing schemes (https://pubmlst.org/ abaumannii/) to assign the sequence type (ST). Clonal complexes (CCs) are assigned using the BURST function available at pubmlst.org. The $bla_{\text{OXA-51-like}}$ variant combined with the CCs derived from both schemes and cgMLST analysis, using the Ridom SeqSphere+ v. 7.0.4 software, will be used to assign the isolate to an IC (28).

- Regarding *P. aeruginosa*, the reagents, primers, and reaction conditions used for MLST will be identical to those described on the *P. aeruginosa* MLST website (http://pubmlst.org/paeruginosa/). Sequence type (ST) assignment will be performed on the *P. aeruginosa* MLST website, START2 software generated a UPGMA dendrogram of the allelic profiles, and the eBURST algorithm inferred clonal relationships between each ST.(28,29)

- As for *K. pneumonia*, MLST will be conducted according to the method of Diancourt *et al*. (30) and sequence types (STs) were assigned through the Institute Pasteur database (http://bigsdb.pasteur.fr/ klebsiella/klebsiella.html). Clonal groups were defined based on STs using eBURST. (29)The clonal relationship of these STs with those in the database of the Institute Pasteur using eBURST will be obtained.

6- Plasmid detection experiments

To determine the plasmid content of the resistant isolates, plasmid DNA will be obtained using commercial kits (such as QIAprep Spin Miniprep Kit (Qiagen)) following the manufacturer's instructions. Finally, plasmids will be visualized by electrophoresis on 0.7% agarose in 0.5% TBE buffer. Size of plasmid will be determined by comparison with control isolates using a Gel Doc EZ Image (Bio-Rad)

7- Cell line (31-32):

Cell line could be used to explore the interactions between tested isolates and host cells, including binding, invasion, and cytotoxicity. In addition, it will be used to study biofilm formation by different tested isolates. The bacterial isolates will be allowed to infect different cell lines for 3 hours and the monolayer confluences' with and without bacteria were compared using automated imaging of 96-well plates. The remaining cells in the wells were visualized with nuclear staining and detected as a retained monolayer.

Anticipated Results and Evaluation Criteria

- The resistance profile of the tested isolates against different antimicrobial agents will be initially determined by the VITEK 2 compact system. It will be subsequently confirmed by agar diffusion assay and determination of the minimum inhibitory concentrations (MICs) according to the Clinical and Laboratory Standards Institute (CLSI) or the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

- Production of carbapenemases and different virulence factors of tested isolates will be phenotypically detected by performing suitable reported techniques and the results will be interpreted according to published data.Regarding the virulence factors, Biofilm production and motility test will be performed to analyze the virulence of the resistant isolates. To detect biofilms isolates will be cultivated in liquid media using a 24 well plate and stained with crystal violet and the amount of dye (proportional to the density of adherent cells) will be determined at 620 nm using a microplate reader. For motility we will observe the shape of the colonies and the characteristics of the growth on motility agar plates.

- The molecular typing of *P. aeruginosa*, *A. baumannii* and *K. pneumoniae* will be determined by multi-locus sequence typing (MLST) technique and the data will be analyzed using the corresponding MLST websites, the Ridom SeqSphere+ v. 7.0.4 software and the Institute Pasteur database, respectively.

- The sequence of the amplified genes encoding different carbapenemases and virulence factors will be evaluated according to National Center for Biotechnology Information (NCBI) database. The sequences of the amplified genes will be submitted to obtain GenBank accession numbers.

- The infection of Gram negative bacteria on cell lines leads to loss of cell membrane integrity and release of cytoplasmic contents which finally results in cell detachment that overall is known as cytotoxicity. The disruption of mammalian cell monolayers was interpreted as directly proportional to the bacterial cytotoxicity.

- The obtained results will be statistically analyzed by SPSS version 20.

Expected Project Outcomes and Impact to AASTMT

I- Technical output and Impact:

Expected project's outputs:

a- At least one peer-reviewed original research paper accepted for publication (received a DOI) in a Q1-Q2 journal or its equivalent,

b- The project will facilitate the practical work of a MSc thesis of Pharmacist Aya El-Kholy, Demonstrator, College of Pharmacy, AASTMT (Alamein Campus) in collaboration with the Faculty of Pharmacy, Alexandria University, under supervision of Prof. Elsayed Aboulmagd Elsayed (one of the team members).

c- The Egyptian isolates will be part of PhD and Master Thesis supervised by Prof. Lucía Gallego, Faculty of Medicine and Nursing, University of the Basque Country.

II- Financial feasibility & Socio-economic Impact:

At a societal level, disease burden, health system development, pharmaceutical regulations and enforcement, health insurance or national healthcare coverage, and access to and quality of medicines all affect antimicrobial use and are affected by socioeconomic factors. Individuals, both high- and low-income, operate in their own societal conditions, and their individual behavior also impacts patterns of antimicrobial use and ultimately, resistance. Drug-resistant infections also affect patients' social and economic status by increasing healthcare costs, mortality and morbidity, and decreasing productivity. When infections can no longer be treated by first-line antibiotics, more expensive medicines must be used. A longer duration of illness and treatment, often in hospitals, increases health care costs as well as the economic burden on families and societies. Antibiotic resistance is putting the achievements of modern medicine at risk. Organ transplantations, chemotherapy and surgeries such as caesarean sections become much more dangerous without effective antibiotics for the prevention and treatment of infections. Finally, the control of AMR will support achieving SDGs stated by the WHO and related to the field of work

III – Publication:

The expected outcome is at least one peer-reviewed original research paper accepted for publication (received a DOI) in a Q1-Q2 journal or its equivalent.

Resources

Resources	
Laboratory Space	- Microbiology laboratory, College of Pharmacy, Arab Academy for Science, Technology and Maritime Transport (AASTMT), Abu Qir Campus: Well-equipped laboratory is available. The laboratory contains good space for carrying out most of the microbiological experiments. The laboratory is well aerated with fire extinguishers. Multiple benches with basic reagents for disinfection and sterilization are present. The basic needs of different culture media, stains and biochemical reagents are available. - Antibiotics & Molecular Bacteriology Laboratory, Faculty of Medicine & Nursing, University of the Basque Country UPV/EHU, Basque Country, Spain. Laboratory equipped with the infrastructure for microbiological experiments and molecular biology techniques. Contains good space and follows all security regulations for a C2 level laboratory.
Personnel	**Dr. Mohammed El-Khouly, PI** He will be responsible for implementation and follow up the different phases of the project and writing the technical reports. He will help in performing different microbiological and molecular experiments, interpretation of the results and writing the manuscript. **Prof. Evan Saad (Co-PI)** She will follow up the implementation of the study plan, participation of writing the technical reports, supervision and interpretation of results of the cell line experiments related to biofilm formation and interaction with host cells, and writing and editing of the manuscript. **Prof. Elsayed Aboulmagd:** He will participate in the following tasks: implementation of the study plan, interpretation of antimicrobial susceptibility testing, supervision and interpretation of the molecular characterization experiments and results, writing and editing of the manuscript. **Prof. Lucía Gallego:** She will be responsible for molecular typing of *A. baumannii* clinical isolates, interpretation of the results related to molecular characterization of the virulence factors and revision of the technical reports and manuscript. She will also supervise molecular methodology with *P. aeruginosa* and *K. pneumonia* clinical isolates. **Pharmacist. Aya Elkholy:** She will be responsible for isolation and identification of the tested microorganisms, determination of the antibiogram of the tested isolates, performing the experiments related to phenotypic detection of virulence factors and antibiotic resistance genes and the PCR procedures for amplification of the target genes. **Technician:** She will help responsible for preparation culture media and reagents; sterilization of glassware; and will help in carrying out some microbiological experiments.
Equipment	Microbiology laboratory, College of Pharmacy, Arab Academy for Science, Technology and Maritime Transport (AASTMT), Abu Qir & Al Alamein Campuses: Most equipment and lab facilities required to perform microbiological

	experiments are available in the laboratory such as: Biological safety cabinet (Laminar flow) – Autoclave - Hot air oven – Incubator – Refrigerator – Spectrophotometer - Light microscopes - Shaking Incubator – Centrifuge - Real-Time PCR - Digital balance - Deep Freezer (-20 ℃). Antibiotics & Molecular Bacteriology Laboratory: Incubators - Biological safety cabinet – Refrigerators - Deep Freezer (-20 ℃) - Thermocycler Perkin Elmer – Centrifuge - Eppendorf centrifuges - Shaking incubator - Hybridization oven - Electrophoresis equipment: power supplies, DNA electrophoresis cells - Thermoleader Dry Block Heat Bath GeneQuant - Gene Pulser Xcell - McFarland Densitometer - Electronic balance - Microwave oven - Gel Doc EZ Image - Chef-DR III. Planned Equipment (will be oriented for El-Alamein Campus): - Conventional PCR (for detection of genes and sequencing). - Gel Electrophoresis Set (for visualizing and documenting the identified/separated genes). - Heat Block - Densitometer (suspension turbidity detector) (McFarland reader) for adjusting bacterial concentrations.
Office and Computer Facilities	- An office equipped with computer attached to the internet is available. - Computer laboratories are available in college of pharmacy-AbuQir campus. - Different programs are supplied by AASTMT: (Endnote – Turnitin). - An office equipped with computer attached to the internet is available and computer equipment in the Antibiotics & Molecular Bacteriology Laboratory with Image Lab 5.1 and Graph Pad Prism 6 programs. - Additional 207 programs are supplied for the staff by the University of the Basque Country at https://ehuappstore.ehu.eus/login

Team Information: The team is composed of 5 members:

1- Dr. Mohammed Abd El-Karim Abd El-Mohsen El-Kholy (PI)
Assis. Prof. - Microbiology and Biotechnology Department, Clinical and Biological Sciences Division, College of Pharmacy, Arab Academy for Science, Technology and Maritime Transport (AASTMT) – Abu Qir Campus.

2- Prof. Evan Ibrahim Saad (Co-PI)
Prof. - Pharmacology and Biochemistry Department, Clinical and Biological Sciences Division.
Dean of College of Pharmacy, Arab Academy for Science, Technology and Maritime Transport (AASTMT) – Alamein Campus.

3- Prof. Elsayed Aboulmagd Elsayed
Prof. - Microbiology and Immunology Department, Faculty of Pharmacy, Alexandria University.

4- Prof. Lucía Gallego
Prof. of Medical Microbiology & Immunology, Head of the Antibiotics & Molecular Bacteriology laboratory, Faculty of Medicine & Nursing, University of the Basque Country UPV/EHU, Spain.

5- Ph. Aya Tarek Moustafa Amin Elkholy
Demonstrator - Microbiology and Biotechnology Department, Clinical and Biological Sciences Division, College of Pharmacy, Arab Academy for Science, Technology and Maritime Transport (AASTMT) – Alamein Campus.

1- Dr. Mohammed Abd El-Karim Abd El-Mohsen El-Kholy (PI)

- Mohammed El-Kholy was graduated in 2008 from the faculty of Pharmacy, Alexandria University, Egypt, with an "Excellent" grade. He got his master's degree in molecular and diagnostic microbiology at the medical research institute, Alexandria Egypt in 2013.
- The master's thesis was about the “Detection of Multidrug Efflux System in Multidrug Resistant *Escherichia coli* Clinical Isolates” evaluating the role of AcrAB TolC efflux pump gene expression using qRT-PCR. Also, the thesis explored the effect of the use of an agent "Mefloquine" as efflux pump inhibitor to be combined with the used ineffective antibiotics.
- He was enrolled in the PhD program in 2013 within the same institute focusing on the same field – antimicrobial resistance – as it is widely spread in my community due to the lack of the rational use of antimicrobial agents. The PhD thesis was about "Virulence Factors, Antifungal Susceptibility Profile and Possible Mechanism of Azole Resistance among *Candida tropicalis* Clinical Isolates" detecting (phenotypically) the presence of different virulence factors (biofilm production – hemolysins – phospholipases and proteinases) within the clinical isolates as well as investigating the possible molecular mechanisms of azole resistance through evaluating the role of two efflux pumps (CDR1 and MDR1) by gene expression and detecting the possible mutations in Erg11 gene by gene sequencing analysis.

Publications and Presentations

Journal Articles

1- Rania Abo Zahra, **Mohammed A. El-Kholy**, Kholoud Baraka. “Virulence genotyping of drug resistant *Pseudomonas aeruginosa* clinical isolates in Egypt using multiplex PCR”, Gene Reports, 2021(22). https://doi.org/10.1016/j.genrep.2020.101000

2- Sherine M. Shawky, Ahmed H. Gaballah, Amr Abdallah, Shady Fadel, **Mohammed A. El Kholy**. “Automated Identification and Antifungal Susceptibility Testing of *Candida* Species using Vitek 2 Compact System in ICUs and Pediatric Oncology Unit, Alexandria, Egypt”, Egyptian Journal of Medical Microbiology, 2017(26). http://ejmm-eg.com/e/index.php/published-issues/81-vol-26/vol-26-no-2-april-2017

3- Ghada F. Helaly, Sherine Shawky, Rania Amer, OlaAbdel- kader, Gamal El-Sawaf, **Mohammed A. El Kholy**. “Expression of AcrAB Efflux Pump and Role of Mefloquine as Efflux Pump Inhibitor in MDR *E. coli*”, American Journal of Infectious Diseases and Microbiology, 2016(4). http://pubs.sciepub.com/ajidm/4/1/2/index.html

4- Sherine M.Shawky, Amr Abdallah, **M. Khouly** “Antimicrobial activity of Colistin and Tigecycline against carbapenem- resistant *Klebsiella pneumoniae* clinical isolates in Alexandria, Egypt”, Int.J.Curr.Microbiol.App.Sci (2015) 4(2): 731-742. https://www.ijcmas.com/vol-4-2/Sherine%20M.Shawky,%20et%20al.pdf

Research Talks and Poster Presentations

1- **Mohammed A. El-Kholy**, Akram M. Fayed, Sherine M. Shawky, Jacques F. Meis “*Candida auris* blood stream infection in Egypt.” 9th Trends in Medical Mycology, Nice, France, conference abstracts. Journal of Fungi, Oct. 2019. https://www.mdpi.com/2309-608X/5/4/95

2- **El-Kholy MA**, Shawky SM, Helaly GF, Gaballah AH, El Ghazzawi EF, Elsawaf GA “Virulence factors, antifungal susceptibility profile and possible mechanisms of azole resistance among *Candida tropicalis* clinical isolates, Alexandria, Egypt”, First Balkan Fungus, Timisoara, Romania, conference abstracts. Revista Română de Medicină de Laborator Supliment 26: 3, Sept. 2018.http://www.rrml.ro/articole/2018/2018_3_supliment.pdf

2- Prof. Evan Ibrahim Saad (Co-PI)

Evan Ibrahim Saad graduated as a pharmacist from the Faculty of Pharmacy, Alexandria University in 1979; she obtained her Master's in 1983 and PhD degree in 1990 from her alma mater studying some biochemical and toxicological aspects on experimental animals. She worked as an Assistant, Associate then Full Professor of Pharmacology at the Faculty of Pharmacy, Alexandria University between 1990 and 2007.
Throughout her career, she acted as Head of Quality Assurance Unit between 2008 and 2010, Vice-Dean for Graduate Studies and Research at the Faculty of Pharmacy, Alexandria University between 2010 and 2013 then as the Faculty Dean between 2014 and 2017. She is now the Dean of the College of Pharmacy, Arab Academy for Science, Technology and Maritime Transport (AASTMT), Alamein Campus.
She is also an accredited reviewer for the National Authority for Quality Assurance and Accreditation of Education (NAQAAE). During her academic career, she taught biochemistry, clinical biochemistry, toxicology, clinical pharmacy and pharmacology to Professional and postgraduate Pharmacy students at Alexandria University, Beirut Arab University and AASTMT.
She supervised 12 Master's and PhD students and has more than 30 important peer-reviewed publications in international journals.

Publications:

1- Alaaeddine R, Elkhatib MAW, Mroueh A, Fouad H, **Saad El**, El-Sabban ME, Plane F, El-Yazbi AF. Impaired Endothelium-Dependent Hyperpolarization Underlies Endothelial Dysfunction during Early Metabolic Challenge: Increased ROS Generation and Possible Interference with NO Function. J Pharmacol Exp Ther. 2019 Dec;371(3):567-582. doi: 10.1124/jpet.119.262048. Epub 2019 Sep 11. PMID: 31511364.
2- El-Gowelli HM, **Saad El**, Abdel-Galil AG, Ibrahim ER. Co-administration of α-lipoic acid and cyclosporine aggravates colon ulceration of acetic acid-induced ulcerative colitis via facilitation of NO/COX-2/miR-210 cascade. Toxicol Appl Pharmacol. 2015 Nov 1;288(3):300-12. doi: 10.1016/j.taap.2015.08.002. Epub 2015 Aug 12. PMID: 26276312.
3- Senbel AM, **Saad El**, Taha SS, Mohamed HF. Different mechanisms for lead acetate, aluminum and cadmium sulfate in rat *corpus cavernosum*. Toxicology. 2016 Jan 18;340:27-33. doi: 10.1016/j.tox.2015.12.004. Epub 2015 Dec 23. PMID: 26723573.
4- El-Yazbi, A., El-Khatib, M., Fouda, M., Sleiman, F., **Saad, E**., Fouad, H. and Eid, A. (2017), High-calorie Diet Induces Vascular and Hemodynamic Abnormalities in Absence of Change in Blood Glucose or Insulin Levels: Modulation by Oral Anti-hyperglycemic Drugs. The FASEB Journal, 31: 1068.3-1068.3.
5- Alaaeddine, R., El-khateeb, M., **Saad, E.I**., Fouad, H.H., El-Sabban, M., Plane, F. and El-Yazbi, A.F. (2018), Endothelial Dysfunction as a result of Hypercaloric Intake: Underlying Mechanism in Absence of Hyperglycemia. The FASEB Journal, 32: 837.2-837.2.

3- Prof. Elsayed Aboulmagd Elsayed

He is a professor of Microbiology, Faculty of Pharmacy, Alexandria University. He obtained the bachelor degree in pharmaceutical sciences in June 1989 and he joined the department of Microbiology as a demonstrator in October 1989. In 1995, he obtained the master degree in the field of drug interactions of fluoroquinolones antimicrobial agents with different pharmaceutical adjutants and dosage forms and the effect of such interactions on the bioavailability of these antimicrobial agents. In 2002, he obtained the PhD from the Institute of Molecular Microbiology and Biotechnology - Westfälische Wilhelms University - Münster – Germany under supervision of Prof. Dr. Alexander Steinbüchel in the field of genetic engineering and Biotechnology. The main topic of the PhD was the cloning and heterologous expression of genes coding biosynthesis of biopolymer isolated from cyanobacteria. In 2007, he joined the College of Medicine, King Faisal University, Saudi Arabia, as a visitor assistant professor and he returned back to join the department of Microbiology and Immunology, Faculty of Pharmacy in 2014. In 2008, he promoted to be associate professor while in March 2015, he became a full professor. He supervised seven master theses and two PhD theses. The main areas of research are: (i) Drug interactions, (ii) Antibiotic combinations, (iii) Molecular characterization of antibiotic resistances, (iv) Phenotypic and genotypic characterization of pathogens (v) Heterologous expression of genes and (vi) Biotechnological production of fermentation products. He has more than 30 publications most of them were published in international journal.

Recent Publications:

1- Al-Sultan AA, **Aboulmagd E**, Amin TT. 2013. ESBL-producing *E. coli* and *K. pneumoniae* in Al-Ahsa, Saudi Arabia: Antibiotic susceptibility and prevalence of *bla*SHV and *bla*TEM. J. Infect. Dev. Ctries. 7(12):1016-1019.

2- Kassem AA, Ismail FA, Ismail FA, Naggar VF, **Aboulmagd E**. 2014. Preparation and evaluation of periodontal films based on polyelectrolyte complex formation. Pharm. Dev. Technol. 2014 Jan 17. [Early Online: 1-9]

3- Aboulmagd E, Alsultan AA. 2014. Synergic bactericidal activity of novel antibiotic combinations against extreme drug resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. Afr. J. Microbiol. Res. 8(9):856-861.

4- Kassem AA, Ismail FA, Ismail FA, Naggar VF, **Aboulmagd E**. 2014. Comparative study to investigate the effect of meloxicam or minocycline HCl in situ gel system on local treatment of periodontal pockets. AAPS Pharm. Sci. Tech. 15(4): 1021-1028.

5- Kaliyadan F, **Aboulmagd E**, Amin TT. 2014. Antimicrobial activity of commercial “antibacterial” handwashes and soaps. Indian Dermatol. Online J. 5(3): 344-346.

6- Al-Sultan AA, Al-Eknah MM, Al-Dougym AM, **Aboulmagd E**. 2014. Detection of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and extended spectrum β-lactamase-producing *Escherichia coli* associated with ovarian hydrobursitis syndrome in female camels (Camelus dromedarius). Asian J. Anim. Vet. Adv. 9(8): 499-505.

7- Alsultan AA, **Aboulmagd E**, Evans BA, Amyes SGB. 2014. Clonal diversity of *Acinetobacter baumannii* from diabetic patients in Saudi Arabian hospitals. J. Med. Microbiol. 63:1460-1466.

8- AL-Sultan AA, Evans BA, **Aboulmagd E**, Al-Qahtani AA, Bohol MFF, Al-Ahdal MN, Opazo AF, Amyes SBG. 2015. Dissemination of multiple carbapenem-resistant clones of *Acinetobacter baumannii* in the Eastern District of Saudi Arabia. Front Microbiol. 6: 634.

9- Abouelfetouh A, Torky AS, **Aboulmagd E**. 2019. Phenotypic and genotypic characterization of carbapenem-resistant *Acinetobacter baumannii* isolates from Egypt. 20;8:185.

10- Abouelfetouh, A., Torky, AS, **Aboulmagd E.** 2020. Role of plasmid carrying blaNDM in mediating antibiotic resistance among *Acinetobacter baumannii* clinical isolates from Egypt. 3 Biotech 2020;10(4):170.

4- Prof. Lucía Gallego

- Dr. Lucía Gallego joined the Antibiotics Section of Microbiology Service (Hospital of Basurto, 1986) as a researcher on antibiotic resistance. In 1996 she started the Antibiotics and Molecular Bacteriology Laboratory (Faculty of Medicine & Nursing, UPV/EHU). She took over the research line "*Acinetobacter baumannii*: study of the mechanisms of resistance to antibiotics and persistence in the nosocomial environment", leading the "*Acinetobacter baumannii* Research Group".
- Dr. Gallego is an expert on antibiotic resistance in nosocomial bacteria, in particular in beta lactamases as mechanism of resistance to β-lactam antibiotics and molecular biology as tools for detection and identification of resistant isolates. Her group has contributed significantly to the knowledge about OXA-type carbapenemases and plasmids as its genetic support and the spread of world-wide multidrug-resistant clones."
- Relevance in relation with the proposal: "Dr. Lucía Gallego is an expert in molecular epidemiology of multidrug resistant clinical isolates producing OXA-type carbapenemases, plasmids and other mobile elements as genetic support and transfer of resistance to antibiotics and the behaviour and survival of multidrug resistant isolates in the nosocomial environment. She holds long-standing collaborations with hospitals in Bolivia studying the resistance profiles and genetic features of *A. baumannii* isolates, demonstrating the high prevalence of multidrug-resistance and plasmids among clinical isolates. The group has also described first different carbapenemase genes such as *bla*OXA40, *bla*OXA-23, *bla*OXA-58, and *bla*NDM-6 and the corresponding mapping of the genetic region. By Multi Locus Sequence Typing they identified the high prevalence in Bolivia of isolates of the International Clone (IC) 7 clone containing the *bla*OXA-23 gene in a transposon-like structure whereas in European isolates Clones IC1 and IC2 with a plasmid *bla*OXA-40 gene were the most frequent. The high prevalence of XDR *A. bau*mannii clones confers increasing risk to patients and is of major concern due to the fatal infections they cause and the lack of therapeutic alternatives for treatment. Her motivation and commitment are very high since this is the central subject of her scientific career."

 # Peer-review publications related to the topic of the Proposal.

1- Kyriaki Xanthopoulou; Mikel Urrutikoetxea-Gutiérrez, Matxalen Vidal-García, José Luis Diáz de Tuesta del Arco, Sandra Sánchez, Julia Wille; Harald Seifert; Paul G Higgins, **Lucia Gallego**. First Report of New Delhi Metallo-B-Lactamase-6 in a Clinical *Acinetobacter baumannii* Isolate From Northern Spain. Frontiers in Microbiology November 2020 vol 11:article 589253, (2020) Significance: we describe for the first time in *A. baumannii* a new carbapenemase named NDM-6 that confers high level of resistance to carbapenems and that is spreading all over the world.

2- Kyriaki Xanthopoulou, Julia Wille, Oleg Krut, Harald Seifert, **Lucia Gallego**, Paul G Higgins. Mobile Genetic Elements Harboring Antibiotic Resistance Determinants in *Acinetobacter baumannii* Isolates From Bolivia. Frontiers in Microbiology, vol 11, article 919:1-11 (2020) Significance: first mapping of plasmids containing multiple antibiotic resistance genes from bolivian isolates.

3- Monica Cerezales, Kyriaki Xanthopoulou, Julia Wille, Zulema Bustamante, Harald Seifer,; **Lucia Gallego**, Paul G Higgins. *Acinetobacter baumannii* analysis by core genome MLST in two hospitals in Bolivia: endemicity of international clone 7 isolates

(CC25). International Journal of Antimicrobial Agents, June 2019:: 53, 6: 844-849 (2019) Significance: development of MLST technique for the identification of the genetic relatedness of the *A. baumannii* clinical isolates from different hospitals in Bolivia.

4- Monica Cerezales, Kyriaki Xanthopoulou, Julia Ertel, Alexandr Nemec, Zulema Bustamante, Harald Seifert, **Lucia Gallego**, Paul G Higgins. Identification of *Acinetobacter seifertii* isolated from Bolivian hospitals. Journal of Medical Microbiology 2018, Vol/páginas: 67 (6): 834-837 (2018) Significance: Genome sequence to identify *A. seifertii*, a species closely related to *A. baumannii*, for the first time in Bolivia.

5- Mónica Cerezales, Alain Ocampo-Sosa, Laura Alvarez, Catalina Díaz, Zulema Bustamante, Jazmín Santos, Luis Martínez-Martínez, Paul G Higgins, **Lucía Gallego**. High prevalence of extensively drug-resistant *Acinetobacter baumannii* at a Children Hospital in Bolivia. The Pediatric Infectious Diseases Journal Feb 22, 37 (11):1118-1123, (2018) Significance: the study showed the high level of resistance of the isolates causing infections among children in hospitals of the city of Cochabamba, Bolivia, and the presence of multidrug-resistant clones.

 # Most relevant grants.

1- *Acinetobacter baumannii*: new targets to control the resistance to antibiotics and persistance in the nosocomial environment. Grupo Consolidado del Sistema de Investigación Funded: Basque Government & University of the Basque Country UPV/EHU, 2017-18 (PI)

2- "Molecular analisis of multidrug-resistance *Acinetobacter baumannii* and its relation with the presence of the *bap* gen in isolates from Hospitals of the city of Cochabamba" Funded: ASDI-UMSS Programm, Dirección de Investigación Científica y Tecnológica (DICyT) Bolivia € 2016-18

3- Development of molecular biology techniques in a "Módulo de bacteriología", Maestría de Microbiología Clínica de la Universidad Mayor de San Simón en Cochabamba, Bolivia Funded: Cooperation Office, University of the Basque Country UPV/EHU 2014 (PI)

4- "Iberoamerican Project to design useful tools to control the resistance to antibiotics in clinical isolates of *Acinetobacter baumannii*" Funded: University of the Basque Country 2014-2015 (PI)

5- "Development of genetic techniques to detect and control carbapenem resistant isolates obtained from Bolivian hospitals", Funded: Spanish Agency of International Cooperation, Ministry of Foreign Affairs, € 2008-10 (PI)

 # Major significant research outputs.

- Title*:* Representative of the Faculty of Medicine & Nursing in the National Plan Against Resistance to Antibiotics (Spanish Agency of Medicines and Health Products) for educational and research affairs. From 2015 to present

-Title*:* "Microbiota and liver disease: a challenge for the future" The Second Symposium within the MOU between Theodor Bilharz Research Institute (TBRI) & University of the Basque Country UPV/EHU. El Cairo, Egypt. 2019

-Title: "Plasmids as new targets to control the spread of MDR determinants among nosocomial isolates of *Acinetobacter baumannii*" The First Symposium Challenge of Carbapenem Resistance in Gram Negative Bacteria. El Cairo, Egypt, 2019

-Title: "Plasmid Content of a clinical IC4 *Acinetobacter baumannii* isolate from Bolivia"12TH International Symposium on the Biology of *Acinetobacter*. 2019

- Title: "OXA-23 carbapenemase in clinical isolates of *Acinetobacter baumannii* from Cochabamba, Bolivia" 5TH General Assembly and International Conference Organization for Women in Science for the Developing World. 2016

-Tittle: "Putting gender perspective on the agenda of infectious diseases" IV Encuentro Vasco-Chileno de Investigación Biomédica, Leioa Biscay 2016

-Tittle: “Redes de Colaboración en Investigación Europa-América Latina” III Encuentro Vasco-Chileno de Investigación Biomédica, Santiago de Chile, Chile 2015

Recent accepted projects:

1- “Exacerbation of the Antibiotic Resistance Health Crisis Associated with the Covid Pandemic” Euskampus Resilience COVID-19 Programme. 2021

2- “Emergency and dissemination of resistance to antibiotics: links between human, animal health, food and the environment” Basque Government ELKARTEK Program 2020 KK-2020/00007. From 01/01/2020 to 31/12/2021

3- “Development of methodological tools for assessment of the impact and risk of antibiotic resistance in the environment” Collaborative Projects UPV/EHU (2019) COLAB19/08. From 01/01/2020 to 31/12/2021

4- “Control of the dissemination of resistance to antibiotics among bacteria from different sources through the development of conjugation inhibitors” Research Groups from the University of the Basque Country UPV/EHU (2018) GIU 18/229 From 01/01/2019 to 31/12/2021

5- “Joint Research Laboratory on Environmental Antibiotic Resistance” Laboratorios Transfronterizos UPV/EHU (2019) LAB19/04 From 01/01/2019 to 31/12/2019

5- Ph. Aya Tarek Moustafa Amin Elkholy

- Aya was part timer demonstrator at the Microbiology and Biotechnology Department, , College of Pharmacy, Arab Academy for Science, Technology and Maritime Transport (AASTMT) – Abu Qir Campus for three years before moving to the new branch in Alamein starting from the academic year fall 2020/2021.
- She is a master degree student at faculty of pharmacy Alexandria university, she has already finished her general courses and about to finish my special courses too.
- Aya started to prepare the protocol of my thesis under the supervision of Prof. Elsayed Aboulmagd who is also a team member and planning to consider Dr. Mohammed El-Kholy as an external supervisor.
- The title of the thesis will be oriented to carbapenem resistance ad virulence factors in one of the Gram negative pathogens and she is willing to carry out the practical work in order to detect carbapenemases and virulence factor to get my master degree.
- By applying for the current project grant and gaining the planned, Aya will be able to upgrade her practical skills and perform all the practical works in the favor of her degree, project and the publication of the work in a highly reputable journal.
- Aya's work, experience gained and expected degree obtained will be for the sake of College of Pharmacy, Arab Academy for Science, Technology and Maritime Transport (AASTMT) – Alamein Campus.

Research Team Information Table

Name of Res. Team Member in English	Name of Res. Team Member in Arabic	University / Institute In English	Position / Title	% of time spent on project	No. of months	Incentive per month (LE)	Number of other projects and their IDs	Total % of time spent on other projects	Contact No
Mohammed Abd El-Karim Abd El-Mohsen El-Kholy	د. محمد عبدالكريم عبدالمحسن الخولى	AASTMT/ College of Pharmacy (PI)	Lecturer, College of Pharmacy, AASTMT (Abu Qir)	50	12	2000	-	-	mohammed.elkholy@aast.edu +201003576685
Prof. Evan Ibrahim Saad	ا.د. إيفان سعد	AASTMT/ College of Pharmacy (Co- PI)	Dean, College of Pharmacy, AASTMT, (Alamein)	50	12	1700	-	-	eisaad@yahoo.com +201227311309
Prof. Elsayed Aboulmagd Elsayed	ا.د. السيد ابو المجد السيد	Faculty of Pharmacy, Alexandria University	Prof. of Microbiology & Immunology	30	12	1400	-	-	elsayed20@hotmail.com +201001631869
Prof. Lucía Gallego	ا.د. لوسيا جاليغو	University of the Basque Country/ Faculty of Medicine & Nursing	Prof. of Antibiotics & Molecular Bacteriology	30	12	1400	-	-	lucia.gallego@ehu.eus +34946012778
Aya Tarek Moustafa Amin Elkholy	ص. آية طارق مصطفى أمين الخولى	AASTMT/ College of Pharmacy (Alamein).	Demonstrator, College of Pharmacy, AASTMT	80	12	600	-	-	+201281044280

Project Management

DETAILED PLAN ON PROJECT'S ACTIVITIES (*GANTT CHART*):

Activity Name	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12
1. Collection of clinical isolates from different specimens												
1.1. Isolation and purification of the isolates.												
1.2. Identification of the isolates by conventional microbiological methods.												
1.3. Confirmation of the identification by Vitek.												
2. Antibiotic susceptibility testing												
2.1. Agar diffusion method.												
2.2. Determination of Minimum inhibitory concentration.												
3. Phenotypic and molecular detection of:												
3.1. Carbapenemases												
3.2. Virulence factors.												
4. Sequencing of carbapenemases encoding gene.												
4.1. DNA isolation.												
4.2. DNA sequencing.												
5. Molecular typing of the isolates (MLST)												
6. Cell Line												
7. Preparation of manuscript and publication												

Main Task 1: Responsibility of:
1- Prof. Elsayed Aboulmagd Elsayed.
2- Dr. Mohammed El-Kholy.
3- Ph. Aya El-Kholy.
Main Task 2: Responsibility of:
1- Prof. Elsayed Aboulmagd Elsayed.
2- Dr. Mohammed El-Kholy.
3- Ph. Aya El-Kholy.
Main Task 3: Responsibility of:
1- Prof. Elsayed Aboulmagd Elsayed .
2- Dr. Mohammed El-Kholy.
3- Ph. Aya El-Kholy.
Main Task 4: Responsibility of:
1- Prof. Elsayed Aboulmagd Elsayed .
2- Prof. Lucía Gallego
3- Dr. Mohammed El-Kholy.
4- Ph. Aya El-Kholy.
Main Task 5: Responsibility of:
1- Prof. Elsayed Aboulmagd Elsayed .
2- Prof. Lucía Gallego.
3- Dr. Mohammed El-Kholy.
4- Ph. Aya El-Kholy.
Main Task 6: Responsibility of:
1- Prof. Evan Saad
2- Ph. Aya El-Kholy
Main Task 7: Responsibility of:
Result analysis: Each member is concerned with the analysis of the assigned task.
Scientific writing: Mainly Dr. Mohammed El-Kholy and Ph. Aya El-Kholy with the coordination of the other members
Reviewing: All members

To undertake this project, we have a multidisciplinary team that works in complementary research areas. This collaboration will allow us to undertake more ambitious and innovative projects than those that we could address individually. The governance will be carried out in a shared leadership fashion. The Coordinator will be the ultimately responsible for the appropriate development of the project but there will be a direct communication between the researchers of each part to review periodically the progress of the project to make decisions accordingly. For each task defined partners will be responsible for directing the scientific strategy to achieve the objectives. Regardless that each member of the group will keep the timely follow-up of the tasks and the achievement of objectives, the consortium will set periodic follow-up meetings in which the achievements will be evaluated and the tasks of the next period will be planned.

Allowable Project Costs: Breakdown of Costs Other Grant(s):

Table of Eligible Cost

Eligible costs	Break downs	AASTMT support (L.E.)
(A) Staff Cost	Dr. Mohammed Abd El-Karim El-Kholy. (PI)	24000
	Prof. Evan Ibrahim Saad. (Co-PI) Prof. Elsayed Aboulmagd Elsayed. Prof. Lucía Gallego. Ph. Aya Tarek Moustafa Amin Elkholy.	20400 16800 16800 7200
	Technicians and/or Labor	3600
	Consultation fees	
	Total	**88800**
(B) Equipment	**Equipment**	
	- Conventional PCR (for detection of genes and sequencing).	**75000**
	- Gel Electrophoresis Set (for visualizing and documenting the identified/separated genes).	**45000**
	- Heat Block	**30000**
	- Densitometer (suspension turbidity detector) (McFarland reader) for adjusting bacterial concentrations.	**55000**
	Spare parts	
	Total Equipment	**205000**
(C) Expendable Supplies & Materials	**Stationary**	**5000**
	Miscellaneous Laboratory, Field supplies, Materials	
	PCR Primers	**6000**
	PCR Master Mix	**4000**
	qRT-PCR Master Mix	**5000**
	DNA Extraction Kit	**5000**
	RNA Extraction Kit	**3500**
	Gel Extraction Kit	**2000**
	Plasmid Isolation kit	**4000**
	Restriction Enzymes	**10000**
	DNA Ladder(s)	**1500**
	Micropipettes Set (for PCR)	**7000**
	ATCC bacterial reference strains	**7500**
	Enrichment + Selective + Chrome Agar Culture Media	**29000**
	Plastic Micro Titer Plates	**1500**
	Disposable sterile Petri dishes – Loops - Swabs	**2500**
	Micro-centrifuge Tube Ice Racks	**300**
	Antibiotic discs	**1500**
	Antibiotic Powders	**3000**
	Plastic filter tips	**5000**
	Alcohol (Ethanol)	**900**
	Disinfectants (Soap – Chlor – Dettol)	**1000**
	Others: Glycerol – Tris HCl - PBS	**3000**
	Cryo-tubes / Plastic Racks	**700**
	Cooler box/Ice Packs	**1000**

Eligible costs	Break downs		AASTMT support (L.E.)
	Ethidium Bromide		**1500**
	Boric Acid + Acetic Acid + Sulphuric Acid		**1200**
	Falcon tubes		**350**
	Eppendorfs		**250**
	EDTA		**300**
	Agarose		**1500**
	Total expendable Supplies & Materials		**115000**
(D) Travel	**Internal Transportation:** 1 Visit to Cairo for the PI and MSc. Candidate		**1000**
	Accommodation 2 persons (PI & MSc. Candidate) 3 days		**6000**
	Total travel		**7000**
(E) Other Direct Costs	**Services**	Manufacture of specimens & prototypes	
		Acquiring access to specialized reference sources databases or computer software	
		Computer services	
	Report preparation		**5000**
	Publications & patent Costs		**20000**
	Workshops organization or Training - 1 Training workshop for the PI & MSc. Candidate regarding Molecular Biology Techniques, Cairo University (University Research Park). - 2 seminars organization (1 every 6 months)		**4000**
	Others: Sequencing, Whole genome sequence, Microbial typing, Microbial identification, Electron microscope imaging, etc		**55000**
	Total other direct costs		**84000**
(G) Total Costs			**499800**

Plans for Disseminating Research Results / Sustainability of the action

Dissemination at scientific community level of the project results will be done through communications in international conferences and publications in high impact journals. Likewise, the results will be disseminated through thematic networks. The groups will also be very active at networking, taking part in scientific meetings with external researchers not only from Egypt and Spain but also from other countries to promote both current and new collaborations. Additionally, the internationalization of the results will be supported by our collaborations with leading groups from Germany, Bolivia and United Kingdom. Throughout these years our research groups have developed a large number of national and international collaborations with very relevant groups in the fields of interest for this proposal. Now, in this consortium the network of collaborators will be shared and new synergies will arise. Finally, we will expand our net of collaborators by organizing periodical meetings in which we will invite researchers of interest to improve the results of the Project. Moreover, it is essential that Society receive training and awareness about the problem of the increase of multidrug resistant infections. For this reason, presentations will be done in schools and institutes as well as public talks for citizens. Dissemination articles will be produced in newspapers/magazines, together with talks on radio and TV stations.

The main scientific contribution is that we will generate new knowledge about the factors that determine antibiotic resistance dynamics among bacteria in humans, animals and the environment. This new knowledge will be the fruit of synergy between two interdisciplinary research groups (AAST & UPV/EHU) and the collaborative work with other experts within an integrated approach. However, the medium- and long-term main contribution of this proposal is focused on improving the health and well-being of citizens with the help of the knowledge generated to explore the factors and mechanisms that contribute to the emergence of MDR bacteria and their spread all over the world. The tremendous increase of infections caused by multidrug resistant bacteria affects many areas of modern medicine and future achievements, and it constitutes a major problem of public health. Also importantly, this situation worries our health systems (WHO, national and local government Institutions) and due to the multifactorial nature of the problem (clinical, agricultural-livestock and urban environment) the impact of this proposal is high as indicated by all international institutions as a Challenge "Health, demographic change and well-being". On the other hand, the possibility of applying inhibitors of coupling proteins to agricultural soils opens up the possibility of tackling the transmission of antibiotic resistance via the food chain, thus contributing to Challenge 2 "Safety, food quality; productive and sustainable agricultural activity; sustainability of natural resources, marine and maritime research".

Additionally, the training capacity of the consortium by the formation of young researchers in this topic from a multidisciplinary point of view (pharmacy, microbiology, molecular biology etc.) is undoubtedly one of the impacts to be highlighted in this project. It is worth mentioning that the trajectory of the members of the consortium, documented by the scientific production, leadership and participation in projects and collaborations with numerous national and international groups on subjects related to our project, constitutes a guarantee of the projection of the scientific-technical results that will be generated.
Specific added value is achieved by transnational collaboration between Egypt and Basque Country (Spain) extended not only to research but also for educational activities.

Also, the results of the current project will participate in raising awareness through targeted communications and campaigns aimed at the general public, policymakers, and healthcare providers. It will aid in educating health professionals on appropriate prescribing, and decrease antimicrobial consumption in the health sectors through establishing or improving antibiotic stewardship programmes in healthcare facilities. It will help in developing and distributing educational materials for in-service and pre-service training; and building antimicrobial resistance into national curricula. The project will integrate laboratory and surveillance capacity building for antibiotic sensitivity testing and reporting across human health.

Key Publications and references

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Declaration of original submission and Other Grant(s)

This proposal did not and will not be submitted in whole or part for funding; twice within the same cycle, or to other funding programs within AASTMT, or other funding agencies.

The corresponding PI did not receive any fund or project grants over the last three years.

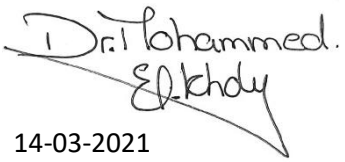
Acknowledgment Form

By signing below, I acknowledge that I have read, understand and accept to comply with all the terms of the foregoing application, mentioned in AASTMT general conditions and guidelines for submitting a research proposal, including, but not limited to:

- The total number of the application pages should not exceed **30 pages** excluding a cover page, as well as all sections of the proposal (as mentioned in AASTMT General Conditions and Guidelines for Submitting Research Proposal). **(From Page 3 to Page 32)**
- At any time, a contracted AASTMT project team member should only be participating in a maximum of 3 projects (or a maximum of 2 projects as a PI).
- Allowable budget maximum limit should be strictly adhered to in the project proposal. In all cases, requested budget has to be justified in detail.
- AASTMT guidelines, IPR rules, code of ethics, etc. (www.aast.edu), should be read carefully and adhered to. These are integral parts of the contract.
- All proposals – in addition to PI and other data - must be uploaded to the AASTMT website by the designated deadline. Uploaded PI data should conform to the corresponding data in the application form. The PI must be a PhD holder.

Applications will not be considered eligible and will be discarded in the following cases:

- Proposals submitted by e-mail or sent as hard copies or uploaded to the AASTMT website after the deadline.
- Proposals not conforming to the designated format.
- Proposals whose uploaded PI data does not conform to PI data in the proposal file.
- Proposals in which the allowable budget maximum limit has been exceeded.
- Proposals in which maximum allowable contracted AASTMT project participation limit has been exceeded.
- Proposal letter does not include a scanned copy of the signed and stamped PI institution endorsement letter in case of team member work outside AASTMT.
- Submitted applications will be evaluated and the applicant will be informed with the evaluation result of his/her proposal within 3-4 months.
- AASTMT technical decisions made by remote reviewers are final.
- Proposal does not include a scanned copy of the signed acknowledgment form.

Date & Signature: 

14-03-2021

Endorsement Letters

Universidad del País Vasco Euskal Herriko Unibertsitatea
NAZIOARTEKO BIKAINTASUN CAMPUSA
CAMPUS DE EXCELENCIA INTERNACIONAL

University of the Basque Country UPV/EHU
Barrio Sarriena s/n
48940 Leioa
Bizkaia, Spain

January 11, 2021

To whom it may concern,

This letter confirms the support of the University of the Basque Country (UPV/EHU) to collaborate with Dr. Mohammed El Kholy from the Faculty of Pharmacy of the Arab Academy for Science, Technology & Maritime Transport (AASTMT), Alexandria (Egypt), in research projects under the Call for Collaboration Research and Innovation Project.

The University will be represented in this activity by the researcher:
Dr Lucía Gallego,
Acinetobacter baumannii Research Group
Antibiotics & Molecular Bacteriology Laboratory
Faculty of Medicine & Nursing
University of the Basque Country UPV/EHU

The researcher has carefully reviewed the application, agrees to the objectives of the project and UPV/EHU´s role and level of participation in it, and is willing and able to complete the activities assigned. It is envisaged that the principal terms of the proposed collaboration would be substantially as follows to achieve the objectives of the AASTMT Grant Call:

- Promote multidisciplinary collaborative research and development projects in the area of antibiotic resistance.
- Support research outputs exploitation and innovation with closer links to regional industries to develop new therapeutic options for treatment of infections caused by resistant bacteria
- Promote the key issues of competitiveness and collaboration
- Support applied research projects that contribute to the achievement of sustainable development
- Raise awareness on the importance of research and innovation in developing new technologies and their economic and social impacts.

Approval of the researcher:	**Approval of Employer: UPV/EHU**
Name: Lucía Gallego	Name: Prof. José Luis Martín
Place/Date: Leioa, 11/01/2021	Place/Date: Leioa, 11/01/2021
Signature: 	Signature: 78865001W JOSE LUIS MARTIN (R: Q4818001B) Firmado digitalmente por 78865001W JOSE LUIS MARTIN (R: Q4818001B) Fecha: 2021.01.11 14:07:51 +01'00'

الأكاديمية العربية للعلوم والتكنولوجيا والنقل البحري
Arab Academy for Science, Technology & Maritime Transport

Alexandria University
Faculty of Pharmacy

جامعة الإسكندرية
كلية الصيدلة

جامعة الإسكندرية
ALEXANDRIA UNIVERSITY
كلية الصيدلة

Date: 08.03.2021

To: Arab Academy for Science, Technology & Maritime Transport

Subject: Endorsement of the project entitled: Co-existence of Antibiotic Resistance and Virulence Factors in Carbapenem Resistant Gram Negative Clinical Isolates from Alexandria, Egypt.

Dear Sir/Madam,

This is to certify that we fully endorse the above mentioned project in which Dr. Elsayed Aboulmagd, Professor of Microbiology, Faculty of Pharmacy, Alexandria University, will serve as a co-investigator. We hereby confirm our complete support of the project and the investigator for the fulfillment of the project following the faculty rules.

Faculty Dean

Prof. Dr. Mervat Amin Kassem

الأكاديمية العربية للعلوم والتكنولوجيا والنقل البحري
Arab Academy for Science, Technology & Maritime Transport

جامعة الإسكندرية
ALEXANDRIA UNIVERSITY

معتمدة بقرار رقم ١٥٥بتاريـــخ ٢٠١٦/٦/٢٧
من الهيـئة القومية لضمان جودة التعليم والاعتماد

كلية الصيدلة
الدراسات العليا والبحوث

إفـــــادة

تفيد كلية الصيدلة – جامعة الاسكندرية بأن الصيدلانية / اية طارق مصطفى امين الخولى (معيدة بكلية الصيدلة – الاكاديمية العربية للعلوم والتكنولوجيا فرع العلمين) مسجلة لدرجة الماجستير في العلوم الصيدلية (الميكروبيولوجيا والمناعة) بتاريخ ٢٠١٩/٨/١٩ واعتمدت الجامعة ذلك بكتابها رقم ٢٨٤ في ٢٠٢٠/٤/٢٧ وتم تسجيل موضوع البحث بتاريخ ٢٠٢١/٢/١٦

موضوع البحث :

"التعايش بين مقاومة المضادات الحيوية وعوامل الضراوة فى عزلات الكليبسيلا نيمونيا السريرية المقاومة للكاربابينيم المعزولة من الاسكندرية "

تحت اشراف:

السيد الأستاذ الدكتور / السيد ابوالمجد السيد (المشرف الرئيسي) استاذ بقسم الميكروبيولوجيا والمناعه كلية الصيدله - جامعة الاسكندرية

السيدة الأستاذ الدكتور / هدى محمد جمال الدين عمر استاذ ورئيس مجلس قسم الميكروبيولوجيا والمناعه كلية الصيدله - جامعة الاسكندرية

السيد الدكتور / محمد عبدالكريم الخولى مدرس بكلية الصيدلة – جامعة الاكاديميـة العربية للعلوم والتكنولوجيا فرع العلمين

وقد اعطيت لها هذه الإفادة بناء علي طلبها وذلك لتقديمها لجهة الأختصاص

وكيل الكليـة للدراسات العليا والبحوث

أ.د هالة مصطفي حمودة

تحريرا في ٢٠٢١/٣/١١
الموظف المختص :

ميدان الشهيد – الأزاريطة – الإسكندرية – جمهورية مصر العربية – صندوق بريد ٢١٥١١ المنشأة شحون : 4871668 /203+ – فاكس : 4873273 /203+
Web Site: www.alexu.edu.eg E-Mail: Postgraduate department@gmail.com

جامعة الإسكندرية
ALEXANDRIA UNIVERSITY
كليـــة الصيـــدلة
قسم الميكروبيولوجيا والمناعة

السـيدة الاسـتاذ الدكتور / وكيـل الكليـة لشئـون الدراسـات العليـاوالبـحوث

تحيـــة طيبـــة وبعـــــد،،،

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برناسة أ.د. هدى محمد جمال الدين عمر

علـي تسجيـل برتوكـول ص/ آية طـارق مصطفـى أميـن الخـولى

معـيدة بكليـة الصيـدلة – الاكاديميـة العربيـة للعلـوم والتكنولوجيـا فـرع العلميـن لدرجـة الماجستيـرفـى العلـوم الصيدليـة (ميكروبيولوجيـا ومنـاعة) . تحت عنـوان :-

باللغـة العربيـة :

" التعايش بين مقاومة المضادات الحيوية وعوامل الضراوة فى عزلات الكليبسيلا نيمونيا السريرية المقاومة للكاربابينيم المعزولة من الاسكندرية "

باللغـة الانجليزيـة :

"Co-existence of antibiotic resistance and virulence factors in carbapenem resistant *klebsiella pneumoniae* clinical isolates from Alexandria "

لجنة الاشراف :

1-أ.د/ السيد ابو المجد السيد استاذ بقسم الميكروبيولوجيا والمناعة كلية الصيدلة - جامعة الاسكندرية (المشرف الرئيسى)

2- أ.د/ هدى محمد جمال الدين عمر استاذ ورئيس مجلس قسم الميكروبيولوجيا والمناعة كلية الصيدلة – جامعة الاسكندرية

3- د./ محمد عبد الكريم الخولي مدرس كلية الصيدلة - الاكاديمية العربية للعلوم والتكنولوجيا فرع العلمين

وتفضلـوا بقبـول فائـق الاحتـرام والتقديـر ...

أ.د. رئيـس مجـلس القسـم

أ.د. هدى محمد جمال الدين عمر

١ ميدان الخرطوم - الأزاريطة - الإسكندرية - جمهورية مصر العربية- بريد السلة 21521
تليفون / فاكس :002/03/4871668
Website: www.alexu.edu.eg Email: phar-vde@alexu.edu.eg