ANTIBACTERIAL AND ANTIBIOFILM PROPERTIES OF *Salvadora persica* EXTRACTS AGAINST Carbapenem-resistant *Acinetobacter baumannii* ISOLATED FROM ENDOTRACHEAL TUBE SAMPLES

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by

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LIST OF SYMBOLS

%	Percentage
>	More than
<	Less than
≥	More than or equal to
≤	Less than or equal to
°C	Degree Celsius
=	Equal to
-	Minus
+	Addition
±	Plus – minus
/	Division or ‘or’
µ	Micro
x	Times or multiplication
ɣ	Gamma

LIST OF ABBREVIATIONS

ANOVA	Analysis Of Variance
AST	Antibiotic Susceptibility Test
ATCC	American Type Culture Collection
BLAST	Basic Local Alignment Search Tool
bp	Base pair
CFU	Colony Forming Unit
CHX	Chlorhexidine
CLSI	Clinical & Laboratory Standard Institute
CV	Crystal violet
CRAB	Carbapenem-resistant *Acinetobacter baumannii*
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
DMSO	Dimethyl sulfoxide
et al.	*Et al*ia (and others)
EDTA	Ethylene-diamine-tetra-acetic acid
ETT	Endotracheal tube
g	Gram
GC-MS	Gas Chromatography-Mass Spectrometry
HUSM	Hospital Universiti Sains Malaysia
ICU	Intensive Care Unit
IHR	International Health Regulations
IMP	Imepenem
IMR	Institute for Medical Research
IPC	Infection prevention and control
JEPeM	Jawatankuasa Etika Penyelidikan Manusia
l	Liter
mg	Milligram
ml	Milliliter
MIC	Minimum inhibitory concentration
MBC	Minimum bactericidal concentration
MBIC	Minimum biofilm inhibitory concentration
MBEC	Minimum biofilm eradication concentration

MDR	Multidrug-resistant
MEM	Meropenem
MHA	Meuller Hinton Agar
NaCl	Sodium Chloride
NCBI	National Center for Biotechnology Information
NIH	National Institutes of Health
NIST	National Institute of Standards and Technology
NP	Nanoparticle
NSAR	National Surveillance for Antibiotic Resistance
OD	Optical density
pH	Potential of Hydrogen
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PRN	Pusat Racun Negara
RT	Retention time
rpm	Revolution per minute
spp.	Species
SPSS	Statistical Package for Social Science
Taq	Thermus aquaticus
TBE	Tris-Borate EDTA
UK	United Kingdom
UMK	Universiti Malaysia Kelantan
UMMC	University of Malaya Teaching Centre
USA	United States of America
USM	University Science Malaysia
UV	Ultraviolet
VAP	Ventilator-Associated Pneumonia
WHO	World Health Organization
±SEM	Standard Error of the Mean
CuO	Copper (II) oxide
H_3PO_4	Phosphoric acid
SiO_2	Silicon dioxide

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Appendix A List of consumables and laboratory equipment

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CIRI-CIRI ANTIBAKTERIAL DAN ANTIBIOFILM EKSTRAK *Salvadora persica* TERHADAP *Acinetobakter baumannii* Rintang carbapenem ISOLASI DARIPADA SAMPEL TIUB ENDOTRAKEAL

ABSTRAK

Acinetobacter baumannii rintang carbapenem (CRAB) adalah salah satu patogen yang sukar dibasmi dari persekitaran dan menentang semuaantibiotik. Kesannya, CRAB boleh menyebabkan jangkitan yang serius kepada pesakit yang sedang dirawat di hospital. Akar *Salvadora persica* telah digunakan sebagai bahan untuk menjaga kebersihan mulut dan Organisasi Kesihatan Sedunia (WHO) telah mengesyorkan penggunaannya. Ia mempunyai potensi untuk digunakan dalam penyelidikan dan kajian yang berkaitan dengan perubatan. Kajian ini bertujuan untuk menunjukkan sifat antibakteria dan antibiofilem keatas ekstrak pelarut yang paling berkesan dari *S. persica* terhadap CRAByang diasingkan daripada sampel tiub endotrakea (ETT). Tiga pelarut yang berbeza (heksana, kloroform, dan methanol) digunakan dalam *Soxhlet apparatus* untuk mengekstrak *S. persica*. Kromatografi gas-spektrometri jisim (GC-MS) digunakan untuk menganalisis setiap ekstrak, dan dicirikan oleh pangkalan data perpustakaan. Asid benzoat, asid lemak, alkohol, dan vitamin Eadalah antara sebatian fitokimia yang ditemui dalam pelbagai ekstrak pelarut *S. persica* yang telah dianalisis oleh GC-MS. Asid benzoik adalah sebatian utama yang ditemui dalam ekstrak heksan dan kloroform, manakala gliserol adalah sebatian utama yang ditemui dalam ekstrak metanol. Ujian mikrodilusi kaldu digunakan untukmenilai aktiviti antimikrob ekstrak terhadap CRAB untuk menentukan nilai kepekatan perencat minimum (MIC). Ujian kepekatan bakterisida minimum (MBC) digunakan untuk menentukan kepekatan yang diperlukan untuk membasmi CRAB. Sementara, ujian kepekatan minimum perencat biofilem (MBIC) dan ujian kepekatan minimum pembasmian biofilem (MBEC) digunakan untuk

mengenal pasti aktiviti antibiofilem CRAB. Kemudian, ultrastruktur biofilem dianalisis menggunakan mikroskopi elektron imbasan (SEM). Heksan telah dipilih sebagai pelarut yang paling efisien kerana nilai MIC dan MBC ekstrak heksan menunjukkan nilai terendah, iaitu masing-masing 1.56-3.13 mg/ml dan 6.25-12.50 mg/ml. Sementara itu, nilai MBIC dan MBEC masing- masing bernilai 6.25 mg/ml dan 62.5-125.00 mg/ml. Aktiviti antimikrob danantibiofilem dari semua CRAB dipilih (n=20) telah dikenal pasti (MIC=1.56-6.25 mg/ml; MBC=3.13-12.50 mg/ml; MBIC=6.25-25.00 mg/ml; MBEC=12.50-62.50 mg/ml). Ujian SEM juga menunjukkan kesan pengurangan struktur selepas dirawat dengan ekstrak. Kesimpulannya, pelbagai kompaun fitokimia yang mempunyai sifat antibakteria diekstrak daripada pelarut yang berbeza. Dari kajian ini, ekstrak yang mengandungi heksan telah menunjukkan sifat antibakteria dan antibiofilem yang paling berkesan terhadap CRAB. Dengan menggunakan pelarut heksan, hasil menunjukkan sifat antibakteria dan antibiofilem yang signifikan terhadap CRAB.

ANTIBACTERIAL AND ANTIBIOFILM PROPERTIES OF *Salvadora persica* EXTRACTS AGAINST Carbapenem-resistant *Acinetobacter baumannii* ISOLATED FROM ENDOTRACHEAL TUBE SAMPLES

ABSTRACT

Carbapenem-resistant *Acinetobacter baumannii* (CRAB) is one of the pathogens that is difficult to eradicate from the surrounding and resist all antibiotics. As a result, CRAB may cause serious and deadly infections in patients under treatment in hospitals. *Salvadora persica (S. persica)* roots have been used for maintaining oral hygiene and the World Health Organization (WHO) has recommended them. It has the potential to be used in research and studies related to medicine. This study aimed to elucidate the antibacterial and antibiofilmproperties of the most effective solvent extract of *S. persica* roots against CRAB isolated from endotracheal tube (ETT) samples. Three different solvents (hexane, chloroform and methanol) were used in the Soxhlet apparatus to extract *S. persica*. Gas chromatography-mass spectrometry (GC-MS) was used to analyze each extract, and characterized by library databases. Benzoic acid, fatty acids, alcohols, and vitamin E were among the phytochemical compounds found in the various solvent extractsof *S. persica* that had been analyzed by GC-MS. Benzoic acid was the main compound found in hexane and chloroform extract, while glycerol was main compound found in methanol extract. Broth microdilution assay was used to evaluate the antimicrobial activity of extracts against CRAB todetermine the minimum inhibitory concentration (MIC) values. While, minimum bactericidal concentration (MBC) assay was used to determine the concentration needed to eradicate CRAB. The Minimum biofilm inhibition concentration (MBIC) assay and minimum biofilm eradication concentration (MBEC) assay were used to identify the antibiofilm activity of CRAB. Then, the

ultrastructure of the biofilm was analyzed using scanning electron microscopy (SEM). Hexane was selected as the most efficient solvent due to their lowest MIC and MBC values (1.56-3.13 mg/ml and 6.25-12.50 mg/ml, respectively). While, MBIC and MBEC values were 6.25 mg/ml and 62.5-125.00 mg/ml, respectively. Antimicrobial and antibiofilm activity of all selected CRAB (n=20) were identified (MIC=1.56-6.25 mg/ml; MBC=3.13-12.50 mg/ml; MBIC=6.25- 25.00 mg/ml; MBEC=12.50-62.50 mg/ml). SEM shows reduction effect after treated with the extract. In conclusion, various phytochemical compounds that have antibacterial properties were extracted from different solvents. From this study, extracts containing hexane demonstrated the most effective antibacterial and antibiofilm properties against CRAB. By using hexane solvent, the results show significant antibacterial and antibiofilm properties against CRAB.

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Plants or herbs were extracted and used as traditional medicine for the treatment of many diseases because they have performed a biological effect including antimicrobial and antibiofilm activity (Haque & Alsareii, 2015; Jasim *et al.*, 2018). Various bioactive phytochemicals of the plants were extracted and have been identified. One of the plant parts that has been widely used is roots of *Salvadora persica* (Miswak). Studies on the activity of miswak extract could provide some novel antibiofilm compounds to fulfill the need for therapies for hospital-acquired infections such as pneumonia and ventilator- associated pneumonia (VAP) caused by CRAB which colonized the endotracheal tube (ETT) of patients with VAP. CRAB tends to produce biofilm which protects them from the action of antibiotics.

Roots of *S. persica* extracts are known to have activity against cariogenic bacteria but little is known on CRAB. The phytochemicals of *Salvadora persica (S. persica)* have potent antibacterial and antibiofilm activities, however, the phytochemical compounds' antibacterial and antibiofilm activities are influenced by the extraction solvents. Therefore, this study aims to elucidate the antibacterial and antibiofilm properties of hexane, chloroform, and methanol extract of *S. persica* roots against CRAB. *S. persica* extracts were prepared using various solvents. The active phytochemical compoundswere determined by GC-MS.

The antibacterial and antibiofilm activities against CRAB (ATCC samples as control and clinical isolates) were performed. The antibacterial activities were determined by the broth microdilution method to determine the minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC). The antibiofilm

activities were determinedby minimum biofilm eradication concentration (MBEC) assay and minimum biofilminhibition concentration (MBIC) assay (Avila-Novoa *et al*., 2019).

Acinetobacter is a genus of bacteria that is commonly found in the environment, including soil and water. Among *Acinetobacter* species, the most frequent source of infections is *Acinetobacter baumannii (A. baumannii)* which is responsible for the majority of Acinetobacter infections in humans (Antunes *et al*, 2014; Khoshnood *et al.,* 2023). *A. baumannii* shown in Figure 1.1 is an opportunistic Gram-negative, aerobic, bacillus, and non-motile *pathogen*. This pathogen is ubiquitous, free-living, and small with the ability to colonize healthy or damaged tissues in the human body (Howard *et al*., 2012; Lee *et al*., 2017; Khoshnood *et al.*, 2023). Thus, they are among the most significant nosocomial pathogens, especially in immunocompromised hosts. It is also responsible for a widerange of diseases, the most prevalent of which include ventilator-associated pneumonia (VAP), urinary tract infections, and bloodstream infections. Depending on the type of infection and the genetic makeup of the bacterial strain, the death rateis alarming and can reach up to 35% (Cavallo *et al*., 2023).

Streptococcus mutans (S. mutans) is a facultatively anaerobic, coccus, and Gram-positive bacterium. This pathogen is naturally present in human oral microbiota. Colonization of *S. mutans* on the dental surface will cause damage to the tooth structure in the presence of fermentable carbohydrates like sucrose and fructose. *S. mutans* shown in Figure 1.2 are associated with pyogenic and other infections in the mouth, heart, skin, central nervous system, and muscle. Since it is present in the oral cavity and the multispecies biofilms on the tooth surface, it is also known as a cariogenic bacterium.

S. mutans is a dominant species in dental biofilms than other Streptococcus species (Ahrari *et al.*2015). Due to the *S. mutans* acid tolerance and capability of this pathogen to live in the environment of oral cavities with low pH, *S. mutans* ATCC 25175 was used as apositive control strain since it is the main oral pathogen related to oral infections. The initiation of infections and tooth decay occurs when *S. mutans* make contact with sugar-containing products in the mouth. *S. mutans* can be an opportunistic pathogen,initiating disease and damaging the host. The previous study has been tested for activities against *S. mutans* (Veloz *et al*., 2019).

Acinetobacter baumannii (A. baumannii) ATCC 19606 and *Streptococcus mutans (S. mutans)* ATCC 25175 were used as positive controls. *A. baumannii* ATCC 19606 were purchased as the standard test strains for CRAB. *A. baumannii* ATCC 19606 genome encodes 69 virulence genes, including the resistance genes being studied (*blaOXA-23* and *blaNDM-1*) and genes associated with biofilm formation (*bap, ompA,* and *csuE*). This organism has been used as a quality control organism to study anti biofilms and antibacterial activity of CRAB. It has been fully characterized by Yan Zhu *et al*., 2020. The strains were only used for a maximum of five passages. The other control strains that were used were isolated from two clinical strains of CRAB from the Microbiology Laboratory, School of Medical Sciences.

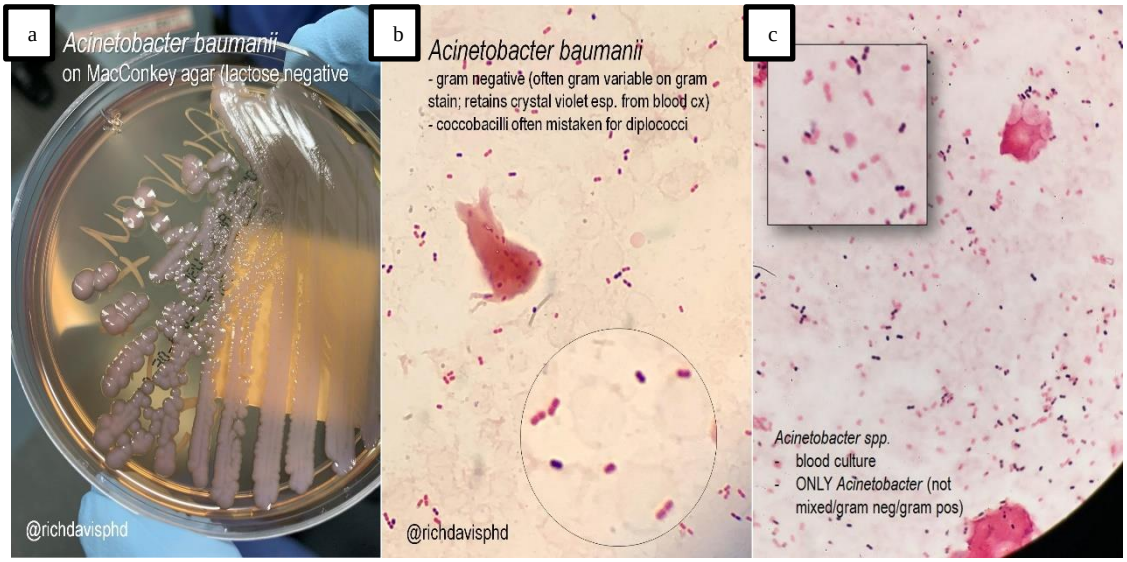


Figure 1.1 *A. baumannii;* (a) Complex streak overnight growth on MacConkey agar at 37°C. (b) Gram-stained *A. baumannii* cells. (c) Blood culture. (Adopted from GrepMed/ Rich Davis, PhD)

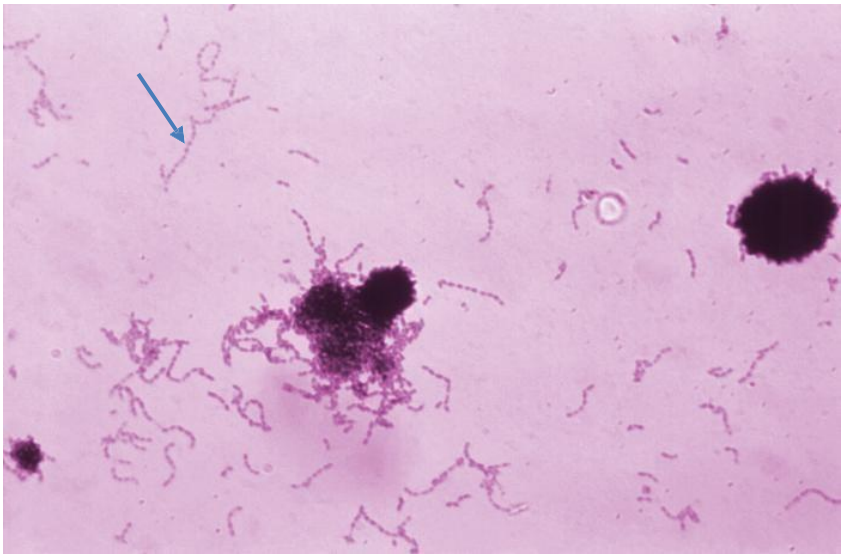


Figure 1.2 *Streptococcus mutans*. Gram-stained in thioglycolate broth culture specimen, Gram-positive, streptococcus bacteria (arrow). (Adopted from CDC/ Dr. Richard Facklam)

Mouthwash or oral rinse is a liquid product that is used as a supplementary cleansing tool other than a toothbrush and toothpaste. It is used to rinse teeth, gums, and mouth for dental hygiene practice. Mouthwash is also used to fight against bad breath, freshen the breath, whiten the teeth, and prevent tooth decay. Moreover, mouthwash usually contains antiseptic and disinfectants like chlorhexidine (CHX) that can kill harmful bacteria in the mouth. There are plenty of mouthwashes on the marketplace currently, however, CHX has been identified as the most effective chemical agent for reducing plaque (Amoian *et al*., 2017).

CHX mouthwash was used as a positive control treatment. It is primarily prescribed by dentists to treat the bleeding, swelling, and inflammation associated with gingivitis. CHX can be categorized as an antibacterial medication. CHX works by reducing the amounts of bacteria in the mouth, it helps to lessen gum inflammation, bleeding when you brush, and swelling. The strong efficacy of CHX, a cation bisbiguanide compound, has been related to its bactericidal and bacteriostatic properties as well as its high substantivity in the oral cavity. Due to its low absorption from the digestive system, CHX does not result in systemic toxicity.

Scanning electron microscopy was utilized to visualize the ultrastructure effect of biofilm. Scanning electron microscopic (SEM) is a type of electron microscope that creates images of the samples by scanning the sample's surface using a concentratedelectron beam. The interaction of the atoms and the electrons in the sample producedvarious signals that carry information about the surface topography and chemical composition of the sample (Koga *et al*., 2021). The electron beam is scanned in a raster scan pattern, and the position of the electron beam is combined with the strengthof the signal to create an image.

1.2 Significance of Study

Biofilm forms a biological scaffold that protects bacteria from antimicrobials. Bacteria in the biofilm are 1000-fold more resistant to antibiotics compared to their planktonic state. The formation of biofilm is shown in Figure 1.3.

CHX mouthwash at a concentration of 0.2% - 2% has been used to reduce the organism burden of CRAB which commonly colonized the ETT of patients. However, previous studies differ in opinion on its efficacy in reducing bacterial colonization (La Combe *et al*., 2018; Rabello *et al*., 2018). According to Tran & Butcher's comprehensive review in 2019, nosocomial pneumonia and VAP could not be reliably prevented with CHX, regardless of strength, preparation, or frequency of use.

There was insufficient data to establish a clear correlation between CHX and shorter the durations of ventilation, shorter ICU patient stays, antibiotic sensitivitiesor indicators of oral health (Gunasekera & Gratrix, 2016; Waters & Muscedere, 2015). Disorders of the oral cavity were a common side effect linked to CHX. Considering the uncertainties surrounding the risk-benefit balance of CHX dental care, current guidelines do not offer any explicit advice. Hence, the need to find analternative mouthwash is inevitable.

Antibiotic resistance is a widespread phenomenon. Excessive use of antibiotics can lead to an increase in antibiotic resistance among microorganisms. Governments, the healthcare industry, and the general public need to pay attention to this growing threat to global public health. When bacteria can grow in inhibitory antibiotic concentrations, antibiotic resistance occurs. Thus, the development of novel antimicrobials become a significant challenge.

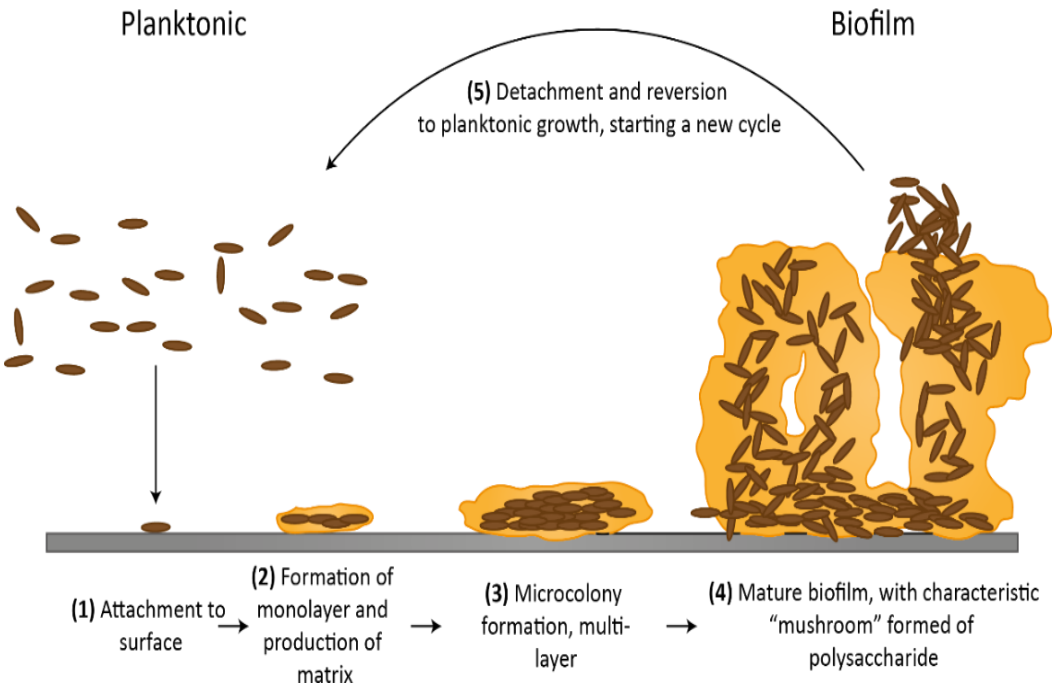


Figure 1.3 Formation of biofilm. (1) Reversible attachment between the planktonic bacteria (brown ovals) and the substrate through a specific interaction with the surface (grey). (2) The bacteria form a monolayer and irreversibly attach by producing an extracellular matrix component. (3) Multilayer appears as the formation of microcolony. (4) As the colony grows and the biofilm matures, it forms characteristic mushroom-like structures due to polysaccharides. (5) Biofilm capable of dispersing into the environment as some cells start to detach. (Adopted from Trafny, 2008).

Herbal medicine has been practiced by 80% of the world's population for centuries for various treatments (Ekor, 2014; Siddeeqh *et al*, 2016; Al-Judaibi, 2020). A crucial element of the traditional medicinal system remains to be the utilization of plants as therapeutic agents. The study of medicinal plants in order tocreate such chemicals has gained popularity in recent years.

S. persica is one of the plants that has high potential in research for novel antimicrobials for infectious diseases. *S. persica* twigs and roots also known in the general population mostly among Malay and Arabic as miswak, have been used as toothbrushes for more than 1000 years (Haque & Alsareii,2015; Aumeeruddy *et al.*, 2018). Nowadays, it is also widely used as an herbal medicine for the treatment of human diseases that are used by 80 % of the global population (Al-Judaibi, 2020).

This medical plant can be used for protection against pathogenic dental biofilms of cavity-causing bacteria. Several studies have been reported on its traditional uses, phytochemistry, pharmacological properties, and potential bio application in various fields. In the previous studies extracts of *S. persica* possess a lot of biological activities, including significant antimicrobial and anti-inflammatory properties and lack of toxicity (Ibrahim *et al*., 2011; Al-Sohaibani & Murugan, 2012; Balto *et al.*, 2014; Balto *et al*., 2017). To prepare the extraction of *S. persica*, various methods have been used especially aqueous and alcohol extracts (Al-Bayati & Sulaiman, 2008). Different *S. persica* extract preparation methods, concentrations,and bacterial species affected the results of the experiment.

Chemical profile study is crucial for the purpose of identifying the pharmacological effects, recognizing between geographical sources, and assessing the quality of herbal medications (Hendriks *et al.*, 2005; Lee *et al*., 2016). Research on *S. persica* found the following bioactive compounds: sulfur, chloride, fluorides, alkaloids, phosphorus, tannins, vitamin E, silica, butanediamide, and benzyl

isothiocyanate (Akhtar *et al.*, 2011; Balto *et al*., 2017).

In a normal population, significant reductions in the cariogenic number of bacteria and plaque score were seen upon the application of 7 % *S. persica* extract, however, this concentration is inferior to 0.2 % CHX mouthwash (Jassoma *et al.*, 2019). Balto *et al* study in 2017 discovered that the ethanol extract exhibited strong antibacterial and antibiofilm properties against cariogenic bacteria (Balto *et al*., 2017). Abdel-Kader *et al*, 2019 found that cold chloroform is the best method to preserve benzyl isothiocyanate, the major active antibacterial metabolite for both Gram-negative and Gram-positive (Sofrata *et al*., 2011; Abdel-Kader *et al*., 2019).

A mouthwash of aloe vera gel extract combined with *S. persica* ethanol extract works better than CHX in lowering the gingival inflammation score of intubated patients who are hospitalized in the ICU (Rezaei *et al.*, 2016). An in vitro study shows that ethanol extracts inhibit the growth of selected Gram-negative organisms. However, *A. baumannii* was not included in the study (Al-Judaibi, 2020). Until today, we still lack knowledge on how *S. persica* extracts will affect the multiple drug-resistant Gram-negative organisms that commonly colonized the ETTincluding CRAB. This study aims to elucidate the antibacterial and antibiofilm activities of *S. persica*. Hexane, methanol, and chloroform extracts against CRAB that colonize the ETT.

On the other hand, drug resistance is a major global concern, as it makes the treatment of these infections much more difficult, leading to higher mortality rates. Healthcare workers in hospitals are particularly at risk due to contact with infected patients, contaminated hospital environments, and inadequate infection control practices, as well as a high rate of abuse of medical instruments and devices. Therefore, it is important to identify new supplement to current treatment options that can help mitigate the risk of infections caused by *A. baumannii*.

1.3 Rationale of Study

The main concern with antibiotic treatments for bacterial infections is the development of drug resistance. This has led to a rise in the incidence of infections driven by drug-resistant bacterial strains, including CRAB, which make it harder for conventional antibiotics to treat them. Antibiotic side effects have been reported in addition to the development of drug resistance (Howard *et al.*, 2012; Lee *et al.*, 2017; Manchanda *et al.*, 2010). Antibiotics are commonly used to treatbacterial infections.

Symptoms of diarrhea, vomiting, and nausea are the most common side effects of antibiotic use, and they are frequently brought on by changes in the digestive tract's natural microbes. Antimicrobials can also cause changes in liver function by causing a decrease in the production of certain enzymes, which can have a direct influence on the liver's ability to filter impurities from the blood. Inaddition to these side effects, allergic reactions to certain antibiotics may occur in some individuals. The most serious side effect of antibiotic use is toxicity or anaphylaxis, which occurs when a person's immune system rejects the antibiotic (Lee *et al*., 2017).

Antibiotic use also can result in changes to the amounts of good bacteria in the human body as well as the development of antibiotic-resistant bacterial strains. This is of special concern regarding formerly drug-susceptible pathogens,such as CRAB, as the antibiotic resistance of these bacteria means that conventional antibiotics are no longer effective against them.

Even though antibiotic resistance is a normal phenomenon, antibiotic-resistant bacteria can evolve more easily when antibiotics are overused or misused (Sherif *et al.* 2021; Whiteway *et al*., 2022). In recent years, the use of *S. persica* extract has been studied as an alternative treatment option for treating CRAB infections. Originating in North Africa and the Middle East, *S. persica* is a medicinal plant that has been used for centuries as an antiseptic, a soothing agent, and a form of diuretic (Halawany, 2012; Haque & Alsareii, 2015).

The development of CRAB strains has raised global concern, as therapeutic options are limited (Sherif *et al.*, 2021). Drug resistance is generally achieved by mutational changes in the chromosome and plasmids and by the adaptability of the bacteria to frequently changing environmental conditions (Avila-Novoa *et al*., 2019; Manchanda *et al*., 2010; Sherif *et al*., 2021) The emergence of Carbapenem resistance strains of *A. baumannii* has been linked to the high selective pressure of improper antibiotic use and the increased number of immunocompromised patients in the hospital. This widespread introduction of the resistant strain will only continue to increase, resulting in a significant increase in mortality due to severe infections.

1.4 Research Questions

1. How does type of solvents affect the phytochemical properties of *S. persica* extracts?
2. How does the type of solvents and concentration of *S. persica* extracts affect their antibacterial and antibiofilm properties against CRAB?
3. How does *S. persica* extract affect different microbiological profiles of CRAB clinical isolates?

1.5 Objectives of Study

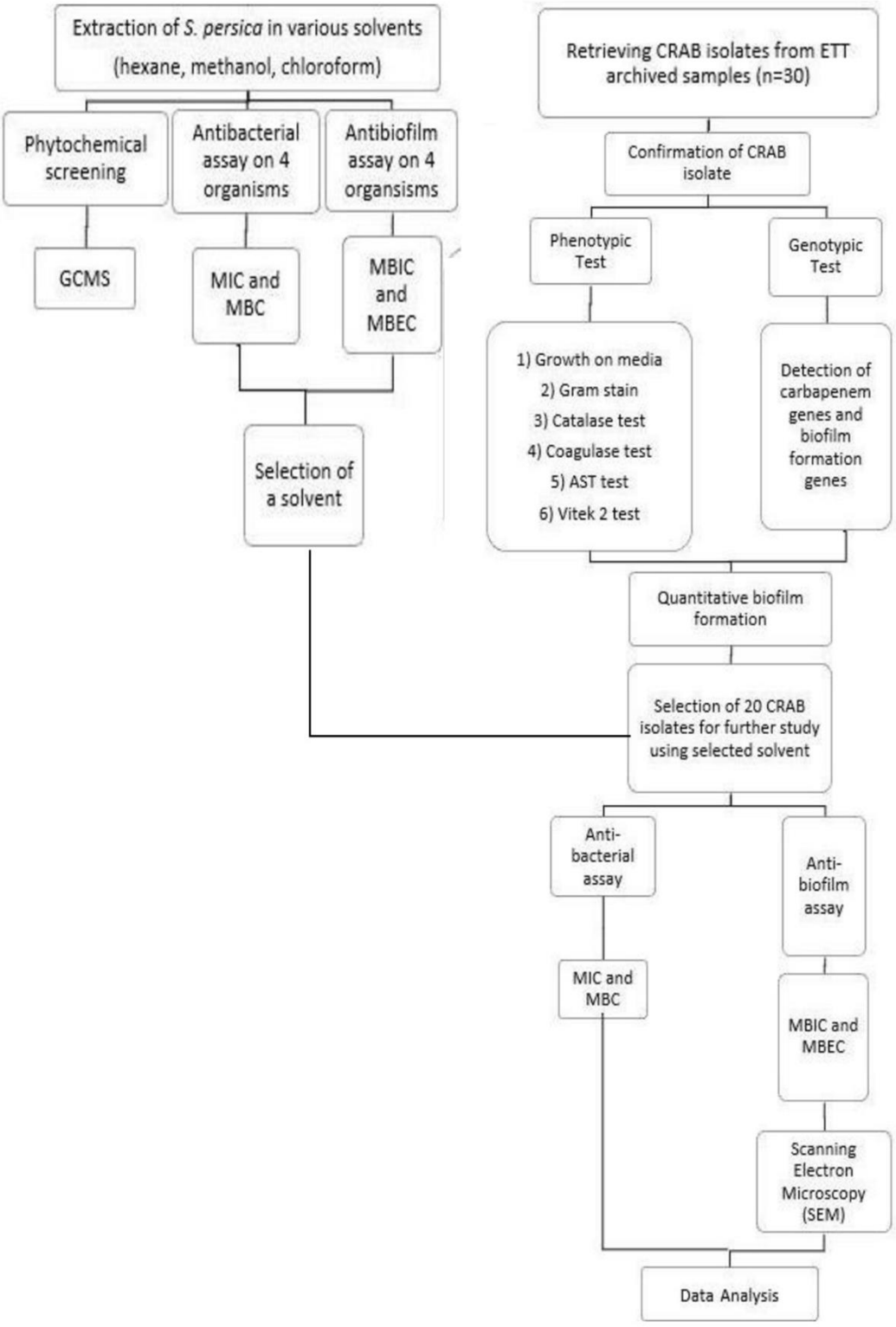
1.5.1 General Objective

To elucidate the antibacterial and antibiofilm properties of hexane, chloroform, and methanol extract of *S. persica* against CRAB isolated from ETT samples.

1.5.2 Specific Objectives

1. To elucidate phytochemical properties of *S. persica* extract using different solvents.
2. To determine the effect of antibiofilm and antibacterial activity of *S. persica* extract using different solvents against CRAB.
3. To determine the antibiofilm and antibacterial activity of selected *S. persica* extract against various clinical strains of CRAB.

1.6 Flow of Study



CHAPTER 2

LITERATURE REVIEW

2.1 *Acinetobacter baumannii (A. baumannii)*

A. baumannii is a Gram-negative, rod-shaped bacterium that is found in various environmental settings. It is an opportunistic pathogen and a major causative agent of serious nosocomial infections worldwide (Howard *et al*., 2012).

A. baumannii is capable of surviving in hostile environments, displaying multiple drug resistance and an extraordinary ability to persist within healthcare-associated environments. *A. baumannii* is considered an "intelligent survivor" due to its ability to respond to environmental fluctuations and rapidly transmit plasmid-associated-resistance determinants which contribute to its high prevalence in both community as well as hospital settings (International Infectious Diseases Society for Clinical Microbiology and Infectious Diseases, 2018).

A. baumannii was previously, a benign organism that was mainly found in our surroundings, especially in soil and water-wet areas such as hospital sinks. Nowadays this microorganism developed as a crucial nosocomial pathogen causing multiple problems in the healthcare system, particularly in the intensive care unit (ICU) (Chang *et al*., 2015). The microorganism is especially adept at colonizing the sites of colonization (tubing used in medical procedures, ventilatory equipment, and catheters) leading to an increased risk of nosocomial infections (Jasim *et al.,* 2018; Whiteway *et al*., 2022). *A. baumannii* can form biofilm.

Increased prevalence of drug-resistant *A. baumannii*, including CRAB, has become a major clinical threat due to the intrinsic of the bacteria and acquired resistance mechanisms. The ability of *A. baumannii* to resist several drugs is due to

various resistance factors, such as the existence of extended-spectrum β-lactamases and biochemical enzymes which expressed by the bacteria, along with efflux pumps which help the removal of harmful substances from the bacterial cell (Rice, 2006). Furthermore, CRAB can exist as biofilms increases its resistance to antimicrobials, and serves as a protective mechanism against potential elimination (Nguyen & Joshi, 2021).

Recent studies have focused on strategies for combating drug-resistant strains of *A. baumannii*. Such strategies include the use of alternative antibiotics, plant extracts, and bacteriophages. Antibiotic monotherapy is often ineffective because of the highly complex microbiota of nosocomial infections and is increasingly losing its efficacy. Therefore, the problem of the resistance of *A. baumannii* towards antibiotics often necessitates the search for effective alternative therapies (Al-Ayed *et al*., 2016).

2.1.1 Taxonomic Classification

Taxonomically, the genus *Acinetobacter* belongs to the family known as Moraxellaceae. The details taxonomy hierarchy of *A. baumannii* is shown in Table 2.1 (Howard *et al*., 2012).

Table 2.1 Taxonomy and classification of *A. baumannii*

Rank	Name
Kingdom	Bacteria
Phylum	Proteobacteria
Class	Gammaproteobacterial
Order	Pseudomonadales
Family	Moraxellaceae
Genus	*Acinetobacter*
Species	*Acinetobacter baumannii*

2.1.2 Morphology of *A. baumannnii*

A. baumannii is a Gram-negative coccobacillus bacterium found in the soil and water, and it prefers moist environments. *A. baumannii* is a hospital-acquired pathogen. (Clinical Microbiology Reviews, 2019). This microorganism was able to grow well at 37 °C on routine solid media, such as sheep blood agar. *A. baumannii* is a non-motile, non-spore-forming organism that shows poor cultural and biochemical characteristics. Unlike many bacteria, *A. baumannii* can survive in diverse and hostile climates, exhibiting extreme resilience against temperatures, pH, detergents, and antiseptics (Manchanda *et al*., 2010). When grown on culturalplates, *A. baumannii* colonies are smooth and circular, measuring 1-2 mm in diameter (Howard *et al*., 2012). The colonies are shown in Figure 2.1.

A. baumannii also contains a wall-less LPS (Lipopolysaccharide) layer that is surrounded by another outer capsule layer composed of lipopolysaccharide and teichoic acid (Antunes *et al*., 2014). Cellular components such as enzymes and toxins are also contained in the outer capsule layer. This type of capsule layer allows the bacterium to adhere to the surfaces of endotracheal tubes, providing an additional protective layer against environmental stresses (Antunes *et al*., 2014).

Cell surface structures such as fimbriae, flagella, and pili are also important components of *A. baumannii* and help enable the bacterium to move around and adhere to surfaces (Singhi & Srivastava, 2020). Fimbriae are small protein-based structures that allow the bacterium to adhere firmly to surfaces. Flagella are filamentous organelles that allow the bacteria to move around. Pili are short appendages that are involved in specific interactions between bacteria, with the most common type being the sex pili which are responsible for interbacterial conjugation (Singhi & Srivastava, 2020).

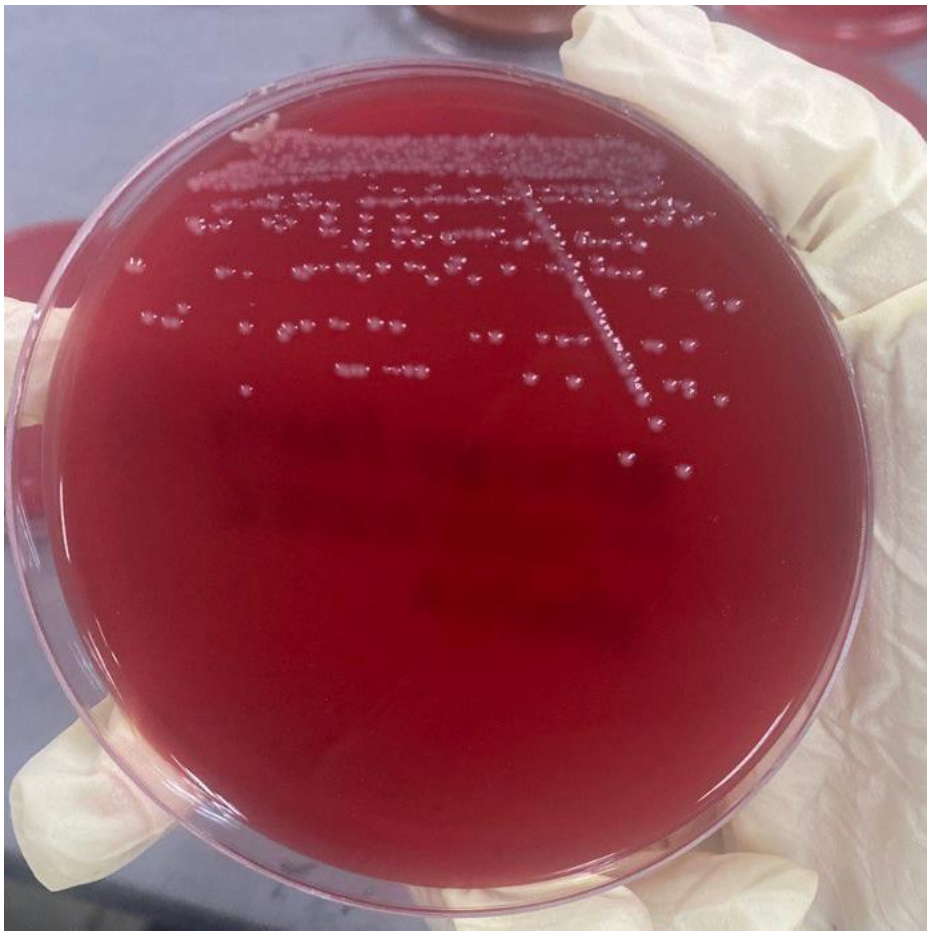


Figure 2.1 *A. baumannii* colonies grow on sheep blood agar.

2.1.2(a) Ultrastructure analysis of *A. baumannii*

Scanning Electron Microscopy (SEM) analysis is the most common way used to visualize *A. baumannii* at the cellular level (Figure 2.2). SEM images indicate collapsible egg-shaped rods with a capsule layer of carbohydrate material around their exterior (Sherif *et al*., 2021). An effective method for analyzing the surface features of biological materials is SEM. An SEM analysis showed that extracellular appendages joined *A. baumannii* cells to each other. The effects of antibiotic therapy on biofilm formation were investigated, and the surface structures of biofilms formed on the MBEC test were examined using SEM.

The Gram-negative coccobacillus can be visualized by SEM analysis, the former revealing egg-shaped rods with a capsule layer of carbohydrate material and the latter showing that the cells are organized in a propeller-like arrangement.

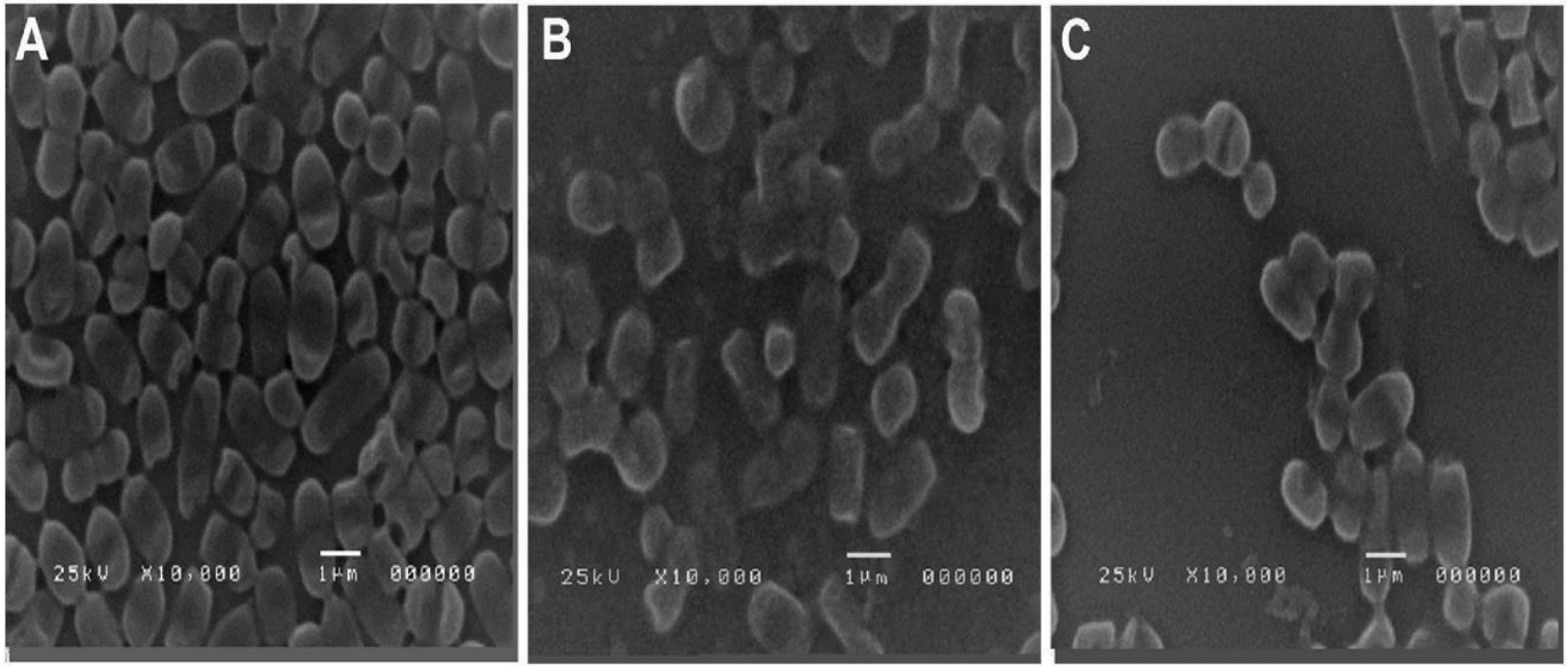


Figure 2.2 Scanning electron microscopy images; (A) untreated strong biofilm producer *A. baumannii* isolates, after (B) a 24-hour treatment with sub-MIC cinnamic acid, and (C) a 24-hour treatment with sub-MIC gallic acid. Magnification 10,000× (Adopted Sherif *et al*., 2021)

2.1.3 History of *A. baumannii* and the emerging strain of CRAB

The *Acinetobacter* bacteria was initially isolated in 1911 by Dutch M. W. Beijerinck from soil using minimum media that was enhanced with calcium acetate (Whiteway *et al*., 2022). *Acinetobacter* is a genus of bacteria that was first discovered in the early 20th century, but it was acknowledged in the last decade as a common pathogen. The most prevalent species of *Acinetobacter* involved in clinical infections is *A. baumannii*, which makes up 73% of all *Acinetobacter* clinical isolates and is one member of the *Acinetobacter calcoaceticus-A. baumannii* complex. *A. baumannii*, formerly known as *Acinetobactercalcoaceticus*, is a waterborne and soil-based pathogenic organism (Kuo *et al*.,2004).

From 1992 to 1996, annual susceptibility reports showed that all isolates of *A. baumannii*, whether endemic or epidemic, were susceptible to colistin, sulbactam, and carbapenem, but resistant to two or more antibiotic families, always including gentamicin and *b*-lactams. Nonetheless, three main patterns of antibiotic sensitivity in the *A. baumannii* population could be identified based on the varying susceptibilities to tobramycin, amikacin, ciprofloxacin, and tetracycline (Corbella *et al*., 2000).

A. baumannii was vulnerable to standard antibiotics in the 1970s, but it has since evolved into an MDR bacterium that can acquire resistance genes. The first hospital-wide outbreak of *A. baumannii* infections in New York City in 1991 raised initial concerns on multi-resistant, CRAB infections (Corbella *et al*., 2000). In 1991, CRAB, one of the earliest strains of *A. baumannii* resistant to antibiotics, was also discovered in the United States (Kuo *et al*., 2004). In the 1,000-bed hospital in Barcelona, Spain, a persistent outbreak of multi-resistant *A. baumannii* infections was observed starting in 1992. This led to a significant overuse of imipenem, to which the organisms were universally susceptible. CRAB strains first appeared in

January 1997 and spread quickly throughout the ICU (Corbella *et al*., 2000).

Subsequently, reports of worldwide outbreaks and CRAB infections emerged from a number of countries, including Brazil, Cuba, France, England, Hong Kong, Singapore, Argentina, and Spain (Corbella *et al*., 2000; Kuo *et al*., 2004). The possibility that the world might be approaching the post-antimicrobial era is raised by the current global concern over the increasing number of resistant organism populations in medical environments (Corbella *et al*., 2000).

2.1.4 Epidemiology of CRAB

The increasing emergence of CRAB strains has become another worrisome truth in recent years and a major problem in a hospital environment that can cause elevated morbidity and mortality rates due to treatment difficulties (Corbella *et al*., 2000; Lin, 2014; Manchanda *et al*., 2010; Sherif *et al*., 2021). Between 2005 and 2009, *A. baumannii* from a global collection developed imipenem resistance rates that exceeded 50% (Lin, 2014).

About two out of every three isolates in Brooklyn, New York, were found to be resistant to the antibiotic carbapenem, according to citywide surveillance. After being established in a university hospital in Chicago in 2005, one of the CRAB strain-types has become predominant. Furthermore, it was revealed by molecular epidemiological studies of successive *A. baumannii* outbreaks in ICU that carbapenem resistance first appeared in Italy between 1999 and 2002 (Corbella *et al*., 2000; Lin, 2014).

In China, it has been observed that imipenem-resistant *Acinetobacter* spp. is spreading clonally, and *OXA-23* carbapenem genes are widely dispersed as well. The percentage of healthcare-associated infections in Taiwan caused by CRAB increased significantly from 14% in 2003 to 46% in 2008, compared to infections by all *A. baumannii* (Lin, 2014).

According to the Malaysia National Surveillance of Antibiotic Resistance (NSAR), Malaysia's overall rate of carbapenem resistance grew from 49% in 2008 to61% in 2016, after that it remained largely stable at 60% annually (Woon *et al*., 2021).The rate of CRAB in the general ICU at the University of Malaya Teaching Centre (UMMC) has remained high, at approximately 0.5 per 100 admissions annually, according to confidential hospital surveillance statistics. This percentage exceeds thenational KPI for MDR *A. baumannii* incidence in Malaysia, which is 0.3 per 100 admissions. 74% of the isolates in research carried out in a tertiary-care hospital in Johor, Malaysia, demonstrated dominant genotypes (Woon *et al*., 2021).

The rates of carbapenem resistance in *Acinetobacter* spp. isolates from Malaysia (1987–2016) are shown in Figure 2.3. Meropenem is MEM, and imipenem is IMP. Apurple font is used to indicate the NSAR data, along with the acronym "NSAR". The information in Figure 2.3 is derived from the other studies: Hospital Selayang (H. SLYG) in 2010, UMMC from 2008 to 2009, HUSM from 2003 to 2006 and 2005 to 2009, UKMMC from 2010 to 2011, and UMMC from 1987 to 1987 and 1996 to 1998. A variety of data was acquired in 2010 and 2011 from various hospitals in the state of Perak, mostly in the area of the town of Ipoh; in 2011 from HSNZ; and in 2011 and 2012 from Hospital Sultanah Aminah (HSA) (Rani *et al*., 2017).

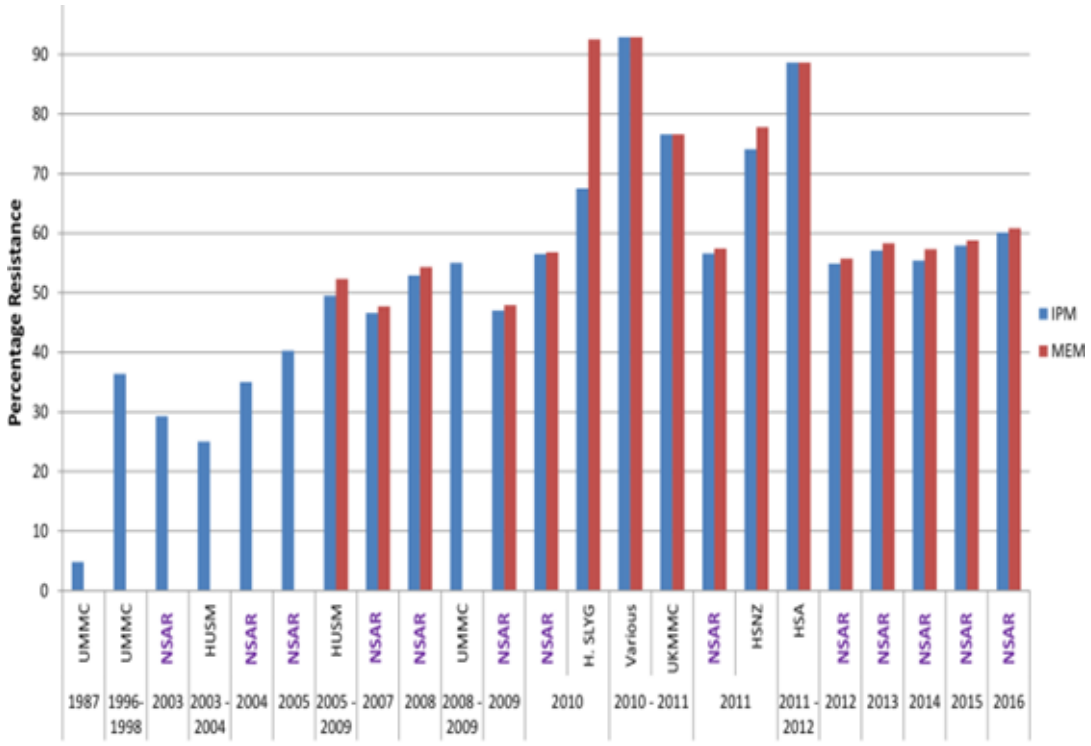


Figure 2.3 Carbapenem resistance rates for Malaysian *Acinetobacter* spp. isolates (1987–2016) (Adopted from Rani *et al*., 2017)