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Antimicrobial activities of some selected plants and honey against some clinical microorganisms

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ABSTRACT

Background: Honey and natural plants are used for treatment of infections by bacteria as *Klebsiella*, *Pseudomonas*, *Acinetobacter*, *Enterobacter* and *Escherichia coli*. The objective of this study was to investigate the antimicrobial activities of honey and some natural plants as garlic, onion, Portulaca oleracea (rigla) against *MRSA* and some Gram-negative bacteria, to compare their potency with commercially used antibiotics. **Methods**: In study 60 clinical specimens were collected from hospitalized patients with gastrointestinal, wound and urinary tract infections. All isolates were identified using microbiological standard procedures. Antimicrobial activity of honey, garlic, onion and rigla plant was investigated at concentrations of 100%, 50%, 25%, and 12.5% by measurement of inhibition zones. The antimicrobial activity of mixture of natural plants extracts and commercial antibiotics was also investigated. **Results**: At a 12.5% concentration, garlic showed inhibition zones of 12 mm and 20 mm against *Klebsiella* and *Enterobacter*, respectively, while Citrus honey was more effective on *Pseudomonas* and *Acinetobacter* with an inhibition zone of 10 mm. Mixture of commercial antibiotics and plant extracts at concentrations was more effective on *Enterobacter*. Onion at 12.5% concentration with ceftazidime showed 10 mm inhibition zone, while against *Pseudomonas* the inhibition zone was 12 mm by clover honey at 12.5% concentration with ceftazidime. Mixture of ethanol extract at 12.5% with ceftazidime was active against *Acinetobacter* with12 mm inhibition zone. **Conclusion**: Importance of study is identification of multidrug resistance bacteria in human patient at hospitals. Using natural extracts of plants and honeys are safe, efficient and low cost for treatment resistant bacteria.

Introduction

Antimicrobial agents are substances known to have therapeutic effect against pathogenic microorganisms as either prevention or treatment [1].

The main reason of infectious diseases is natural development of bacterial resistance to various antibiotics due to accumulation of different antibiotic residues inside the same strain. Multidrug resistant bacteria cause financial and economic implications, treatment failure and spread of pathogenic bacteria from person to person.

Pathogenic bacteria as *MRSA*, *Klebsiella, Enterobacter*, *Pseudomonas*, *Acinetobacter* and *E. coli*. Most methicillin-resistant *Staphylococcus aureus (MRSA)* infections occur in people who have been in hospitals or other health care settings. *Klebsiella pneumoniae* are bacteria that normally

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live in intestines and feces. They also have a high tendency to become antibiotic resistant. These bacteria are harmless when they are in intestines or stool. Also, *Enterobacter* can cause any of a variety of conditions, including eye and skin infections, meningitis, bacteremia.

Acinetobacter is a type of bacteria that is resistant to many types of antibiotics. It is usually present in wet environments, such as soil and mud ponds wetlands wastewater fish farms seawater.

Healthy people can also carry the *Acinetobacter* bacteria on their skin, particularly if they work in a healthcare setting. It can survive for a long time on dry surfaces. That is resistant to almost all types of antibiotics, including a powerful group of antibiotics called carbapenems, *Escherichia coli* normally live in the intestines of healthy people and animals. Most types of *E. coli* are harmless or cause relatively diarrhea. Most pathogenic bacteria are resistance to chemical antibiotics and overuse of antibiotics effect on immune system of patients that cause more death people. In addition to the side effects of overuse and misuse of antibiotics which can harm vital organs like liver, kidney, pancreas and spleen as well as the immune system [2] and thus the situation has forced he attention of scientists towards natural and herbal products in the search to develop better quality drugs with improved antibacterial activities as alternative cure [3] Recently, in 2023, the global prevalence of antibiotic-resistant bacteria has become a cause for widespread concern. This disconcerting trend is further compounded by the absence of new antibiotic classes being developed, ultimately giving rise to what is commonly referred to as the "antibacterial crisis [4] Therefore, we can use natural products to overcome antimicrobial resistant bacteria. Honey, garlic, onion and rigla plant have been used as medicine in different cultures. They have proved to be a good antimicrobial, antiviral, anti-inflammatory, antitumor and antioxidant agent [5].

Methods:

Samples collections: 60 clinical samples were collected from hospitalized patients with gastrointestinal, wound, and urinary tract infections at Kasr El-Ainy and Elsheikh Zayed Hospitals. The age range of patients was 16 to 60 years. Main bacterial species were identified using microbiological standard procedures under microscope and biochemical reactions according to [6], Methicillin-Resistant *Staphylococcus aureus* (MRSA), *Klebsiella* sp, *Enterobacter* sp, *Pseudomonas* sp, *Acinetobacter* sp and *Escherichia coli*.

Antibiotic sensitivity testing: The inoculum was standardized using 0.5 McFarland's standard as described by Isunu et al [7] Antibiotic susceptibility test was performed using Kirby-Bauer disc diffusion method described by Cheesbrough [6]. The diameter of zones of inhibition was measured and interpreted using standard interpretative charts as recommended by the Clinical and Laboratory Standards Institute [8].

Antibiotics: Used commercial antibiotics against gram positive (MRSA) and gram negative as Vancomycin, Cefoxitin, Imipenem, Amikacin, Levofloxacin and Ceftazidime.

Natural extracts: Honey from Ministry of Agriculture (clover and citrus). All honey samples stored in the dark at room temperature until further use then were purchased and used with different concentrations. Samples were filtered with manual Seitz filter attached with Syringe. Garlic: Matured fresh garlic bulbs were purchased from Egyptian market and cleaned well.

Onion: which used in this study was egyptian red onion (*Allium Cepa*), fresh bulbs will be rinsed in distilled water and air-dried.

Portulaca oleracea plant: Used egyptian plant known as rigla from elsharkia in Egypt. Extractions of rigla by distilled water and extraction by ethanol 95%. Different concentrations of sterile extract *Portulaca oleracea* plant by two ways of extracts with distal water as aqueous extract and with ethanol 95% as ethanolic extract. All natural extracts with different concentrations (100%, 50%, 25%, and 12.5%) in sterile tubes with distal water, incubated at 37°C for 24h-48h. Then repeated that at 45°C.

Media preparation:

This medium was used to culture bacteria as nutrient agar media, macconkey's agar and blood agar, broth media and muller hinton agar. identification of the infecting organism, all samples were cultured on nutrient agar and macconkey's agar plates. Incubation aerobically at different temperatures 37°C and 45 °C for 24 and 48 h.

The filter paper discs (6 mm) were prepared using Whitman filter paper were obtained by punching and putting in bottle and sterilizing in

hot air oven at 170˚C for 30 minutes. the discs were impregnating with 20 µL of each separate plant extracts, were placed on the surface of the bacteria seeded agar plates by a sterile forceps and it was allowed to diffuse for 5 min then these plates were incubated at 37°C then at 45 ˚C for 24 h ,48h, [9], we prepared different concentrations of clover honey, citrus honey, garlic, onion and *Portulaca Oleracea* (rigla) in sterile tubes with distal water. The different discs of filter papers were prepared and impregnating with 20µl of extracts at muller hinton agar, which inoculated with 0.5 McFarland of pathogenic bacteria, then incubated at 37˚C for 24h-48h. Then repeated that at 45˚C.

Determination of low concentration of extracts that gave high inhibition zone against pathogenic bacteria and determination of inhibition zones of gram-positive bacteria (MRSA), gram negative bacteria which more resistant against commercial antibiotics, then mixing natural extracts of honey or plants that had high inhibition zone and commercial antibiotics, compared results.

MIC determination and MBC

The MIC assay was carried out using broth dilution method as described by Kone et al [10]. Test tubes containing different concentrations of the extracts of ranging from 100%,50%,25%,12.5% were inoculated with the standardized bacteria and incubated for 18 – 24 hours. The lowest concentration of natural extracts that shows low visible turbidity or growth of the bacterial isolates was recorded as the MIC. The MBC assay of the extracts was determined using the method described by Kone et al [10]. The test tubes from the MIC test that did not show visible growth were aseptically inoculated on different sterile muller-hinton agar plates and incubated at 37 °C for 24 hours. The MBC was chosen as the lowest extracts concentration that resulted in no visible growth of the bacterial isolates on the plate.

Data analysis using SPSS:

All data were analyzed using SPSS to determine difference between inhibition zones of natural extracts from honey, plants and pathogenic bacteria

Results:

Identification of bacterial isolates

The distribution of sample in relation to age and sex, by biochemical characteristics of the bacteria. These include *Escherichia coli (E. coli*), *Klebsiella* sp, *Pseudomonas*, *Staphylococcus aureus* (MRSA) and *Enterobacter*.

Antibacterial activity of natural extracts: The antibacterial activities of extracts are shown in tables (1,2,3 and 4) at 100%,50%.25%,12.5% concentrations. **At 100%** was high inhibition zone of clover honey and citrus honey by range (11mm to 30mm) as most effective on bacteria, then garlic had inhibition zones by range (14mm to 20mm), Onion was less effective on bacteria.

Pseudomonas and *Acinetobacter* and *E. coli* were sensitive to rigla plant (ethanolic, aqueous) by range (6 to 25mm). **At 50%** clover honey and citrus honey had high inhibition zones on bacteria by range (10 to 15mm), then garlic affected by range (10 to 14 mm) but *MRSA* and *Pseudomonas* were resistant. Then rigla plant inhibited bacteria by range (8 to 10mm) except *MRSA* was resistant. onion less effective with 50% concentration by range (6 to 10mm).

At 25% clover honey was more effective on all bacteria then citrus honey on all bacteria except *MRSA* and most effective on *E. coli* by 20mm. Garlic was effective on bacteria by range (7 to 15mm) and onion inhibited bacteria by range (7 to 9mm).

At 12.5% concentrations, garlic was the most effective on *MRSA* by 11mm, on *Klebsiella* by12mm, on *Enterobacter* by 20mm, then rigla ethanolic plant inhibited bacteria by 16mm on *Enterobacter*, on *Acinetobacter* and *E. coli* by 8mm, but onion less effective on bacteria.

That was no results at 45 °C or 25°C, the best temperature for bacterial growth was 37°C.

Results mean that clover honey and citrus honey are most effective antibacterial products, then garlic and rigla plant, but onion low effective product on bacteria.

Antibiotic sensitivity pattern of bacterial isolates

The antibiotic sensitivity pattern for gram-positive and gram-negative bacteria are shown in tables (5). The isolates were highly resistant to Ceftazidime then low inhibition zone by Levofloxacin, when mixture resistant antibiotics and natural extracts with low concentrations, gave high inhibition zones on resistant bacteria and bacteria became sensitive. in table (6), *Klebsiella* was the most resistant to that mixture and antibiotics, then MRSA and *E coli*, But *Enterobacter*, *Acinetobacter* and *Pseudomonas* were sensitive to antibiotics and mixture of natural extracts that in figure (1)

At concentration12.5%of onion + Ceftazidime gave 10mm on *Enterobacter,* clover honey with concentration12.5%+ Ceftazidime gave 12mm on *Pseudomonas.*

And ethanolic rigla with concentration 12.5%+ Ceftazidime gave 12mm on *Acinetobacter*.

Analysis of lowest concentrations of natural products alone can increase activity of commercial antibiotics that mean natural products can use alternative natural antimicrobial agents.

Minimum inhibitory and bactericidal concentration The MIC and MBC values are given in table (7). The MIC values ranged from 3.12 to 25% v/v and MBC ranged from 1.56 to 12.5% v/v. across all extracts and organisms tested that in table (7) and table (8): garlic had the least MIC and MBC for most bacteria. tested honeys and natural plants showed varied bacteriostatic and bactericidal activities.

Klebsiella was the most resistant to that mixture and antibiotics, then MRSA and *E coli*, But *Enterobacter, Acinetobacter* and *Pseudomonas* were sensitive to antibiotics and mixture of natural extracts

MIC of natural products: (% v/v)

The lowest concentration of natural extracts that shows low visible turbidity or growth of the bacterial isolates was recorded as the MIC.

MBC of natural products:(% v/v)

The MBC was chosen as the lowest extracts concentration that resulted in no visible growth of the bacterial isolates on the plate.

Table 1. Inhibition zone of 100% concentration on bacteria.

		Clover honey	Citrus honey	Garlic	Onion	Rigla ethanolic	Rigla aqueous
MRSA	24h 48h	26mm 26mm	20mm 20mm	7mm 7mm	7mm 7mm	6mm 6mm	6mm 6mm
Klebsiella	24h 48h	30mm 30mm	30mm 30mm	14mm 14mm	7mm 7mm	7mm 7mm	7mm 7mm
Enterobacter	24h 48h	12mm 12mm	19mm 19mm	20mm 20mm	10mm 10mm	9mm 9mm	8mm 8mm
Pseudomonas	24h 48h	20mm 25mm	15mm 18mm	9mm 9mm	8mm 8mm	25mm 25mm	10mm 10mm
Acinetobacter	24h 48h	11mm 11mm	10mm 10mm	7mm 7mm	6mm 6mm	28mm 28mm	10mm 10mm
E. coli	24h 48h	9mm 9mm	15mm 15mm	8mm 8mm	5mm 5mm	6mm 6mm	4mm 4mm

Table 2. Inhibition zone of 50% concentration on bacteria

	incubation time	Clover honey	Citrus honey	Garlic	Onion	Rigla ethanolic	Rigla aqueous
MRSA	24h	10mm	9mm	0	0	0	0
Klebsiella	24h	15mm	12mm	14mm	10mm	10mm	11mm
Enterobacter	24h	10mm	10mm	8mm	9mm	11mm	10mm
Pseudomonas	24h	13mm	9mm	0	9mm	7mm	7mm
Acinetobacter	24h	10mm	12mm	10mm	8mm	10mm	8mm
E. coli	24h	11mm	9mm	5mm	6mm	10mm	8mm
	incubation time	Clover honey	Citrus honey	Garlic	Onion	Rigla ethanolic	Rigla aqueous
MRSA	48h	9mm	0	0	0	0	0
Klebsiella	48h	0	0	7mm	8mm	0	8mm
Enterobacter	48h	0	0	0	7mm	9mm	0
Pseudomonas	48h	0	0	0	8mm	6mm	4mm
Acinetobacter	48h	10mm	11mm	7mm	6mm	10mm	8mm
E. coli	48h	9mm	7mm	5mm	0	0	0

Table 3. Inhibition zone of 25% of concentration on bacteria

		Clover honey	Citrus honey	Garlic	Onion	Rigla ethanolic	Rigla aqueous
MRSA	24h	10mm	0	7mm	0	0	0
Klebsiella	24h	9mm	8mm	8mm	7mm	7mm	7mm
Enterobacter	24h	9mm	10mm	8mm	9mm	9mm	8mm
Pseudomonas	24h	8mm	8mm	9mm	8mm	9mm	7mm
Acinetobacter	24h	10mm	10mm	7mm	6mm	9mm	8mm
E. coli	24h	9mm	20mm	15mm	10mm	0	7mm
		Clover honey	Citrus honey	Garlic	Onion	Rigla ethanolic	Rigla aqueous
MRSA	48h	0	0	0	0	0	0
Klebsiella	48h	0	0	0	0	0	0
Enterobacter	48h	0	0	0	0	0	0
Pseudomonas	48h	0	0	0	0	0	0
Ainetobacter	48h	0	0	0	0	0	0
E. coli	48h	0	15mm	13mm	8mm	0	6mm

Table 4. Inhibition zone of 12.5% concentration on bacteria

		Clover honey	Citrus honey	Garlic	Onion	Rigla ethanolic	Rigla aqueous
MRSA	24h	0	0	11mm	0	0	0
Klebsiella	24h	0	0	12mm	0	0	0
Enterobacter	24h	9mm	9mm	20mm	9mm	16mm	8mm
Pseudomonas	24h	8mm	10mm	0	0	0	0
Acinetobacter	24h	0	10mm	0	10mm	8mm	0
E. coli	24h	0	0	8mm	0	8mm	10mm
		Clover honey	Citrus honey	Garlic	Onion	Rigla ethanolic	Rigla aqueous
MRSA	48h	0	0	10mm	0	0	0
Klebsiella	48h	0	0	0	0	0	0
Enterobacter	48h	0	0	0	0	0	0
Pseudomonas	48h	0	0	0	0	0	0
Acinetobacter	48h	0	0	0	0	0	0
E. coli	48h	0	0	0	0	0	0

Table 5. Inhibition zones of commercial antibiotics against *MRSA* and gram negative bacteria.

	VA (Vancomycine)	FOX (Cefoxitin)	IPM (Imipenem)	LEV (Levofloxacin)	CAZ (Ceftazidime)	AK (Amikacin)
MRSA	20mm	14mm	40mm	40mm	10mm	30mm
Klebsiella	20mm	10mm	42mm	40mm	8mm	30mm
Pseudomonas	20mm	10mm	44mm	42mm	0	36mm
Enterobacter	36mm	10mm	40mm	42mm	0	30mm
Acinetobacter	36mm	10mm	40mm	42mm	0	30mm
E.coli	36mm	10mm	42mm	40mm	10mm	30mm

Table 6. Mixture of resistant antibiotics and natural extracts with low concentrations can get high inhibition zones on resistant bacteria to convert that bacteria to sensitive

	Mixture		Resistant antibiotic only(FOX)	Resistant antibiotic only(CAZ)
MRSA	Garlic 12.5%+FOX	14mm	14mm	10mm
Klebsiella	Garlic 12.5%+FOX Garlic 12.5%+ CAZ	0 0	10mm	8mm
Enterobacter	Onion12.5%+CAZ	10mm	10mm	0
Pseudomonas	Clover honey12.5%+CAZ Citrus honey12.5%+FOX	12mm 10mm	10mm	0
Acinetobacter	Ethanolic rigla12.5%+CAZ	12mm	10mm	0
E.coli	Aqueous rigla 12.5%+FOX Citrus honey 25%+CAZ	10mm 8mm	10mm	10mm

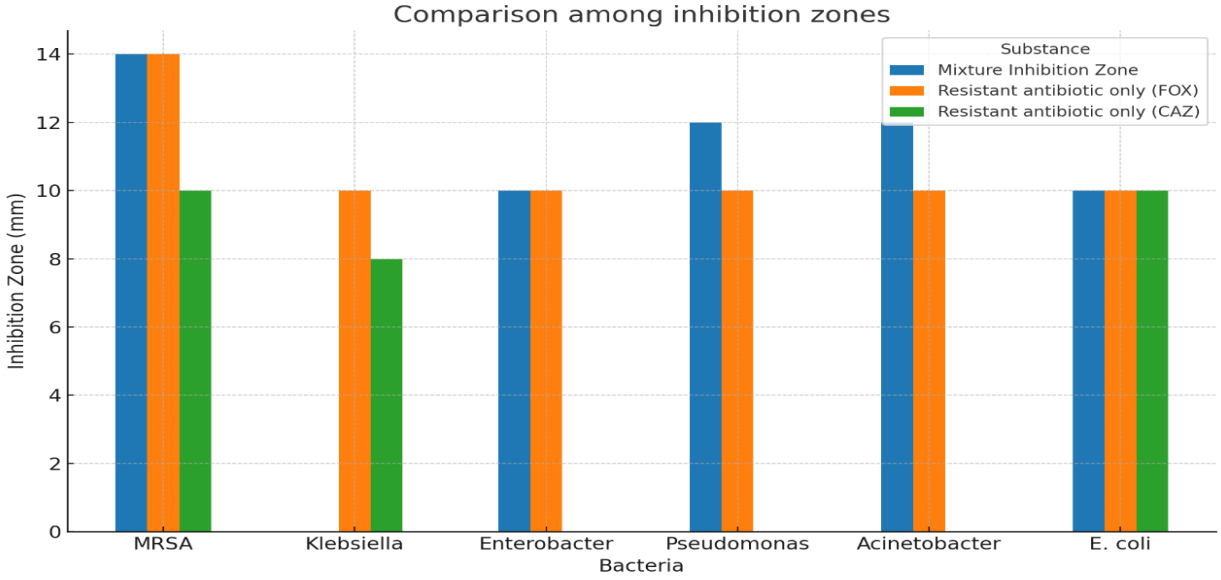
Table 7. MIC of natural products on bacteria.

	Klebsiella	*MRSA*	*Enterobacter*	*Pseudomonas*	*Acinetobacter*	*E. coli*
Clover honey	12.5	12.5	12.5	12.5	12.5	12.5
Citrus honey	3.12	12.5	12.5	12.5	25	12.5
Garlic	3.12	3.12	6.25	12.5	6.25	6.25
Onion	12.5	12.5	6.25	25	25	12.5
Ethanolic rigla	12.5	12.5	25	12.5	25	12.5
Aqueous rigla	12.5	12.5	25	12.5	25	12.5

Table 8. MBC of natural products on bacteria.

	Klebsiella	*MRSA*	*Enterobacter*	*Pseudomonas*	*Acinetobacter*	*E. coli*
Clover honey	6.25	6.25	6.25	6.25	6.25	6.25
Citrus honey	1.56	6.25	6.25	6.25	12.5	6.25
Garlic	1.56	1.56	3.12	6.25	3.12	3.12
Onion	6.25	6.25	1.56	12.5	12.5	6.25
Ethanolic rigla	6.25	6.25	12.5	6.25	12.5	6.25
Aqueous rigla	6.25	6.25	12.5	6.25	12.5	3.12

Figure 1. Mixture of commercial antibiotics and natural extracts



Discussion

As using of antibiotics has caused serious problems such as multidrug-resistant bacteria, antibiotic overuse and antibiotic residues in food, new materials to replace antibiotics to treat bacterial infections is needed. Previous studies have shown that plants extracts can be used to treat a variety of disorders including inflammatory conditions, bacterial infections, cancer and other diseases. In the present study, antibacterial activities of honey and natural plants as garlic, onion and rigla

The results according tables of inhibition zones of different concentration of natural products and honey on pathogenic bacteria show *klebsiella* and MRSA were the most resistant bacteria to some plants and honey.

The lowest concentration 12.5% affected on *klebsiella* by 12mm with garlic only, *MRSA* also inhibited only by11mm with garlic 12.5%concentration as lowest concentration, but zero inhibition zones by other plants, While *Enterobacter* was the lowest resistant bacteria and all natural products inhibited that bacteria by different zones, in 12.5% concentration of clover honey made 9mm, citrus honey 9mm, garlic 20mm, onion 9mm, ethanolic rigla 16mm and by aqueous rigla 8mm.

While 25% that concentration more sensitive on *E coli* by citrus honey 20mm and 15mm by 25% of garlic also, *Pseudomonas* and *Acinetobacter* were more sensitive by 25%concentrations of all plants and honeys by average inhibition zone from 8-10mm.

A high level of resistance of the bacterial isolates to commonly used antibiotics was observed in this study. *Escherichia coli* were highly resistant to most antibiotics tested. *Staphylococcus aureus* was resistant to gentamycin.

In that study, antibacterial activity against *Klebsiella* indicated by having the lowest MIC value as 3.12% (v/v) by citrus honey and also by garlic. *MRSA* indicated by lowest MIC 3.12% by garlic. However, previous study according to [11] showed MIC of *A. mellipodae* honey against *E. coli* was 12.5% and MIC of garlic extract against *E coli, S. aureus* and *Pseudomonas* was 6.25%, as our study MIC of garlic extract against *E. coli* was 6.25% ,6.25%against *Enterobacter*, 6.25% against *Acinetobacter*.

MBC lowest value on *Klebsiella* with citrus honey and garlic by 1.56% and on *MRSA* by 1.56% of garlic in this study, but on *Enterobacter* gave 1.56% by onion.

, But in previous study [12] showed MBC of garlic on *S. aureus* by 12.5% and by honey 6.25%.

Conclusion:

This study revealed the multiple antibiotic-resistant bacteria cause infections and diseases. It further revealed that the different concentrations of natural extracts used in this study possess some bioactive compounds that make natural extracts good antibacterial agents against these multiple antibiotic resistant bacteria. Findings from this study have further established that honey, garlic, onion and rigla plant are effective alternative treatment for

multiple antibiotic resistant bacteria (MARB) that are associated with infections.

Abbreviations

SD: Standard

SPSS: Statistical Package for Social

WHO: World Health Organization

MRSA: Methicillin-resistant Staphylococcus aureus

G: Gram

MDR : Multidrug resistance

E. coli : *Escherichia coli*

WHO : The World Health Organization

min. : Minute

˚C Degree Celsius

MBC minimum bactericidal concentration

MIC Minimum inhibition concentration

VA Vancomycin

FOX Cefoxitin

AK Amikacin

LEV Levofloxacin

CAZ Ceftazidime

IPM Imipenem

MH Muller Hinton

CFU colony forming units

Ethics approval and consent to participate: This study has been carried out in accordance with the code of Ethics of Kasr El-Ainy Hospital, Elsheikh Zayed Hospital. Availability of data and materials.

Authors' contributions: Both authors one and two designed the study, analyzed and made the interpretation of data; and developed the first draft of the manuscript.

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