Available online www.jsaer.com

***Journal of Scientific and Engineering Research*, 2018, 5(3):212-227**

Research Article

ISSN: 2394-2630
CODEN(USA): JSERBR

Natural Extracts As Antibiotics Alternatives against Multi Drug Resistant Bacteria Isolated from Cancer Patients Receiving Chemotherapy

Mohammed Rawway[1]*, Eman M. Zaki[2], Usama M. Abdul-Raouf[1], AbdAllah A. Saleh.[1, 2]

[1]Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Assiut, Egypt
[2]Clinical pathology and hematological malignancies Department, South Egypt Cancer Institute - Assiut University, Egypt

Abstract Fifty blood, urine, pus and sputum samples were collected from patients suffering from malignancies and receiving chemotherapy at South Egypt Cancer Institute. The antibacterial effects of some antibiotics (amikacin, nitrofurantion, imipenem, ciprofloxacin, trimethoprime/sulphamethoxole, oxacillin, gentamycin, ceftazidime, cefuroxime and tobramycin) were tested to estimate the most resistant ten isolates, and minimum bactericidal concentration for some of these antibiotics were estimated. The most common multidrug resistant isolates in patients receiving chemotherapy were identified as *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* according to microscopic and biochemical characters and finally confirmed by VITEK 2 System. Alternatives of antibiotics such as plant extract were tested. The isolated bacteria have been tested against ethanolic and cold water plant extract of (marjoram, thyme, lemon peel, and rosemary). Rosemary ethanolic extracts had the high effect on all bacterial isolates. All tested bacteria were sensitive to rosemary, thyme and marjoram while lemon peel had sensitive effect on Gram-negative isolates and intermediate effect on *Staph. aureus.*

Keywords Multidrug resistant bacteria, Natural extract, Chemotherapy, Cancer patients, rosemary, thyme, lemon peel and marjoram

1. Introduction

Antibiotic-resistant bacteria and prolong hospitalization increase the risk of death and require treatment with toxic and expensive antibiotics. Once the antibiotic profile is available, a narrow-spectrum antibiotic can be recommenced [1]. Patients with chronic disease such as malignant tumors, leukemia have an increased susceptibility to infections with opportunistic pathogens. Immune suppressive drugs may lower resistance to infection. Injuries to skin or mucous membranes bypass natural defense mechanisms [2]. Nosocomial pneumonia is also a significant problem. About 3% of patients on ventilators acquire pneumonia, which in this circumstance, has a very high case-mortality rate. The source of the microorganism is often endogenous but may also be exogenous with transfer of an organism from the respiratory equipment [3]. Nosocomial bacteremia represents about 5% of nosocomial infections. Although they are only a small proportion of nosocomial infections, they have high case-mortality rates, sometimes greater than 50% [4]. Historically, *staphylococci*, *pseudomonas*, and *Escherichia coli* have been the causes of nosocomial pneumonia and surgical wound infections have caused the most illness and death in hospitalized patients; intensive care units was the cause of antibiotic resistance [5]. Hospitalized patients with decreased immunity are more prone to *S. aureus* infections. *S. aureus* infects not only the superficial but also the deep tissues and local abscess lesion [6]. *E. coli* is an emerging nosocomial pathogen causing problems in health care settings. *E. coli* is responsible for a number of diseases including urinary tract infection (UTI), septicemia, pneumonia, neonatal meningitis, peritonitis and gastroenteritis [7]. Three to seven

percent of hospital-acquired bacterial infections are related *to K. Pneumonia*, which is the eighth significant pathogen in healthcare settings [8]. Many Gram-negative pathogens are also multidrug-resistant, including organisms that express extended spectrum beta-lactamases such as *K. pneumoniae*, *K. oxytoca*, and other *Enterobacteriaceae*. Such enzymes can degrade third generation cephalosporins, and organisms expressing these beta-lactamases may be treated only with carbapenem antibiotics such as imipenem and meropenem [9]. In recent years there has been extensive research to determine the antimicrobial activity of some plant essential oils that have been shown to have a wide spectrum of activity against microbes [10].

[11]. investigated that lemon grass essential oil has quite high degree of antimicrobial activity against oral pathogen. Marjoram (*Origanum vulgare*) may have a great potential for industrial applications [10]. The essential oil of the seeds has anti bacterial effects on Gram positive and Gram negative bacteria [12]. Thyme was one of the most active and exhibited greatest inhibition against *Lactobacillus plantarum.* Some other researchers reported that *Thymus vulgaris L.* essential oil at low concentrations (2, 5 and 8%) in a solution of water, propylene glycol and an emulsifying agent, present highly effective antioxidant when used for nile tilapia fillets, at refrigeration temperatures and by placing the fillet immersed in the solution. The reduction of oxidative processes in tilapia fillets by using the essential oil occurred between 5.0 and 96.5%. This demonstrates its high effectiveness, even at low concentration [13,14]. Medicinal plants contain physiologically active principles that over the years have been exploited in traditional medicine for the treatment of various diseases, as they contain anti-microbial properties .These medicinal herbs are components of the traditional medicine worldwide due to the low cost and easy access .Many of these plant extracts contain organic inhibitory for microorganisms [15].

2. Materials and Methods

2.1. Sample collections

Fifty specimens for isolation of MDR bacteria were collected from four different sources include (blood, urine, pus, sputum, swabs and stool) from all departments of south Egypt cancer institute, Assiut University**.** These specimens were received from patients with varying cancer diagnosis include Acute lymphoblastic leukemia (ALL), Acute myeloid leukemia (AML), Chronic myeloid leukemia (CML), Chronic lymphocytic leukemia (CLL), colon cancer, colorectal cancer, stomach cancer, breast cancer, bladder cancer.

2.2. Bacterial isolation and purification

Specimens were cultured primarily on blood agar containing (g/l): peptone 1.0 g, beef extract 3.0 g, agar 1.5 g, after cooling to 50^{o}C, 10% sterile defibrinated sheep blood was added [16]. Then cultured on selective media (a) Eosin methylene blue agar medium [17], containing (g/l): Agar 13.0g, Pancreatic digests of casein 10.0g, Lactose 5.0g, Sucrose 5.0g, K_2HPO_4 2.0g, Eosin Y. 0.4g, Methylene blue 0.065g, (b) Mannitol salt agar medium [18], containing (g/l): Protease peptone No.3 10.0g, Bacto-beef extract 100.0g, Bacto-mannitol 10.0g, Sodium chloride 75.0g, Bacto-agar 15.0g, Bacto-phenol red 0.025g. Separate single colonies grown on the previous media were picked up and streaked to check their purity and get pure separate single colonies. The purified separate single colonies of bacteria were maintained on nutrient agar slants for identification.

2.3. Isolation of bacteria using streak plates as described by [19]

Inocula are usually spread over the surface of agar plate in a standard pattern so that the quantity of bacterial growth can be determined either semi-quantitatively or relatively. The relative numbers of organisms in the original specimen can be estimated based on the extent of growth of colonies. Incubation of cultures plates of MacConkey and Cled agar selective media was done in an inverted position for 24 to 48 hours at 37^{o}C [20].

2.4. Identification of bacterial isolates

Bacterial isolates were identified according to the Bergey's Manual of Determinative Bacteriology [21].

2.5. Confirmation of bacterial isolates by VITEK 2 System (bioMérieux, Inc.)

2.5.1. Procedures

All isolates were identified phenotypically by VITEK 2 System Version: 07.01 in bacteriology laboratory at clinical pathology department at south Egypt cancer institute. GP card includes 43 biochemical tests measuring carbon source utilization, enzymatic activities and resistance. Identification cards were inoculated with microorganism suspensions using an integrated vacuum apparatus. The filled cassette was transported automatically into a vacuum chamber station. After the vacuum was applied and air was reintroduced into the

station, the organism suspension was forced through the transfer tube into micro channels that fill all the test wells.

Each card was removed from the carousel incubator once every 15 minutes and transported to the optical system for reaction readings. Final identification results were available in approximately eight hours.

2.6. Final confirmation of MDR bacterial isolates by Molecular Characterization

Molecular characterization of selected MDR bacterial isolates was done with the help of Solgent Company, Daejeon South Korea. Cultures were sent to the Solgent Company for rRNA gene sequencing. Bacterial DNA was extracted and isolated using Solgent purification bead. Prior to sequencing, the ribosomal RNA gene was amplified using the polymerase chain reaction (PCR) technique in which two universal primers were used for amplification:Forwardprimer:27F(AGAGTTTGATCCTGGCTCA).Reverseprimer:1492R(GGTTACCTTGTTA CGACTT. PCR products were purified and sequenced using a PCR purification kit. The purified PCR products were reconfirmed by gel electrophoreses with 1% agarose gel. Bands were eluted and sequenced with the incorporation of di-deoxy nucleotides (dd NTPs) in the reaction mixture [22].Sequences were further analyzed using BLAST from the National Center of Biotechnology Information (NCBI) website. The percentages of sequence matching were also analyzed and the sequence was submitted to NCB1-GeneBank to obtain accession numbers.

2.7. Antimicrobial susceptibility testing (Kirby-Bauer) disk diffusion agar method [23]

In vitro antibacterial susceptibility was determined for bacterial isolates (n=50) by disk diffusion agar method using Muller-Hinton agar according to Clinical and Laboratory Standard Institute [24]. A loopful of each tested bacterial culture (24 hours) was transferred to a tube containing 5 ml of sterile physiological saline (0.85%), using sterile loop. Addition of sterile saline or other colonies to the tube was applied until the turbidity was adjusted to match a 0.5 McFarland standard tube using adequate light. The inoculated plates were allowed to remain on a flat position and level surface undisturbed for 3-5 minutes to allow the adsorption of excess moisture. The discs were then applied on the seeded plate with a sterile forceps, at a distance of about 20 mm from each other and 15 mm from the edge of the plate, and were pressed firmly into the agar to ensure complete contact.

The plates were incubated inverted at 37˚C for 24 – 48 hours. After incubation, the degree of sensitivity was determined by measuring the easily visible clear zone of inhibited growth due to the diffusion of the antibacterial agents from the discs into the surrounding medium. Results were interpreted according to [24].

In this experiment, the effect of antibiotics was tested on all the bacterial isolates. The antibiotics used were amikacin (Ak30), nitrofurantion (F300), imipenem (Ipm10), trimethoprime + sulphamethoxazole (Sxt1.25/23.75), cefuroxime (Cxm30), oxacillin (Ox1), gentamycin (CN10), ceftazidime (Caz30), ciprofloxacin (Cip5), and tobramycin (Tob10).

2.8. Determination of the minimum inhibitory concentration (MICs) of selected antibiotics

The antibiotics tobramycin and oxacillin were selected according to sensitivity of different bacterial isolates to determine the MIC and MBC against tested bacterial isolates.

A stock solution of these antibiotics were prepared (1000, 500, 250, 125, 62.5, 31.25, 15.625,7.813 and 3.906).The stock solutions of the antibiotics were prepared according to [25].

2.9. Determination of minimum bactericidal concentration (MBCs) of selected antibiotics

0.1 ml taken from each tube (from previous MIC experiment) was spread on nutrient agar plates. All plates were incubated at 37°C for 24 hours. After incubation period the number of colony forming units (CFU) / ml was detected to determine MBC [26].

2.10. Medicinal plant extracts as antibiotics alternatives.

Table 1: The used medicinal plant

English Name	Scientific Name	Family	Used Part
Lemon peel	*Cymbopogon citrates*	*Poaceae*	Fruit
Marjoram	*Origanum majorana*	*Lamiaceae*	Leaf
Rosemary	*Rosmarinus officinalis*	*Lamiaceae*	Leaf
Thyme	*Thymus vulgaris*	*Lamiaceae*	Leaf

Preparation of plant extract and method of extraction

a. Aqueous extract

A total of 50 g of fresh plant was collected. The plant material was washed for any contaminants and then was crushed in a mortar and pestle to expose the inner part. The sample was then soaked in equivalent amount of distilled water at 20 ^{o}C and kept for a total of 3 days. It was then filtered through a porous cloth, the filtrate collected and this procedure was repeated twice. In the end, all of the filtrate was combined, filtered through Whatmann nr.1 filter paper and then concentrated in a rotary evaporator to obtain a thick, brown extract [27].

B. Alcoholic extract

The alcoholic extract was prepared by similar way (50 gram of plant with known amount of ethanol and left 24 hours at room temperature with occasional shaking and filtered to obtain clear extract.

The antimicrobial activity was tested by loaded the prepared extracts on sterile filter paper disc [28].

According to [29]. Values of inhibition zones are (0-9mm) is resistant, (10-15mm) is intermediate and ≥ 16mm is sensitive.

3. Results

3.1. Isolation and characterization of clinical bacterial isolates.

A total of 50 clinical samples (blood, urine, pus, sputum, swabs and stool) were taken from patients suffering from tumors (include Acute lymphoblastic leukemia (ALL), Acute myeloid leukemia (AML), Chronic myeloid leukemia (CML), Chronic lymphocytic leukemia (CLL), colon, colorectal , stomach, breast, bladder cancers) at south Egypt cancer institute. Fifty bacterial isolates were recovered. The results indicated that, bacterial isolates were belonged to five groups including *Staphylococcus aureus*, *pseudomonas* spp., *Klebsiella* spp., *Staphylococcus epidermidis* and *Escherichia coli*.

3.2. Community acquired and hospital acquired.

The obtained result from Table (2) clearly demonstrated the highly percentage of hospital-acquired infection obtained (88%) while the percentage of community acquired were (12%).

Table 2: Type of infection in studied patients

Total	Community acquired		Hospital acquired	
	N	%	N	%
50	6	12	44	88

3.3. Identification of most common bacterial isolates.

The bacterial isolates were subjected to 4 patterns of identification, the staining reactions and the culture characteristics of isolates on simple, enriched and selective media as well as biochemical reactions using VITEK2 system and biochemical tests manually.

3.3.1. Gram-negative bacilli

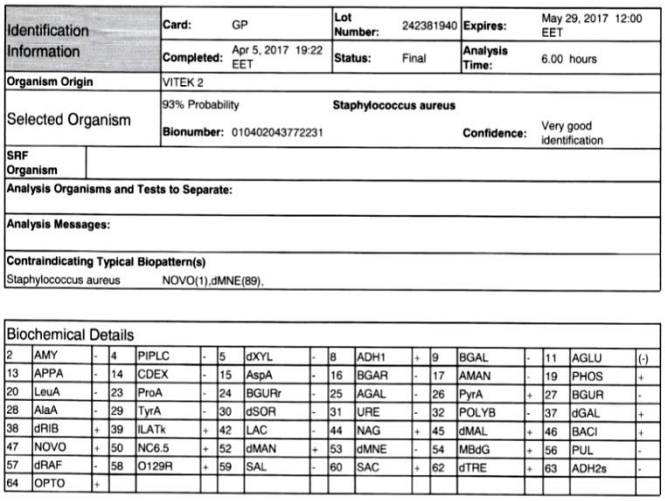
Morphology and Cultural properties: Circular, convex, smooth colonies with distinct edges, Facultative anaerobic, produce rose- pink to red colonies on MacConkey agar media and yellow colonies when cultured on CLED agar.

According to the above characteristics the isolates of the first group of Gram-negative bacilli were identified as *Escherichia coli.* The results obtained by manual biochemical are confirmed by VITEk-2 system technique.

3.3.2. Gram positive cocci

Morphology and Cultural properties: Circular colonies, arranged in irregular grape like clusters, Facultative anaerobic, produce golden yellow colonies on nutrient agar and produce beta (complete) hemolysis on blood agar.

According to the above characteristics the isolates of this group of Gram-positive cocci were identified as *Staphylococcus aureus*. The results obtained by manual biochemical are confirmed by VITEK 2 System technique, as shown in (Figure 1).



Identification Information	Card: GP	Lot Number: 242381940	Expires: May 29, 2017 12:00 EET
	Completed: Apr 5, 2017 19:22 EET	Status: Final	Analysis Time: 6.00 hours
Organism Origin	VITEK 2		
Selected Organism	93% Probability	Staphylococcus aureus	
	Bionumber: 010402043772231		Confidence: Very good identification
SRF Organism			
Analysis Organisms and Tests to Separate:			
Analysis Messages:			
Contraindicating Typical Biopattern(s)			
Staphylococcus aureus	NOVO(1),dMNE(89).		

Biochemical Details																	
2	AMY	-	4	PIPLC	-	5	dXYL	-	8	ADH1	+	9	BGAL	-	11	AGLU	(-)
13	APPA	-	14	CDEX	-	15	AspA	-	16	BGAR	-	17	AMAN	-	19	PHOS	+
20	LeuA	-	23	ProA	-	24	BGURr	-	25	AGAL	-	26	PyrA	+	27	BGUR	-
28	AlaA	-	29	TyrA	-	30	dSOR	-	31	URE	-	32	POLYB	-	37	dGAL	+
38	dRIB	+	39	ILATk	+	42	LAC	-	44	NAG	+	45	dMAL	+	46	BACI	+
47	NOVO	+	50	NC6.5	+	52	dMAN	+	53	dMNE	-	54	MBdG	+	56	PUL	-
57	dRAF	-	58	O129R	+	59	SAL	-	60	SAC	+	62	dTRE	+	63	ADH2s	-
64	OPTO	+															

Figure 1: Identification of S. aureus using VITEK 2 system

Table 3: Morphological, physiological and biochemical characters of bacterial isolates

	Staph. aureus	***E. coli***	***K. pneumonia***	***P. aeurginosa***
Gram stain	+ve	– ve	– ve	– ve
Shape	Cocci	Rod	Rod	Rod
Motility	Non motile	Motile	Non motile	Motile
Spore forming	Non spore	Non spore	Non spore	Non spore
Catalase	+	+	+	+
Coagulase	+	-	-	-
Oxidase	-	-	-	+
Indole	-	+	-	-
KOH test	-	+	+	+
Lactose	-	+	+	-
Glucose	+	+	+	+in O_2
Maltose	-	+	+	-
Sucrose	-	+	+	-
Haemolysis	B	γ	Γ	β
Manitol	+	+	+	-
MR	-	-	-	-
VP	-	-	+	+
Citrate	-	-	+	+
Urease	+	-	+	+
Nitrate reduction		+	+	-

MR = methyl Red Test, V.P = Voges – Proskauer reaction - = Negative, +Positive, In O_2 = in oxidation, β haemolysis = complete lysis of red blood cells, γ haemolysis= non hemolytic activity

3.4. Bacterial count using Uricult method

Growth After inoculation of the disks with 10^9 CFU of *E. coli* per ml, approximately 10^6 CFU/cm^2 adhered to the catheter disk. After 24 h, 10^7 to 10^8 CFU/cm^2 were obtained. Adhesion to the catheter material occurred equally well with an inoculums from an overnight MH agar, Concentrations ranging from 5–300 ug of 10 antibiotics per ml were chosen for testing on the basis of the urinary levels of these agents still attainable approximately 2 h after standard doses.

3.5. Susceptibility of different pathogenic bacterial isolates to different antibiotics drugs

The antibiotics amikacin shows the highest effect against bacterial isolates followed by imipenem and ciprofloxacin respectively. The bacterial isolates are more resistant to ceftazidime, tobramycin, oxacillin and gentamycin.

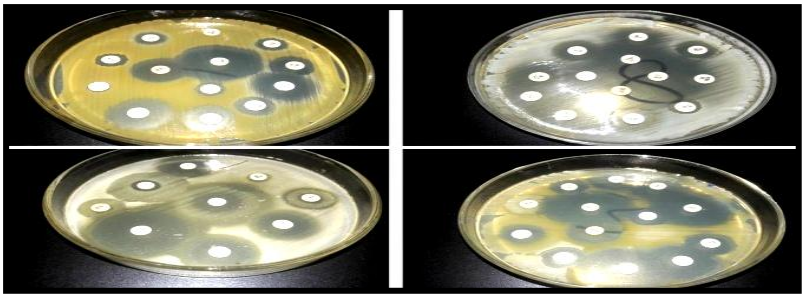


Figure 2: Antibiotic susceptibility testing showing sensitive isolates

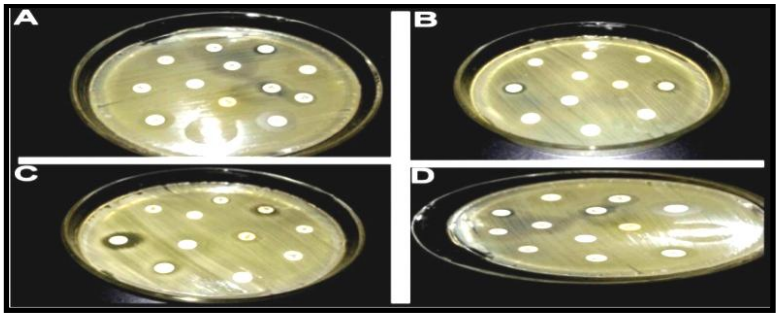


Figure 3: Antibiotic susceptibility testing showing resistant isolates

The sensitivity tests of pathogenic bacterial isolates against different tested antibiotics were illustrated in table (4) and figure (4). These results showed that the antibiotics amikacin has high sensitive effect to different bacterial isolates with percentage 86 % followed by imipenem with percent 82 %, ciprofloxacin with percent 60 % while trimethoprime and cefuroxime with percent 54 %, gentamycin with percentage 48 %, nitrofurantion 42 %, tobramycin 26 %, oxacillin 16% and ceftazidime with percent 10%. The antibiotics nitrofurantion show the highest intermediate effect on most bacterial isolates with percent 24 % followed by ciprofloxacin 20%, trimethoprime 12% while imipenem and ceftazidime with percent 6%, oxacillin with percent 6%, amikacin and gentamycin with percent 4% , and tobramycin with percent 2 %, the lowest intermediate effect was cefuroxime have percent 0 %.

The percentage of resistance organisms reaches 84% with the antibiotic ceftazidime followed by oxacillin with percent 78 %, tobramycin 72 %, gentamycin 48%, cefuroxime 46% while nitrofurantion and trimethoprime 34%, ciprofloxacin 20% , imipenem 12% and amikacin 10 %.

Table 4: Sensitivity of bacterial isolates to different antibiotics drugs

Antibiotics	**Conc.**	**Sensitivity %**					
		S (no)	**S (%)**	**I (no)**	**I (%)**	**R (no)**	**R (%)**
Amikacin (AK)	30	43	86%	2	4%	5	10%
Nitrofurantion (F)	300	21	42%	12	24%	17	34%
Imipenem (IPM)	10	41	82%	3	6%	6	12%
Ciprofloxacin (CIP)	5	30	60%	10	20%	10	20%
Trimethoprim/ Sulphmthaxozole (SXT)	1.25/ 23.75	27	54%	6	12%	17	34%
Cefuroxime (CXM)	30	27	54%	0	0%	23	46%
Oxacillin (OX)	1	8	16%	3	6%	39	78%
Gentamycin (CN)	10	24	48%	2	4%	24	48%
Ceftazidime (CAZ)	30	5	10%	3	6%	42	84%
Tobramycin (TOB)	10	13	26%	1	2%	36	72%

S=Sensitive I= Intermediate R= Resistant

%= Number of sensitive, intermediate or resistant bacteria /total number of bacteria × 100

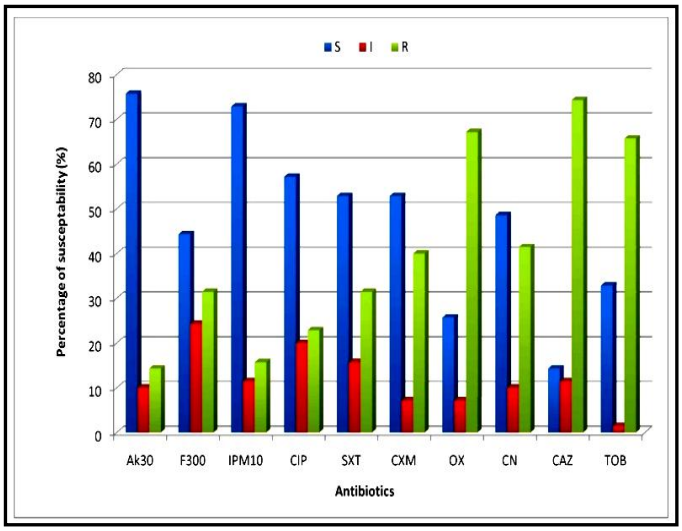


Figure 4: Sensitivity of bacterial isolates to different antibiotics
S=Sensitive I= Intermediate R= Resistant

3.6. Determination of MIC and MBC for most resistant antibiotics against resistant bacterial isolates

In this experiment, the minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of tobramycin and oxacillin, which were determined for *E.coil, P. aeruginosa, K. pneumonia* and *Staph. aureus.*

The obtained results in Table (5) and figure (5) clearly illustrate that the maximum MBC were obtained at tobramycin antibiotic which recorded 250 (Ug /ml) against *E. coil* and *K. pneumonia* and the lowest MBC were obtained 125 (Ug/ml) against *Staph. aureus* and *P. aeruginosa*. MBC equal to MIC which equal to 125 (Ug/ml) against *Staph. Aureus* and 250 (Ug/ml) against *K. pneumonia* .

The obtained results in Table (6) and figure (7) clearly illustrate that the maximum MBC were obtained at oxacillin antibiotic which recorded 250 (Ug/ml) against *K. pneumonia* and the lowest MBC were obtained 62.5 (Ug/ml) against *P. aeruginosa*. MBC equal to MIC which equal to 125 (Ug/ml) against *E.coli*.

Table 5: Minimum inhibitory concentration and minimum bactericidal concentration of tobramycin against resistant bacteria

Bacterial isolates	MIC (ug/ml)	MBC (ug/ml)
Staph. aureus	125	125
E.coli	125	250
K. pneumonia.	250	250
P .aeruginosa	62.5	125

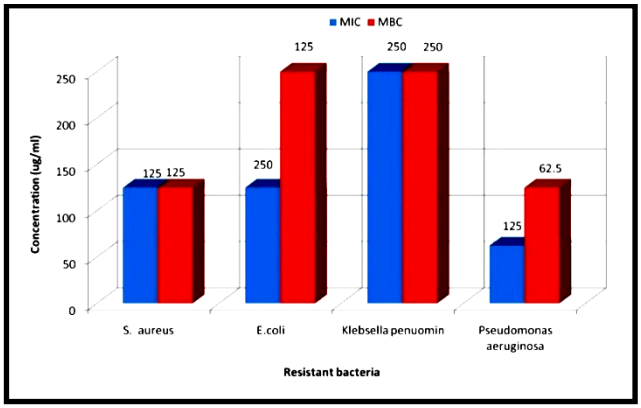


Figure 5: Minimum inhibitory concentration and minimum bactericidal concentration of tobramycin against resistant bacteria

Table 6: Minimum inhibitory concentration and minimum bactericidal concentration of oxacillin against resistant bacteria

Isolate No	MIC (ug/ml)	MBC (ug/ml)
Staph. aureus	62.5	125
E. coli	125	125
K. pneumonia	125	250
P. aeruginosa	31.25	62.5

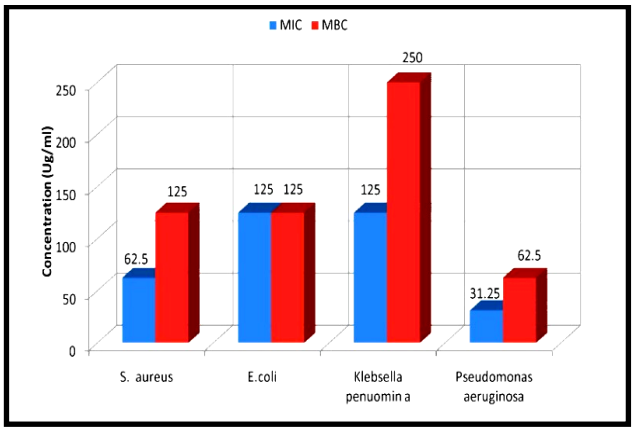


Figure 6: Minimum inhibitory concentration and minimum bactericidal concentration of oxacillin against resistant bacteria

Identification Information	Analysis Time:	4.75 hours	Status:	Final
Selected Organism	91% Probability	Klebsiella pneumoniae ssp pneumoniae		
	Bionumber:	2607735553565611		
ID Analysis Messages				

Susceptibility Information	Analysis Time: 10.75 hours			Status: Final	
Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
ESBL	NEG	-	Meropenem	>= 16	R
Ampicillin	>= 32	R	Amikacin	16	S
Ampicillin/Sulbactam	>= 32	R	Gentamicin	>= 16	R
Piperacillin/Tazobactam	>= 128	R	Tobramycin	>= 16	R
Cefazolin	>= 64	R	Ciprofloxacin	>= 4	R
Cefoxitin	>= 64	R	Levofloxacin	>= 8	R
Ceftazidime	>= 64	R	Nitrofurantoin	128	R
Ceftriaxone	>= 64	R	Trimethoprim/Sulfamethoxazole	>= 320	R
Cefepime	>= 64	R			

+= Deduced drug *= AES modified **= User modified

AES Findings		
Confidence:	Consistent	
Phenotypes flagged for review:	BETA-LACTAMS	IMPERMEABILITY CARBA (+ESBL OR +HL AmpC),CARBAPENEMASE (+ OR - ESBL)
	AMINOGLYCOSIDES	RESISTANT GEN TOB NET AMI (AAC(6')+?)

Figure 7: Multidrug resistant bacteria isolated from patients with malignancies at SECI and confirmed using VITEK2 System

3.7. Confirmation of the Identification of multi-drug resistant isolates

Ten multi drug resistant bacterial isolates were identified phenotypically by conventional biochemical tests as mentioned previously in table (3) , then confirmed by VITEK 2 system and finally the most common confirmed by Molecular Characterization.

Identification Information	Analysis Time:	4.00 hours	Status:	Final
Selected Organism	95% Probability	Klebsiella pneumoniae ssp pneumoniae		
	Bionumber:	6647734753564210		
ID Analysis Messages				

Susceptibility Information	Analysis Time: 9.25 hours			Status: Final	
Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
ESBL	NEG	-	Meropenem	>= 16	R
Ampicillin	>= 32	R	Amikacin	>= 64	R
Ampicillin/Sulbactam	>= 32	R	Gentamicin	>= 16	R
Piperacillin/Tazobactam	>= 128	R	Tobramycin	>= 16	R
Cefazolin	>= 64	R	Ciprofloxacin	>= 4	R
Cefoxitin	>= 64	R	Levofloxacin	>= 8	R
Ceftazidime	>= 64	R	Nitrofurantoin	256	R
Ceftriaxone	>= 64	R	Trimethoprim/Sulfamethoxazole	>= 320	R
Cefepime	>= 64	R			

+= Deduced drug *= AES modified **= User modified

AES Findings		
Confidence:	Consistent	
Phenotypes flagged for review:	BETA-LACTAMS	IMPERMEABILITY CARBA (+ESBL OR +HL AmpC),CARBAPENEMASE (+ OR - ESBL)
	AMINOGLYCOSIDES	RESISTANT GEN TOB NET AMI (AAC(6')+?)

Figure 8: Multidrug resistant bacteria isolated from patients with malignancies at SECI and confirmed using VITEK2 System showing resistant to all tested antibiotics except amikacin

3.8 Confirmation of identification of multi-drug resistant isolates by genotyping identification using 16S Ribosomal RNA Gene Sequencing:

Based on 16S rRNA gene sequence and morphological and biochemical characteristics, two multidrug resistant bacterial strains were identified as *Escherichia coli* AS1, Gene Bank accession No. (MG966417) and *staphylococcus aureus* AS2, Gene Bank accession No. (MG966418).

```
ORIGIN
        1 ATTGAACGCT GGCGGCAGGC CTAACACATG CAAGTCGAAC GGTAACAGGA AGCAGCTTGC
       61 TGCTTTGCTG ACGAGTGGCG GACGGGTGAG TAATGTCTGG GAAACTGCCN GATGGAGGGG
      121 GATAACTACT GGAAACGGTA GCTAATACCG CATAACGTCG CAAGACCAAA GAGGGGGACC
      181 TTAGGGCCTC TTGCCATCGG ATGTGCCCAG ATGGGATTAG CTAGTAGGTG GGGTAACGGC
      241 TCACCTAGGC GACGATCCCT AGCTGGTCTG AGAGGATGAC CAGCCACACT GGAACTGAGA
      301 CACGGTCCAG ACTCCTACGG GAGGCAGCAG TGGGGAATAT TGCACAATGG GCGCAAGCCT
      361 GATGCAGCCA TGCCGCGTGT ATGAAGAAGG CCTTCGGGTT GTAAAGTACT TTCAGCGGGG
      421 AGGAAGGGAG TAAAGTTAAT ACCTTTGCTC ATTGACGTTA CCCGCAGAAG AAGCACCGGC
      481 TAACTCCGTG CCAGCAGCCG CGGTAATACG GAGGGTGCAA GCGTTAATCG GAATTACTGG
      541 GCGTAAAGCG CACGCAGGCG GTTTGTTAAG TCAGATGTGA AATCCCCGGG CTCAACCTGG
      601 GAACTGCATC TGATACTGGC AAGCTTGAGT CTCGTAGAGG GGGGNAGAAT TCCAGGTGTA
      661 GCGGTGAAAT GCGTAGAGAT CTGGAGGAAT ACCGGTGGCG AAGGCGGCCC CCTGGACGAA
      721 GACTGACGCT CAGGTGCGAA AGCGTGGGGA GCAAACAGGA TTAGATACCC TGGTAGTCCA
      781 CGCCGTAAAC GATGTCGACT TGGAGGTTGT GCCCTTGAGG CGTGGCTTCC GGAGCTAACG
      841 CGTTAAGTCG ACCGCCTGGG GAGTACGGCC GCAAGGTTAA AACTCAAATG AATTGACGGG
      901 GGCCCGCACA AGCGGTGGAG CATGTGGTTT AATTCGATGC AACGCGAAGA ACCTTACCTG
      961 GTCTTGACAT CCACGGAAGT TTTCAGAGAT GAGAATGTGC CTTCGGGAAC CGTGAGACAG
     1021 GTGCTGCATG GCTGTCGTCA GCTCGTGTTG TGAAATGTTG GGTTAAGTCC CGCAACGAGC
     1081 GCAACCCTTA TCCTTTGTTG CCAGCGGTCC GGCCGGGAAC TCAAAGGAGA CTGCCAGTGA
     1141 TAAACTGGAG GAAGGTGGGG ATGACGTCAA GTCATCATGG CCCTTACGAC CAGGGCTACA
     1201 CACGTGCTAC AATGGCGCAT ACAAAGAGAA GCGACCTCGC GAGAGCAAGC GNACCTCATA
     1261 AAGTGCGTCG TAGTCCGGAT TGGAGTCTGC AACTCGACTC CATGAAGTCG GAATCGCTAG
     1321 TAATCGTGGA TCAGAATGC
//
```

Figure 9: Partial sequence of 16S rRNA product gene sequence of E. coil AS1

```
ORIGIN
        1 ATCCTGGCTC AGGATGAACG CTGGCGGCGT GCCTAATACA TGCAAGTCGA GCGAACGGAC
       61 GAGAAGCTTG CTTCTCTGAT GTTAGCGGCG GACGGGTGAG TAACACGTGG ATAACCTACC
      121 TATAAGACTG GGATAACTTC GGGAAACCGG AGCTAATACC GGATAATATT TTGAACCGCA
      181 TGGTTCAAAA GTGAAAGACG GTCTTGCTGT CACTTATAGA TGGATCCGCG CTGCATTAGC
      241 TAGTTGGTAA GGTAACGGCT TACCAAGGCA ACGATGCATA GCCGACCTGA GAGGGTGATC
      301 GGCCACACTG GAACTGAGAC ACGGTCCAGA CTCCTACGGG AGGCAGCAGT AGGGAATCTT
      361 CCGCAATGGG CGAAAGCCTG ACGGAGCAAC GCCGCGTGAG TGATGAAGGT CTTCGGATCG
      421 TAAAACTCTG TTATTAGGGA AGAACATATG TGTAAGTAAC TGTGCACATC TTGACGGTAC
      481 CTAATCAGAA AGCCACGGCT AACTACGTGC CAGCAGCCGC GGTAATACGT AGGTGGCAAG
      541 CGTTATCCGG AATTATTGGG CGTAAAGCGC GCGTAGGCGG TTTTTTAAGT CTGATGTGAA
      601 AGCCCACGGC TCAACCGTGG AGGGTCATTG GAAACTGGAA AACTTGAGTG CAGAAGAGGA
      661 AAGTGGAATT CCATGTGTAG CGGTGAAATG CGCAGAGATA TGGAGGAACA CCAGTGGCGA
      721 AGGCGACTTT CTGGTCTGTA ACTGACGCTG ATGTGCGAAA GCGTGGGGAT CAAACAGGAT
      781 TAGATACCCT GGTAGTCCAC GCCGTAAACG ATGAGTGCTA AGTGTTAGGG GGTTTCCGCC
      841 CCTTAGTGCT GCAGCTAACG CATTAAGCAC TCCGCCTGGG GAGTACGACC GCAAGGTTGA
      901 AACTCAAAGG AATTGACGGG GACCCGCACA AGCGGTGGAG CATGTGGTTT AATTCGAAGC
      961 AACGCGAAGA ACCTTACCAA ATCTTGACAT CCTTTGACAA CTCTAGAGAT AGAGCCTTCC
     1021 CCTTCGGGGG ACAAAGTGAC AGGTGGTGCA TGGTTGTCGT CAGCTCGTGT CGTGAGATGT
     1081 TGGGTTAAGT CCCGCAACGA GCGCAACCCT TAAGCTTAGT TGCCATCATT AAGTTGGGCA
     1141 CTCTAAGTTG ACTGCCGGTG ACAAACCGGA GGAAGGTGGG GATGACGTCA AATCATCATG
     1201 CCCCTTATGA TTTGGGCTAC ACACGTGCTA CAATGGACAA TACAAAGGGC AGCGAAACCG
     1261 CGAGGTCAAG CAAATCCCAT AAAGTTGTTC TCAGTTCGGA TTGTAGTCTG CAACTCGACT
     1321 ACATGAAGCT GGAATCGCTA GTAATCGTAG ATCAGCATGC TACGGTGAAT ACGTTCCCGG
     1381 GTCTTGTACA CACCGCCCGT CACACCACGA GAGTTTGTAA CACCCGAA
//
```

Figure 10: Partial sequence of 16S rRNA product gene sequence of S. aureus AS2

3.9.1. Effect of different concentration of rosemary ethanolic extract on the growth of most resistant bacterial isolates

The effect of different rosemary extract concentrations (20,40,60,80,100%) on the growth of both Gram -positive and Gram -negative isolates .The obtained results in table (7) and figure (11) showed that all bacterial isolates are sensitive to rosemary even at low concentration and the highest effect on *Staph. aureus* with diameter more than (20mm) even at 20% concentration .

Table 7: Effect of different rosemary different concentration on the growth of most resistant bacterial isolates

Bacterial isolates	Diameter of inhibition zone of rosemary concentrations (mm)					L.S.D
	20 (%)	40 (%)	60 (%)	80 (%)	100 (%)	
Staph. aureus	22 (S)	25(S)	26(S)	27 (S)	29 (S)	1.25
E. coil	19 (S)	20(S)	21(S)	23 (S)	25 (S)	1.50
K. pneumonia	18 (S)	19(S)	20 (S)	22 (S)	24 (S)	0.78
P. aeruginosa	17 (S)	19 (S)	20 (s)	21 (S)	22 (S)	0.53

L.S.D.: Least significant difference at P ≤ 0.05-0.01
S=Sensitive

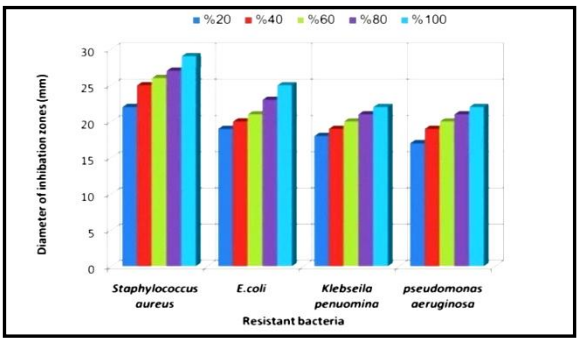


Figure 11: Effect of different rosemary concentrations on the growth of most resistant bacterial isolates

3.9.2. Effect of different concentration of lemon peel ethanolic extract on the growth of most resistant bacterial isolates

The effects of different lemon peel concentration (20, 40, 60, and 80, 100%) on both Gram -positive and Gram negative isolates. The obtained results in table (8) and figure (12) showed that lemon peel had intermediate effect on *Staph. aureus* less than (15mm). Lemon peel had high effect on *E. coil* and *K. Pneumonia* with inhibition zones (21and 22mm) at 100% concentration, while at low concentration had moderate effect on *P. aeruginosa* and sensitive effect from (60%) to 100% concentration less than (20mm).

Table 8: Effect of different lemon peel concentration on the growth of bacterial isolates

Bacterial isolates	Diameter of inhibition zone of lemon peel concentration (mm)					L.S.D
	20 (%)	40 (%)	60 (%)	80 (%)	100 (%)	
Staph.aureus	11 (I)	12 (I)	13 (I)	14 (I)	15 (I)	**0.63**
E.coli	16 (S)	17(S)	19 (S)	20(S)	21 (S)	**1.30**
K.penuomina	16 (S)	17 (S)	18 (S)	21(S)	22 (S)	**1.4**
P.aeruginosa	14 (I)	15 (I)	16 (S)	17 (S)	18 (S)	**0.74**

S=Sensitive I= Intermediate

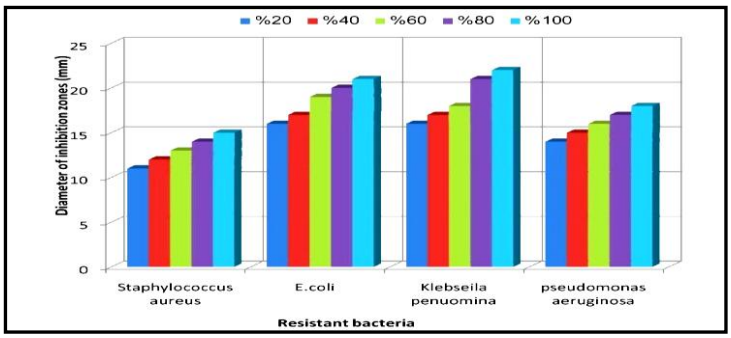


Figure 12: Effect of different lemon peel concentration on the growth of most resistant bacterial isolates

3.9.3. Effect of different concentration of marjoram ethanolic extract on the growth of most resistant bacterial isolates

The effects of different marjoram extract concentration (20, 40, 60, 80 and 100%) on both Gram -positive and Gram –negative isolates. The obtained results in table (9) and figure (13) showed that marjoram had high sensitive effect on *Staph. aureus* reaches (23mm). *E.coil* and *K. pneumonia* were sensitive to marjoram and inhibition zones reach (20 and 21) mm at 100% concentration while at 20% concentration had moderate effect on *p. aeruginosa*

Table 9: Effect of different marjoram concentration on the growth of most resistant bacterial isolates

Bacterial isolates	Diameter of inhibition zone of marjoram concentration (mm)					L.S.D
	20 (%)	40 (%)	60 (%)	80 (%)	100 (%)	
Staph. aureus	19 (S)	20 (S)	21 (S)	22 (S)	23 (S)	**0.75**
E.coli	16 (S)	17 (S)	18 (S)	19(S)	20 (S)	**0.98**
K.pneumonia	17 (S)	18 (S)	19 (S)	20(S)	21 (S)	**0.35**
P. aeruginosa	15 (I)	16 (S)	17 (S)	18(S)	19 (S)	**0.77**

S=Sensitive I= Intermediate

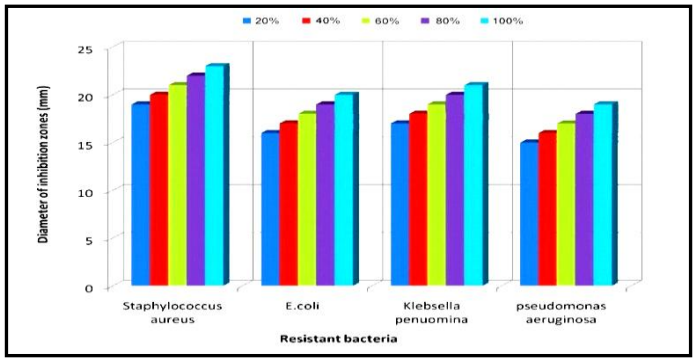


Figure 13: Effect of different marjoram concentration on the growth of most resistant bacterial isolates

3.9.4. Effect of different concentration of thyme ethanolic extract on the growth of bacterial isolates

The effect of different thyme concentration (20, 40, 60, 80, and 100%) on both Gram –positive and Gram negative bacteria. The obtained results in table (10) showed that thyme had high effect on all bacterial isolate. Thyme had inhibition zones (27mm) on *Staph. aureus* at 100% concentration, *E.coil* at low concentration 20% had (30mm) inhibition zones and reaches (35mm) at 100% concentration, *K. penuomina* at low concentration 20% had (26mm) inhibition zones and reaches (31mm) at 100% concentration. *P. aeruginosa* at low concentration 20% had (16mm) inhibition zones and reaches (23mm) in 100% concentration. The high effect of thyme was on *E. coil.*

Table 10: Effect of different thyme concentration on the growth of bacterial isolates

Bacterial isolates	Diameter of inhibition zone of thyme oil concentration (mm)					L.S.D
	22 (%)	44 (%)	66 (%)	88 (%)	100 (%)	
Staph. aureus	22 (S)	23 (S)	25 (S)	26 (S)	27 (S)	**0.87**
E. coli	30 (S)	31 (S)	32 (S)	33 (S)	35 (S)	**1.35**
K. pneumonia	26 (S)	27 (S)	29 (S)	30 (S)	31 (S)	**1.025**
P. aeruginosa	16 (S)	19 (S)	20 (S)	22 (S)	23 (S)	**0.89**

S=Sensitive

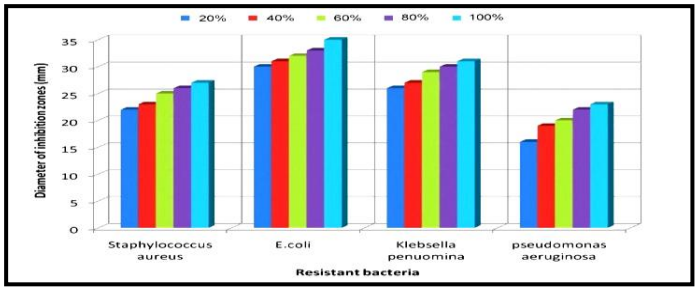


Figure 14: Effect of different thyme extract concentrations on the growth of bacterial isolates

3.10. Effect of different cold water plant extracts on the growth of most resistant bacterial isolates

The results given in table (11) showed that all bacterial isolates are resistant to cold water extract of lemon peel and marjoram while cold water extract of rosemary and thyme had intermediate effect on all bacterial isolates. Rosemary cold water extract was the most effective.

Table 11: Effect of plant extract (extraction by cold water) against most resistant bacterial isolates (mm)

Bacterial isolates	Diameter of inhibition zones of cold water plant extracts against bacteria (mm)			
	Lemon	**Rosemary**	**Marjoram**	**Thyme**
Staph. aureus	0(R)	15(I) a	5(R)	10(I)
E. coil	4(R)	14(I)	0(R)	12(I)
K. pneumonia	5(R)	14(I)	0(R)	10(I)
P. aeruginosa	3(R)	11(I)	0(R)	10(I)

I= Intermediate R= Resistant

4. Discussion

Nosocomial infections are one of the most important causes of mortality and morbidity in hospital in developing countries [30]. Hospital acquired infections continues to be a serious complication of hospitalization. Nosocomial infection rates within neonatal intensive care unit in US hospitals increased by 37% from 1975 to 1995 at a cost of over 4.5 billion dollars in health care [31]. *Klebsiella pneumonia* can also cause less various respiratory infections, such as bronchitis, which is usually a hospital acquired infection .Classically, *K. penuomina* causes a severe, rapid –one set illness that often causes areas of destruction in the lung [32]. *E. coli* infection is one of the major public health problems in developing countries and has contributed exceedingly to morbidity, mortality and increased health costs [33]. *Staphylococcus aureus* can cause a range of illnesses, from skin infections, such scalded skin syndrome, to life-threatening diseases such as pneumonia, toxic shock syndrome (TSS), bacteremia and sepsis [34].

In this study, fifty clinical bacteria were isolated from patients with malignancies at south Egypt cancer institute. The dominant bacterial isolates were *E. coli, Pseudomonas aeruginosa, Staph. aureus and Klebsiella pneumonia.* The occurrence and spread antibiotics resistant bacteria are pressing public health problems worldwide. Many bacteria have become and continue to be resistant nearly against all antimicrobial agents. The resistance rates are higher in developing countries [35].

In our study The percentage of resistance organisms reaches 84% with the antibiotic ceftazidime followed by oxacillin with percent 78 %, tobramycin 72 %, gentamycin 48%, cefuroxime 46% while nitrofurantion and trimethoprime 34%, ciprofloxacin 20%, imipenem 12% and amikacin 10 %. This match with [36]. In case of amikacin and do not match with ciprofloxacin, gentamycin, ceftazidime and imipenem and match with [37], who stated that amikacin and imipenem showed the greatest activity against all isolates, all Gram -negative organisms showed great resistance to second, third and fourth generation cephalosporines [38]. *Klebsiella* sensitive to gentamycin, while another study reported *Klebsiella* to be most resistant to gentamycin and amikacin, and most sensitive to doxycycline [39].

In our study clearly illustrate that the maximum MBC were obtained at tobramycin antibiotic which recorded 250 (Ug /ml) against *E.coil and K. pneumonia*. The maximum MBC were obtained at oxacillin antibiotic which recorded 250 (Ug/ml) against *K.pneumonia*.

In most of developed countries, as Egypt, the antibiotics are provided without prescriptions and there for the need for public awareness against the misuse of antibiotics is important. Simultaneously, some useful but expensive drugs should be used with caution to prevent or slow down the emergence of drug resistance which seem inevitable with wide spread of indiscriminate use [40].

[41], show that MICs and MBCs were determined for ciprofloxacin, amikacin and imipenem. Nine strains were moderately susceptible to ciprofloxacin (MIC, 0.5 to 2, ug/ml), 12 strains were moderately susceptible to imipenem (MIC, 4 to 8, ug/ml), and none was moderately susceptible to amikacin. The ciprofloxacin, imipenem, and amikacin MICs and MBCs for 90% of strains tested were 16 and 128, 32 and 128, and 512 and >512, ug/ml, respectively.

Our results match with [42], who reported that rosemary plants are rich sources of phenolic compounds with high antimicrobial activity against both Gram-positive and Gram-negative bacteria and do not match with [43], who stated that in case of *Klebsiella pneumonia* no antimicrobial effect of rosemary .

In our study lemon grass had intermediate effect on *Staph. aureus* and high effect on *K. penuomina*, *P. aeruginosa* and *E. coli* .Our result match with [44] who reported that lemon grass showed high inhibitory effect on Gram negative and does not match with[45].

In our results marjoram had high sensitive effect on *Staph. aureus, E. coil, K. pneumonia* and *p. aeruginosa*. The present results were in agreement with earlier reports about antimicrobial activity of marjoram essential oils and their main constituents, carvacrol and thymol [46]. Also it is reported that volatile aromatic components in plant kingdoms exhibit more antimicrobial potential than those of non aromatic volatile components of essential oils [47].

The present study showed that thyme had higher effect on all tested bacterial isolate. Our results are in agreement with [48].

In our study clove at 100% concentration had high sensitive effect on *Staph. aureus* and intermediate effect on *E. coli, K. penuomina* and *P. aeruginosa*. At 22 % concentration it was inactive against *P. aeruginosa.* Our results go on line with that of [49].

Thyme and rosemary cold water plant extract had intermediate effect on the growth of all bacterial isolates. Methanolic extracts of lemon and marjoram had intermediate antagonistic effect on *Staph. aureus, K. penuomina, P. aeruginosa* and *E. coli.*

5. Conclusion and Recommendation

Infections acquired in the healthcare setting raise a great risk for patients, leading to high rates of morbidity and mortality. Many of the 90,000 deaths caused by nosocomial infections could be prevented by following evidence-based guidelines and consensus statements on preventive strategies. Several institutions have implemented campaigns to enhance the quality of health care and patient safety by focusing on measures to reduce the four most common nosocomial infections, which comprise approximately 80% of all nosocomial infections. From this study the most bacteria responsible for nosocomial infection were *K. penuomina*, *P. aeruginosa*, *E. coil* and *Staph. aureus* .The most effective antibiotics were amikacin, imipenem, ciprofloxacin, trimethoprime and cefuroxime for different bacterial isolates. Natural extracts gave best results against different bacterial isolates especially rosemary, thyme and marjoram.

References

[1]. Sai-Cheong, L.; Shie-Shian H.; Lai-Chu S., Ming-Han T. and Wen-Ben S. (2011): *In vitro* activities of nine current antibiotics against culprit bacteria in nosocomial infections in an institution in northern Taiwan biomedical journal 34 (6) :580-589 .

[2]. Bearman, G.M.L.; Munro, C.; Sessler, C.N. and Wenzel, R.P. (2006): Infection control and the prevention of noscomial infections in the intensive care unit. SeminRespirCrit Care Med.; (27):310-324.

[3]. Nseir, S.; Povoa P.; Salluh, J.; Rodriguez, A. and Martin-Loeches, L. (2016): Is there a continuum between ventilator-associated trachea bronchitis and ventilator-associated pneumonia? Offi. J. Eur. Soc. Intensive Care Med. (42): 1190-1192.

[4]. Favre, B.; Hugonnet, S.; Correa, L.; Sax, H.; Rohner, P. and Pittet, D. (2005): Nosocomial bacteremia: clinical significance of a single blood culture positive for coagulase-negative staphylococci.J.Infect. Control Hosp.Epidemiol.; 26(8):697-702.

[5]. Mutlu, M.; Aslan, Y.; Saygın, B.; Yılmaz, G.; Bayramoglu, G. and Koksal, M. (2011): Neonatal Sepsis Caused by Gram-negative Bacteria in a Neonatal Intensive Care Unit: A Six Years Analysis. HK. J. Paediatr. (new series); (16): 253-260.

[6]. Lohi, A.; Sipponen, A.; Jokinen, J. and Vanha, K. (2006): Apotential method to treat infected chronic skin ulcer, Kuusenpihkastavalmistetunvoiiteenkayttohaavojenhoidossa. Haava; (3):10-13.

[7]. Darnton, N.C.; Turner, L.; Rojevsky, S.; and Berg, H.C. (2007): On torque and tumbling in swimming *Escherichia coli*. J. Bactoriol.; 189(5): 1756-1764.

[8]. Rayan, K.J. and Ray, C.G. (2004): Sherries Medical Microbiology, 4th ed., McGraw Hill. ISBN 0838585299.
[9]. Abd-El-Rhaman, M.N. (2008): Studies on some clinical bacteria resistance to beta-lactam antibiotics M.Sc. thesis Dep. of Botany, Faculty of Science, Zagazig Univ., Egypt.
[10]. Aridogan, B.; Baydar, C.; Kaya, H.; Demirici, S.; O M.; Zbasar, D. and Mumcu, E. (2002): Antimicrobial activity and chemical composition of some essential oils. Archives of Pharmacal Research., 25: 860-864.
[11]. Go- Yoshimura, M.L.; Theerathavaj, S.; Sroisiri, T. and Suwan, C. (2007): Antimicrobial activity of the midicnal herbal extracts against Mutans *Streptococci* and *Candida albicans* in vitro.
[12]. Hosseinimehr, S.J.; Pourmorad, F.; Shahabimajd, N.; Shahrbandy, K. and Hosseinzadeh, R. (2007): In vitro antioxidant activity of *Polygoniumhyrcanicum*, *Centaureadepressa*, *Sambucusedulus*, *Menthaspicata* and *Phytolacca* Americana. Pakistan J. of Biological Sciences. (10): 637-640.
[13]. Albarracin, H.W.; Alfonso, A.C.; Iván, C.; Sanchez, B. (2012): Application of essential oils as a preservative to improve the self-life of nile tilapia (*Oreochoromisniloticus*). Vitae 19: 3440.
[14]. Khalili, S.T.; Mohsenifar, A.; Beyki, M.; Zhaveh, S. and Rahmani-Cherati, T. (2015): Encapsulation of thyme essential oils in chitosan-benzoic acid nanogel with enhanced antimicrobial activity against *Aspergillusflavus*. Food Sci. Technol.; (60): 502-508.
[15]. Malini, M.; Abirami, G.; Hemalatha, V. and Annadurai, G. (2013): Antimicrobial activity of ethanolic and aqueous ex-tracts of medicinal plants against waste water pathogens. Int. J. Res. Pure Appl. Microbiol.; 3(2):40–42.
[16]. Cruickshank, R.; Duguid, J. P.; Marmion, B. R. and Swain, R. H. A. (1975). Medical Microbiology.12th Ed.; Living stone, London, NewYork, 812-825.
[17]. Atlas, R.M., L.C. Parks (1993). Handbook of Microbiological Media. CRC Press, Inc. London.
[18]. DIFCO Laboratories. (1984). Page 558 in DIFCO Manual, 10th Ed. DIFCO Laboratories, Detroit, MI.
[19]. Baron AJ, Wong TY, Hicks SJ, Gacesa P, Willcock D, Mcpherson MJ (1994). Alginate lyase from *Klebsiella pneumoniae*, subsp. *aerogenes*: gene cloning, sequence analysis and high-level production in *Escherichia coli*. Gene. 1994;143(1):61–66. doi: 10.1016/0378-1119(94)90605-X.
[20]. Cappuccino, J.G. and Sherman, N. (2001), Microbiology: A Laboratory Manual. 6th Edition, Benjamin Cummings, San Francisco.
[21]. Bergey's Manual. (1989). Bergey DH, Krieg NR, Holt JG, Bergey's Manual of Systematic Bacteriology: Williams & Wilkins; 1989.
[22]. Lane D. J. (1991): 16S/23S rRNA sequencing. In Nucleic Acid Techniques in Bacterial Systematics, Eds, Stackebrandt E, Goodfellow M (Wiley, New York), 115-175,(1991).
[23]. Bauer, A. W.; Kirby, W. M. M.; Sherris, J. C. and Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.*, 54(4):493-496.
[24]. Clinical Laboratory Standards Institute (CLSI) (2011). Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard, 10th ed. M2-A10. Clinical Laboratory Standards Institute, Wayne, PA.
[25]. Jorgensen, M. G.; Aalam, A. and Slots, J. (2005): Periodontal Antimicrobials finding the right solutions, J. of Int. Dent. (55):.3-12.
[26]. Moreira, M. R.; Ponce, A. G.; Del Valle, C.E. and Roura, S.I. (2005): Inhibitory parameters of essential oils to reduce a food borne pathogen. LWT.; (38):565-570.
[27]. Hammer, K.A., Carson, C.F. and Riley, T.V. (1999). Antimicrobial activity of essential oils and other plant extracts. Journal of Applied Microbiology 86, 985^990.
[28]. Maslin, M.M. and Pascule , J .V .(2002): Thioproponal S- oxidealachrymatory factor in onion. J. Agric. food Chern.; (19): 269-272.
[29]. Kawther, F.A. (2007): Antimicrobial activity of essential oils of some medicinal plants from Arab Saudi.Saudi J.of Biol.Sci. 14(1):53-60.
[30]. WHO, (2012): Prevention of hospital-acquired infections: A practical guide. 2nd edition. WHO/CDS/CSR/EPH/

[31]. Borguesi A, Stronati M. (2008): Strategies for the prevention of hospital acquired infections in the neonatal intensive care unit. J. Hosp. Infect.; (68): 293–300.
[32]. Lin, Y.T.; Wang, Y.P.; Wang, F.D. and Fung, C.P. (2015): Community-onset *Klebsiella pneumonia* in Taiwan: clinical features of the disease and associated microbiological characteristics of isolates from pneumonia and nasopharynx. Front Microbiol; 9:122.
[33]. Lausch K.R.; Fuursted. K.; Larsen, C.S.; and Storgaard, M. (2013): Colonisation with multi-resistant *Enterobacteriaceae* in hospitalized Danish patients with a history of recent travel: a cross-sectional study. Travel Med Infect Dis; 11(5): 320-3.
[34]. Vandenesch, F.; Lina, G. and Henry, T. (2012): *Staphylococcus aureus* hemolysins, bi-component leukocidins, and cytolytic peptides: a redundantarsenal of membrane-damaging virulence factors? Front Cell InfectMicrobiol; (2): 12.
[35]. Simon, C. F.; Oxman, B. and Nrigu, J. (2009): Prevalance of antibiotics resistance bacteria and treatment. Appl. Environ Microbiol.; (75): 5714-5718.
[36]. Lavakhamseh, H.; Shakib, P.; Rouhi, S.; Mohammad, F. and Ramazanzadeh, R. (2014): A Survey on the Prevalence and Antibiotic Sensitivity of Nosocomial Infections in the Besat Hospital, Sanandaj, Iran Journal NI, 1(2), 1-8.
[37]. El- Sahrigy, S. A.F.; Abdel Rahman, A .M.O.; Youssef,H. ; Talaat, A. A.; Khairy, D.A.; Gomaa, H.E. and Dorgham, S. M. (2015): Nosocomial Infection in an Egyptian Neonatal Intensive Care Unit. Research Journal of Pharmaceutical, Biological and Chemical Sciences (6): 346-352.
[38]. Das, R.N.; Chandrashekhar, T.S.; Joshi, H.S.; Gurug, M.; Shrestha, N. and Shivanada, P.G. (2006): Frequency and susceptibility profile of pathogens causing urinary tract infections at a tertiary care hospital in western Nepal. Singapore Med. J., (47): 281-285.
[39]. Ghaznavi-Rad, E.; Ghasemzadeh-Moghaddam, H.; Shamsudin, M. N.; Hamat, R.A.; Sekawi, Z.; Aziz, M.N.; Tavakol, M.; van- Belkum, A. and Neela, V. (2010): Environmental contamination in the hospital as a possible source for nosocomial infection with methicillin-resistant Staphylococcus aureus. Infect. Control Hosp. Epidemiol., (31): 1302- 1303.
[40]. Ayad, A. (2009): Evalution of antibacterial efficiency of some plant extract to control urinary tract system pathogens. M.S.Sc. thesis, Dep. of Botany, Faculty of Science, Benha Univ., Egypt.
[41]. Haller, I. (1985): Comprehensive evaluation of ciprofloxacin aminoglycoside combinations against Enterobacteriaceae and Pseudomonas aeruginosa strains. Antimicrob. Agents Chemother (28):663-666.
[42]. Moreno, S.; Scheyer, T.; Romano, C.S. and Vojunov, A.A. (2006): Antioxidant and antimicrobial activates of rosemary extracts linked to their polyphenol composition. Free Radical Research, 40 (2): 223-231.
[43]. Abu-Gharbia, M. A.; Michael, N.; El-Mewafy, A.; El-Ghadban, and Abdelmasieh, R. (2015): Antibacterial activity of *cymbopogon citrates* and *rosmarinus officinalis* essential oil against carbapenemsresistant *Klebsiella pneumonia* strains as nosocomial pathogen isolated from intensive care units of two hospitals Global J. Advaanced Research of Medicine and Med. Sci.(1):047-050.
[44]. Kruthi, B.S.; Kruthi, K.; Priya, P.S.; Jyothi, T.H. and Gogte, S. (2012): *In vitro* testing of antimicrobial properties of Lemongrass, Eucalyptus and their synthetic effects. Inter. J. Scientific and Research Publications. 4(2): 1-8.
[45]. Ghaly, M.F.; Shalaby, M.A.; Shash, S.M.S.; Shehata, M.N. and Ayad, A.A. (2009): Synergistic Effect of Antibiotics and plant extract to control clinical bacterial isolates implicted in Urinary tract infections. J. Appl. Sci. Research, 5(10): 1298-1306.
[46]. Bendahou, M.A.; Muselli, M.; Grignon-Dubois, M.; Benyoucef, J. M.; Desjobert, A. F.; and Costa, J. (2008): Antimicrobial activity and chemical composition of *Oiganum glandulosum* Desf. essential oil and extract obtained by microwave extraction: comparison with hydro distillation. J. Food chemistry 106: 132–139.
[47]. Wang, S. Y.; Chen, P.F. and Chang, S. T. (2005). Antifungal activities of essential oils and their constituents from indigenous cinnamon (*Cinnamomum osmophloeum*) leaves against wood decay fungi. bioresource technology. (96): 813–818.

[48]. Nanasombat, S. and Wimuttigosol, P. (2011) : Antimicrobial and antioxidant activity of spice essential oils. Food Sci. Biotechnol., (20): 45-53.

[49]. Saeed, S. and Tariq, P. (2008): *In vitro* antibacterial activity of clove against gram negative bacteria. Pak. J. Bot., 40(5): 2157-2160.