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Epidemiology and Control of Nosocomial Pathogens Recovered from Some Governmental Hospitals, Cairo, Egypt

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A TOTAL of 350 samples were collected from different sites located in three governmental hospitals in Cairo, Handwashing sink was the most contaminated site followed by Computer keyboards and ultrasonic machines. According to viteck, bacterial isolates were identified as *Staphylococcus aureus, S.saprophyticus, Bacillus cereus, B.subtilis, Klebsiella pneumonia, Acnietobacter baumanii, Pseudomonas auerignosa* and *Escherichia coli*. Fungal isolates such as *Aspergillus niger, Aspergillus fumigatus, Rhizopus stolonifer, Fusarium oxysporum, Cladosporium herbarum, Penicillium melanoconidium, and etc.,* were identified by macro and micro morphological characteristics. *S.aureus and K.pneumonia* were the most common isolated bacteria with percentage (44.2&24.7%) respectively, while *A.niger* was the most common isolated fungus with percentage (13%). Synthetic antimicrobial agents are widely used to fight microbial infections with some limitations and toxicity, consequently, many researches focused on new antimicrobial substances from natural plants. Antimicrobial activity of both natural (Eucalyptus leaves and Garlic extracts) and synthetic antimicrobials (Chloramphenicol, Amoxicillin, fluconazole and Amphotericin B) were tested against isolated microbial species using agar disk diffusion method. *B. cereus* was the most sensitive bacterial species to garlic extract with 45mm inhibition zone diameter while *S.aureus* and *B.cereus* were the most sensitive species to Eucalyptus extract with 35&30mm , respecively. On the contrary both of natural extracts possess very weak activity against isolated fungal pathogen.

Keywords: Antimicrobial agents, Bacterial and fungal contamination , Nosocomial infections.

Introduction

The term of nosocomial is used for any disease acquired by patient under medical care during hospital stay (Khan et al., 2015). Nosocomial infections can spread through direct contact from individual-to individual that occur commonly by the hands or by indirect contact through some objects such as common vehicle, liquid and food (Osman et al., 2018). Bacteria, viruses and fungal pathogens are responsible for nosocomial infections depending on based on different populations of patient, medical facilities and differences in the environment underlying which the care is considered (Khan et al., 2017). Commonly, bacteria are responsible for ninety percent of nosocomial infections occurrence, while mycobacterial, viral, fungal or protozoal agents represent the less common microorganisms. Bacteria that frequently cause nosocomial infections include *Staphylococcus aureus, Bacillus cereus, Streptococcus* spp. *Acinetobacter* spp., coagulase negative Staphylococci, enterococci, *Pseudomonas aeruginosa, Legionella* and members of the Enterobacteriaceae family such as *Escherichia coli, Salmonella* spp. *Proteus mirabilis, Serratia marcescens* and *Klebsiella pneumoniae*. However, *E. coli, S. aureus,* enterococci and *P. aeruginosa* are considered the most frequent nosocomial pathogens among bacterial organisms (Bereket et al., 2012). In addition to that, fungal pathogens also cause nosocomial infections but they are considered

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less common than bacteria, From these fungal pathogens; *Asperigillus* species, *Fusarium* species, *Rhizopus* species, *Cladosporium* species and *Penicillium* species (Khan et al., 2017). It was found that plant sources with antimicrobial effect, are considered environmentally friendly to control some of the fungal contamination but these plant sources may not have effect on some of fungal pathogens such as species which produce myctoxins, responsible for fungal contamination (Tiwari et al., 2017). Synthetic antimicrobial agents are widely used to fight microbial infections such as Chloramphenicol which is considered commonly used in the past because of its broad spectrum of activity against many Gram-positive and Gram-negative bacteria (Eliakim-Raz et al., 2015). Also Amoxicillin is considered more effective against gram positive than gram negative microorganisms and it exhibits greater efficiency when compared to other antibiotics, e.g. ampicillin, azithromycin, clarithromycin, cefuroxime and doxycycline in treatment of different infections(Kaur et al., 2011). Amphotricin-B is considered the most active drug used against fungal pathogens (Noor & Pellegrini, 2018). Also fluconazole is considered one of the antifungal drugs which was considered the first azole systematically used for chemoprophylaxis of invasive infections because of its high level of activity and low toxicity, so fluconazole enabled an earlier and prophylactic use of systemic antifungals (Vazquez et al., 2016). It was found that the continuous use of antimicrobial drugs over the years is the main factor responsible for the appearance of bacterial resistance that threat public health (Pesarico AP et al., 2013). In recent years, due to this bacterial resistance to synthetic antimicrobial agents and emerging of multi resistance strains, many researches focused on new antimicrobial substances from natural sources such as plants. Among these plants, Garlic extract (Allicin) (Easa et al., 2001, 2002; Rad et al., 2017), in addition to that Eucalyptus is an aromatic medicinal plant which considered one of the proper choices as antimicrobial agent. The leaf extracts of this plant have many effects as anti-inflammatory, anticancer, antifungal, antiviral and antioxidant (Easa & El-Beih, 2002; Seyyednejad et al., 2014; Saadia et al., 2018; Elsonbaty et al ., 2020). The present study aimed to study the characteristics of the most predominant and pathogenic different microbial organisms causing nosocomial infections and how to control of these infections and reduce them using natural antimicrobials.

Materials and Methods

Sampling procedure

Three hundred and fifty samples were collected from three governmental hospitals in Cairo from January 2016 until Autumn 2016 as shown in (Table 1). Sterile test tubes each containing prepared nutrient broth were labeled appropriately and were taken to the hospital. Swabbing of the surface of each item was made using sterile cotton wool soaked with nutrient broth. In addition to that, samples for isolation of fungi were collected by swabbing the surface of the same sites in the three hospitals using sterile disinfected swab with 70% alcohol solution (Viegas et al., 2016). All the samples were transported back to the laboratory immediately for isolation of pathogenic microorganisms (Muhammad et al., 2017).

TABLE 1. Samples collected from different sites in selected hospitals

Sites of sampling	No. of samples			Total
	Hospital (A)	Hospital (B)	Hospital (C)	
Hand washing sink	11	11	11	33
Computer keyboards	11	10	12	33
Telephone/cell phones	10	10	11	31
Portable radiograph equipment	10	10	11	31
Ultrasound machine	11	11	10	32
Stethoscopes	10	11	11	32
Blood pressure cuffs	11	10	10	31
Suction system switches	11	10	11	32
Ventilator (e.g., buttons, circuits)	10	11	11	32
Bed rails	10	10	11	31
White coats/scrubs	10	11	11	32
Total	115	115	120	350

Isolation and identification of nosocomial pathogens

The streak-plate procedure is used to isolate bacteria on different specific media (Sanders et al., 2012), isolated bacteria were identified by recognizing their culture characteristics on different selective and specific media: MacConkey agar, Cystine Lactose Electrolyte Deficient agar (CLED) agar, Blood agar while Nutrient agar, Mannitol salt agar agar (MYP) agar, Deoxyribonucleic acid agar (DNA agar)according to Bergy's Manual of Systematic Bacteriology (Scardovi et al., 1986; Holt et al., 1984). Microscopic characteristics such as cell morphology, arrangement and gram reaction of bacterial isolates were determined using Gram stain technique (Cruickshank et al., 1975). Biochemical reactions were carried out by Vitek2compact system according to the manufacture's instruction, Vitek 2 Compact is considered an automated microbial identification system that was developed as a result of bioMerieux's long-standing experience with microbial identification (Pincus, 2006). Fungal isolates were identified macromorphologically using different media as: Sabroud's dextrose agar, Potato dextrose agar, Czapek's yeast agar, and yeast extract sucrose agar and micromorphologically by slide culture technique (Gaddeyya et al., 2012).

Antimicrobial assay

Preparation of Eucalyptus leaves extract

Fresh leaves of Eucalyptus were collected from public park. The leaves were thoroughly washed under running tap water and allowed to dry at room temperature for 2 days. The dried material was crushed into fine powder in an electric grinder and stored in airtight bottles (Ammer et al ., 2016). 50g of dried leaves powder was thoroughly mixed with 200mL methanol in triplicate conical flasks. The flasks containing extract were heated on boiling water bath for 1hr, wrapped with aluminum foil and kept at room temperature for5 days with occasional shaking. After stipulated time, the liquid extract of flasks was transferred to falcon tubes and subjected to centrifugation at 5000rpm for 10min. The clear supernatants from the falcon tubes were shifted to pre-weighed beakers, and allowed to evaporate the solvent in hot water bath to get dried methanol free extract of Eucalyptus (Ammer et al ., 2016).

Garlic extract (Allicin)

Garlic extract powder was purchased from SCIENCEMED for Veterinary Drugs. It was dissolved in methanol solution as a solvent, with the concentration of 1mg Allicin/1mL methanol. Methanol was used alone as a negative control throughout this experiment.

Synthetic antibacterial and antifungal drugs

Chloramphenicol was purchased from Sedico Pharmaceutical company. *Amoxicillin* was purchased from GlaxoSmithKline Pharmaceutical Company. *Fluconazole* was purchased from Amoun Pharmaceutical Manufacture. *Amphotricin-B* was purchased from Abbott Pharmaceutical Manufacture.

Bacterial inoculum preparation

A sufficient number of bacterial colonies obtained from well-isolated, morphologically similar colonies were suspended in 4 to 5mL of sterile distilled water to equal a 0.5 McFarland standard (Disha et al., 2018).

Fungal inoculum preparation

The tested fungal species cultured on Sabroud dextrose agar (SDA) in 9cm plate and incubated at 28°C for 7 days. The fungus was harvested aseptically using 5mL of sterile water. The spore suspensions were adjusted to a concentration of 1-2 x 10^6spores/mL in sterile distilled water (Yazdani et al., 2009).

Antimicrobial activity using well diffusion method

According to, Clinical & Laboratory Standards Institute (CLSI) guidelines (Cui et al., 2021) muller Hinton agar plates were inoculated with the tested organisms using a sterile cotton swab rolled in the suspension of bacterial and/or fungal cultures. A sterile cork borer of 5mm diameter was used to make wells on inoculated medium. Then 100µL of each of the plant extracts (natural antimicrobial agents) at concentration 1mg/mL, and synthetic antimicrobial agents were dropped into each appropriate labeled well (Atata et al., 2003; ShahidiBonjar et al., 2004). The inoculated plates were kept in the refrigerator for 1 hour to allow the antimicrobial agents to diffuse on to the agar (Atata et al., 2003). The Mueller Hinton Agar plates were incubated at 37°C for 24h for bacteria and at 28°C for 5-7days for fungi. Antimicrobial activity was determined by measuring the mean value diameter of inhibition zones at mm formed after incubation (Akinsulire et al., 2007).

Statistical analysis

Statisical analysis was carried out depending upon on the method (one sample t-test) which is calculated in order to determine if each bacterial

species has effect on the different sites in the hospital. The mean for each sample is calculated first then standard deviation will be calculated to get (t-test value) which we can calculate p-value from it, according to Krzywinski & Altman (2013) and Lakens (2017). The calculated P-values determine the significance which if it's less than (0.05) P< 0.05, that means bacterial species are statistically significant.

Results

A total of 292 bacterial isolates were obtained from 350 samples collected from 11 places in 3 different governmental hospitals in Cairo, 131 of them were gram negative isolates and 161 were gram positiveas shown in Table 2. The bacterial isolates were identified using Vitek 2 compact system as shown in Fig.1.

Among gram positive and negative bacterial isolates, it was found that the most common bacterial species cause the infection in hospital sites were *Klebsiella pneumonia* &*Staphylococcus aureus* with (30& 20 isolates) respectively in hand washing sink hand (18,14 isolates) of *Staphylococcus aureus* in (stethoscopes) & (pressure cuffs, ultrasound machine & computer keyboards) respectively followed by *Klebsiella pneumonia* with (12 isolates) in computer key boards, This result clarifies that *Staphylococcus aureus & Klebsiella pneumonia* were the most predominant nosocomial bacterial pathogens isolated from different sites in selected hospitals. On the other hand, *Bacillus subtilis* & *Bacillus cereus* were the least isolated nosocomial bacterial pathogens as shown in Table 3. The previous distribution of bacterial species was clarified using statistical analysis based on (one sample t-test for a mean calculation),this study stated that there is a significant effect for (*Staphylococcus aureus*, *Staphylococcus saprophyticus, E. coli, Bacillus cereus & Bacillus subtilis)* which cause contamination of different sites in hospitals, in which this is determined by calculating the p-value of each bacterial species in different sites, where (P-value) less than 0.05 that means that bacterial species are statistically significant.

TABLE 2. Total count of bacterial isolates among different sites

Sites	Total bacterial isolates count	
	Gram negative	Gram positive
Pressure cuffs	8	18
Ventilator (buttons and circuits)	11	10
Suction system switches	5	9
Portable radiograph equipment	10	7
Ultrasound machine	15	16
Bed rails	7	8
Stethoscopes	12	18
White coats/scrubs	5	5
Telephone/cell phones	13	22
Computer keyboards	18	22
Handwashing sink	36	26
Total	131	161

A total of 181 fungal isolates were obtained from all studied sites. Computer keyboards was the most contaminated site with nosocomial pathogenic fungi (120 isolates) with percentage (66.2%) followed by both of (Hand washing sink & Suction system switches) (109 &101 isolates) with percentage (60.2% and 55.8%) respectively followed by Ventilator (92 isolates) with percentage (50.8%) while Ultrasound machine was the least contaminated site with nosocomial pathogenic fungi (20 isolates) with percentage (11%) followed Telephone/cell phones (43 isolates)with percentage (23.7%) followed by pressure cuffs (60 isolates) with percentage (33.1%) as shown in Table 4.

Based on macro and micromorphological characteristics, nosocomial fungal pathogens were identified as: *Asperigillus niger, Asperigillus fumigatus, Asperigillus flavus, Asperigillus terrus, Rhizopus stolonifer, Fusarium oxysporum, Fusarium proliferatum, Fusarium nivale, Fusarium dimerum, Cladosporium herbarum, Penicillium melanoconidium, Penicillium carneum, Penicillium sclerotigenum, Penicillium viridiactum* and *Penicillium flavigenum.*

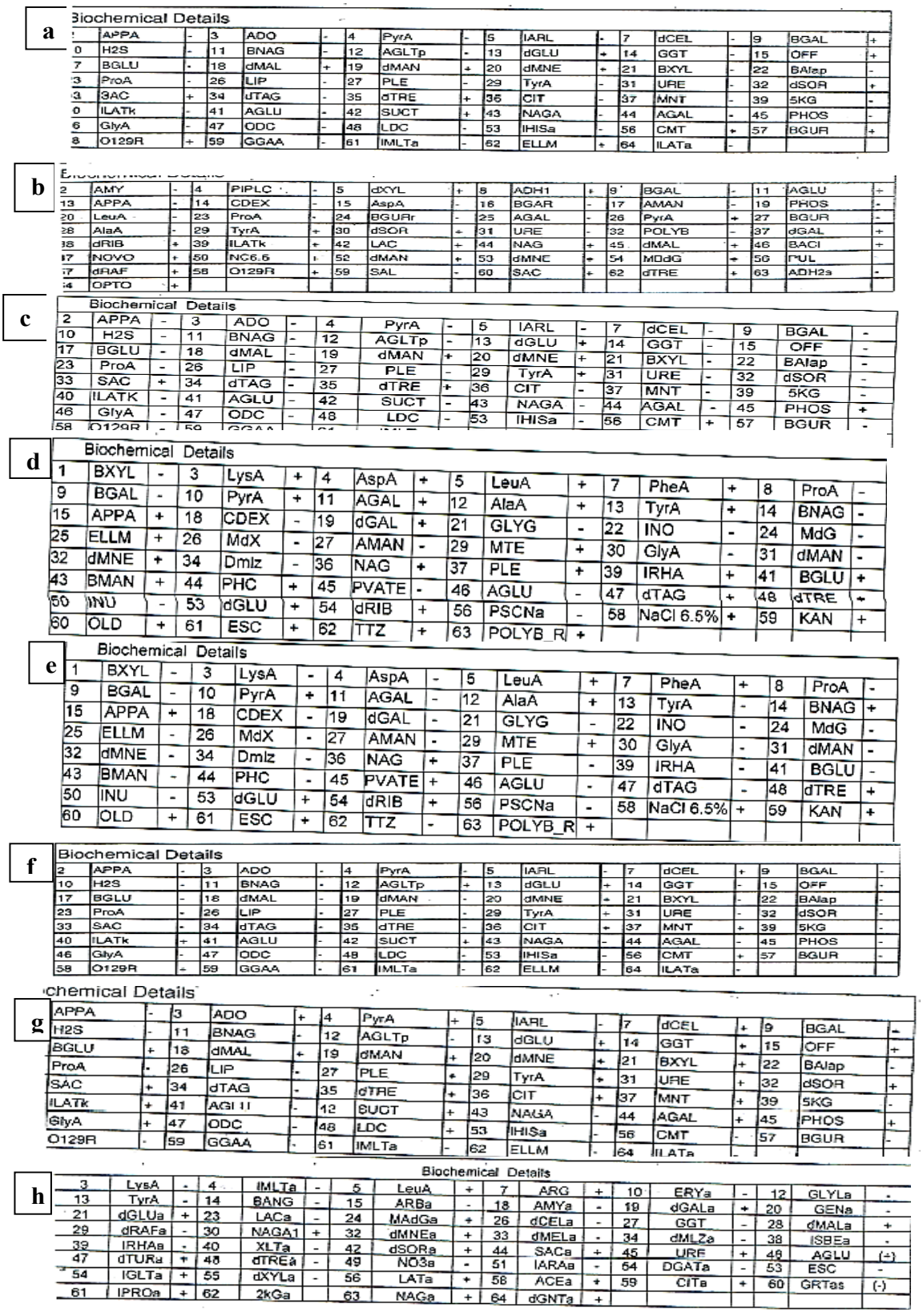

Fig. 1. Bacterial identification using Vitek 2 compact system: a: *Escherichia coli*, b: *Staphylococcus aureus,* c: *Staphylococcus saprophyticus*, d: *Bacillus subtilis,* e: *Bacillus cereus*;, f: *Acnietobacter baumanii,* g: *Klebsiella pneumonia* and h: *Pseudomonas auerignosa*

TABLE 3. Distribution of different bacterial species for each site in hospital

Sites / Bacterial species	Pressure cuffs	Ventilator	Suction system switches	Portable radiograph equipment	Ultrasound machine	Bed rails	Stethoscopes	White coats	Telephone	Computer keyboards	Hand washing sink	Total	**Percentage (%)	P-value
S.aureus	14	10	8	7	14	3	18	5	14	16	20	129	44.2	0.0003
S. saprophyticus	4	0	1	0	0	2	0	0	1	0	6	14	4.8	0.0002
P. auerignosa	1	4	4	4	5	0	1	0	0	1	0	20	6.8	0.146
A.baumanii	0	2	0	6	5	5	4	3	4	0	0	29	9.9	0.943
K. pneumoniae	6	5	0	0	6	0	7	0	6	12	30	72	24.7	0.333
B. cereus	0	0	0	0	1	2	0	0	4	2	0	9	3.1	0.005
B. subtilis	0	0	0	0	1	1	0	0	3	4	0	9	3.1	0.007
E. coli	1	0	0	0	0	5	0	2	2	0	0	10	3.4	0.002
Total	26	21	13	17	32	18	30	10	34	35	56	292	100	
***Percentage (%)	8.9	7.2	4.4	5.8	11	6	10.3	3.4	11.6	12	19.2	100		

[#] *S:Staphylococcus, K: Klebsiella, A: Acnietobacter, P: Pseudomonas, E: Escherichia, B: Bacillus*

** Percentage calculated in vertical column [%]= (Number of each bacterial speices in all sites)/ (Total number of all bacterial species in all the sites)*100

*** Percentage calculated in horizontal column [%]= (Number of all the bacteria in each site)/ (Total number of all bacterial species in all the sites)*100

TABLE 4. Total colony count of fungal isolates on different sites

Sites	Total number of fungal isolates	*Percentage (%)
Pressure cuffs	60	33.1%
Ventilator(buttons, circuits)	92	50.8%
Suction system switches	101	55.8%
Portable radiograph equipment	74	40.8%
Ultrasound machine	20	11%
Bed rails	80	44.1%
Stethoscopes	90	49.7%
White coats/scrubs	63	34.8%
Telephone/cell phones	43	23.7%
Computer keyboards	120	66.2%
Handwashing sink	109	60.2%

*Percentage calculated in vertical column [%]= (Number of colonies of fungal isolates in each site)/(Total number of fungal isolates in all the sites)*100

From all studied sites and fungal isolates, it was found that *Asperigillus niger* was considered the only fungal species isolated from all the sites with the highest contamination occurrence in cell phones and ultrasound machine with 4 and 3 isolate respectively, followed by *Penicillium sclerotigenum* with largest number in ventilator, portable radiograph equipment and bedrails, 3 isolates, each, followed by *Asperigillus fumigatus* and *Asperigillus flavus* with largest number found in pressure cuffs & computer keyboards and stethoscopes & cell phones. On the contrary *Rhizopus stolonifer* was the least isolated fungal species with percentage (1.2%) followed by *Fusarium dimerum* with percentage (3%) and then *Penicillium viridiactum* with percentage (3.7%). It was found that all the *Fusarium sp.* and *Rhizopus stolonifer* species didn't cause contamination for the following sites Ventilator, Suction system switches, Portable radiograph equipment, Ultrasound machine, Bed rails, Stethoscopes and cell phones. (Table 5).

Antimicrobial activity of synthetic and natural extracts against bacterial and fungal nosocomial pathogens:

It was found that Eucalyptus extract and allicin

were very effective on controlling gram positive and gram negative pathogens. *Bacillus cereus* was the most sensitive bacterial species to garlic extract with inhibition zone diameter (45mm) followed by *Bacillus subtilis* (28mm) as shown in Table 6. While *Staphylococcus aureus* and *Bacillus cereus* were the most sensitive bacterial species to Eucalyptus extract with inhibition zone diameters 35mm and 30mm, respectively, followed by *Bacillus subtilis* (25mm). As for synthetic antibacterial agents, *Bacillus cereus* was the most sensitive bacterial species to Amoxicillin with inhibition zone diameter 54mm followed by *Staphylococcus aureus* (41mm) while from the gram negative bacterial species, *E.coli* is considered the most sensitive bacterial species to chloramphenicol with (40mm) inhibition zone diameter. Amphotricin B was considered the most active synthetic drug used against all isolated fungal species and *Penicillium carneum* was the most sensitive fungal pathogen with inhibition zone diameter 28mm followed by *Asperigillus niger* with inhibition zone diameter 25mm as shown in as shown in Tables 6, 7 and Figs. 2-6.

TABLE 5.Distribution of fungal species among different sites on selected hospitals

Sites	A.niger	A.fumigatus	A.flavus	A.terrus	R.stolonifer	F.oxisporum	F.proliferatum	F.nivale	F.dimerum	C.herbarum	P.melanoconidium	P.carneum	P.sclerotigenum	P.viridiactum	P.flavigenum	Total	**Percentage [%]
Pressure cuffs	0	3	1	0	1	2	3	2	1	0	0	0	0	0	0	13	8%
Ventilator(e.g., buttons, circuits)	2	1	0	0	0	0	0	0	0	0	1	1	3	2	1	11	6.8%
Suction system switches	1	2	2	1	0	0	0	0	0	2	3	2	1	1	2	17	10.5%
Portable radiograph equipment	2	2	1	1	0	0	0	0	0	1	2	1	3	2	1	16	9.8%
Ultrasound machine	3	1	2	2	0	0	0	0	0	0	2	0	1	0	1	12	7.4%
Bed rails	2	1	1	2	0	0	0	0	0	2	1	0	3	1	0	13	8%
Stethoscopes	2	0	3	0	0	0	0	0	0	0	1	1	2	0	1	10	6.1%
White coats/ scrubs	1	0	2	1	0	3	4	2	2	0	0	0	0	0	0	15	9.2%
Telephone/cell phones	4	2	3	0	1	0	0	0	0	1	1	2	2	0	1	17	10.5%
Computer keyboards	2	3	0	0	0	2	4	5	2	1	2	1	1	0	0	23	14.1%
Handwashing sink	2	2	1	2	0	0	0	0	0	2	2	1	2	0	1	15	9.2%
Total	21	17	16	9	2	7	11	9	5	9	15	9	18	6	8	162	100%
***Percentage	13%	10.5%	9.8%	5.5%	1.2%	4.3%	6.8%	5.5%	3%	5.5%	9.2%	5.5%	11.1%	3.7%	5%	100%	

**A:Asperigillus, R:Rhizopus, F:Fusarium, C:Cladosporium, P:Penicillium*

**Percentage calculated in vertical column [%]= (Number of fungal species in each site)/(Total number of all fungal species in all sites)*100

***Percentage calculated in horizontal column [%]= (Number of each fungal speices in all sites)/(Total number of all the fungal species in all the sites)*100

TABLE 6. Antibacterial activity of natural and synthetic antimicrobial agents against pathogenic bacterial species

Bacterial species	Diameter of inhibition zone in mm for gram positive and negative bacterial species			
	Eucalyptus	Garlic extract	Amoxacillin	Chloramphenicol
S. aureus	35	24	41	-
S. saprophyticus	19	27	40	-
B.s cereus	30	45	54	-
B. subtilis	25	28	32	-
P. aeurignosa	-	-	-	-
A. baumannii	-	-	-	-
K. pneumonia	16	24	-	34
E. coli	23	23	-	40

[#]*S: Staphylococcus, K: Klebsiella, A:Acnietobacter, P: Pseudomonas, E:Escherichia, B:Bacill*

TABLE 7. Antifungal activity of synthetic and natural antimicrobial agents against pathogenic fungal species

Fungal species	Diameter of inhibition zone in mm			
	Fluconazole	Garlic extract	Eucalyptus plant extract	Amphotricin B
Asperigillus niger	-	-	-	25
Asperigillus fumigatus	-	-	-	20
Asperigillus flavus	-	-	-	17
Asperigillus terrus	-	-	-	-
Rhizopus stolonifer	-	-	-	13
Fusarium oxysporum	-	-	-	17
Fusarium proliferatum	-	-	-	-
Fusarium pnivale	-	-	-	-
usarium pdimerum	-	-	-	-
Cladosporium herbarum	-	-	-	21
Penicillium melanoconidium	-	-	-	17
Penicillium carneum	-	-	-	28
Penicillium sclerotigenum	-	-	-	13
Penicillium viridiactum	-	-	-	15
Penicillium flavigenum	-	-	-	17

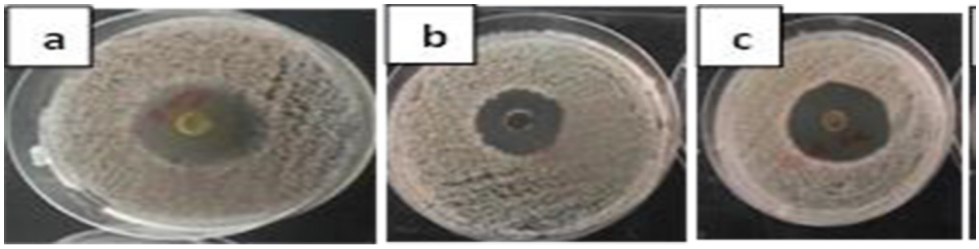

Fig. 2. Antibacterial activity of synthetic and natural antimicrobial agents against *S. aureus*; a: Eucalyptus leaf extract, b: Garlic extract and c: Amoxicillin

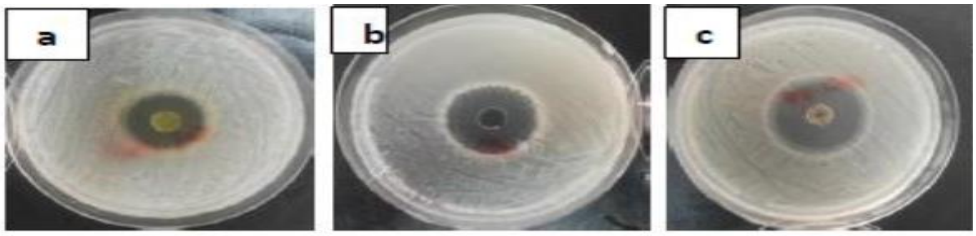

Fig. 3. Antibacterial activity of synthetic and natural antimicrobial agents against *B. subtilis*; a: Eucalyptus leaf extract, b: Garlic extract and c: Amoxicillin

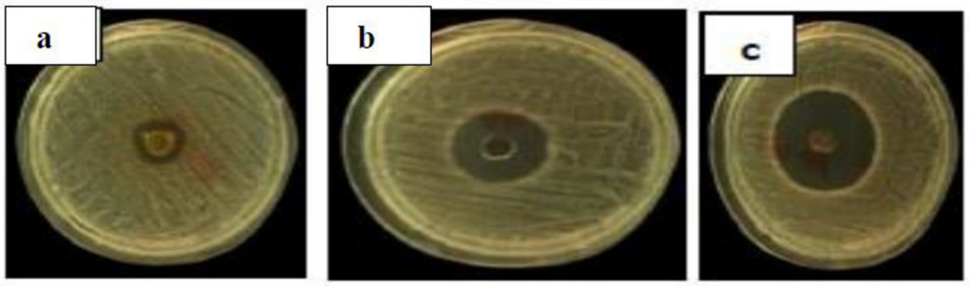

Fig. 4. Antibacterial activity of synthetic and natural antimicrobial agents against *K. pneumoniae*; a: Eucalyptus leaf extract, b: Garlic extract and c: Chloramphenic

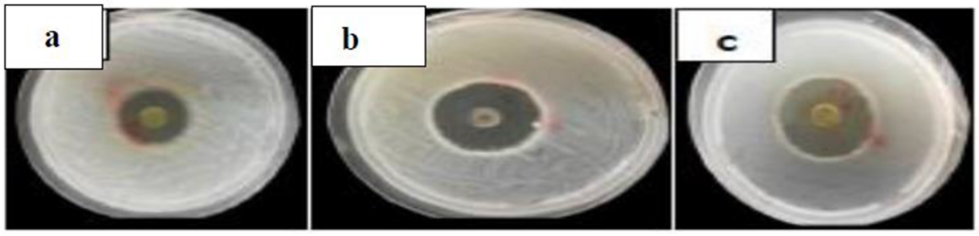

Fig. 5. Antibacterial activity of synthetic and natural antimicrobial agents against *E. coli*; a: Eucalyptus leaf extract, b: Garlic extract and c: Chloramphenicol

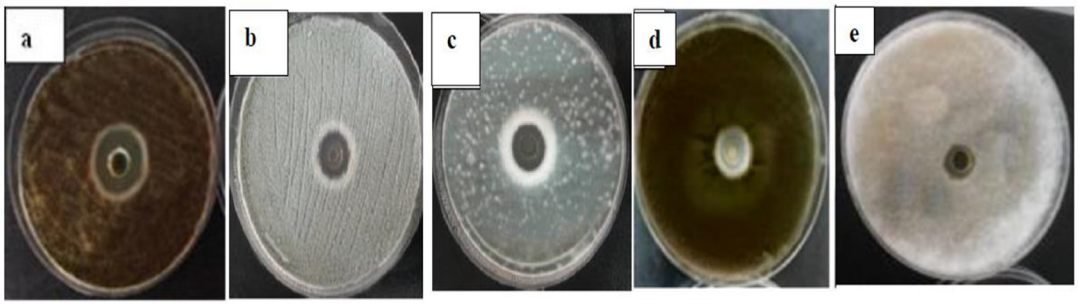

Fig. 6. Antifungal activity of synthetic antimicrobial agents (Amphotrircin. B.) against; a: *Asperigillus niger*, b: *Asperigillus fumigatus*; c: *Penicillium melanoconidium*; d: *Cladosporium herbarum* and e: *Rhizopus stolonifer*

Discussion

Nosocomial infection is considered one of the greatest challenges to health systems in the developed world (Bouza et al., 2019). The present study revealed that Hand washing sink is considered the most colonized site with bacterial species,this may due to factors that may contribute to contamination of hand washing sink, these factors include:visible splashing out of and around the sink when the tap was turned on and the distance between the sink and the patient

ranged between 1 and 2mm. *Klebsiella* species and *Staphylococcus* species are considered the most common species on hand washing sink, this was concur with the results obtained by Roux et al. (2013). While mobile phones are considered the most site contaminated with fungal isolates, this may due to the daily contact of mobile phones with body parts such as: the face, ears and hands, these fungal isolates can survive on the surface of the cell phones causing contamination for it, other studies investigated that it's possible that air contribute to the growth of fungi on mobile phones, these fungal spores float in the air can be attached to the media on the mobile phone, this study is reported by Amanah et al. (2019). It was reported that many of medicinal plants have antibacterial activities like Eucalyptus leaf extract which has antibacterial activity against many bacterial species as: *Staphylococcus aureus* (Easa et al ., 2019). It was found that the inhibition effect produced from Eucalyptus leaf extract differ from bacterial species to another, this depends upon difference in the cell wall of the bacterial organisms and difference in phytochemical composition of the medicinal plants (Roux et al., 2013). In addition to that Eucalyptus leaf extract has antibacterial effect against *Bacillus subtilis* and *Bacillus cereus*, this may due to leaf extract contains phytochemical compounds like (saponins, cardiac glycosides, tannins), tannins have role for its antimicrobial activity which its mechanism depends upon the ability of binding proteins then inhibiting cell protein synthesis (Alghamdi & Ababutain, 2019). On the other hand,it was found that gram negative bacteria are slightly sensitive to essential oils when compared with gram positive bacteria, this may due to the structure of their cellular walls where the cell wall of gram negative bacteria consists mainly of lipoproteins and lipopolysaccharides which form a barrier to hydrophobic compounds so they are less sensitive to antimicrobial agents, this study is reported by Bachir & Benali (2012). While it was found that *E.coli* and *K. pneumoniae* are inhibited by methanol extract of eucalyptus, this may due to eucalyptus leaf extract has high concentration of polyphenols components which include (tannins, steroid, alkaloids, flavonoids and terpenoids) (Amini et al., 2016). Many studies reported that allicin (the main active ingredient of garlic extract) has role as antimicrobial agent through inhibiting DNA and protein synthesis moderately and inhibiting RNA synthesis completely as a primary target. Additionally, garlic extract has many anionic components which have antimicrobial properties , from these components(nitrates, chlorides ,sulfates and other water solube components), this study clarified the role of garlic extract as antmicrobial agent against many bacterial species (*Staphylococcus aureus, Bacillus subtilis*, *Bacillus cereus, E. coli and K. pneumoniae)* (Chand, 2019). Amoxicillin inhibited many of gram positive bacteria, this may due to amoxicillin is considered one of beta lactam antibiotics which act through binding to penicillin binding proteins leading to activation of autolytic enzymes in the bacterial cell wall resulting in lysis of the cell wall of bacterial species then destruction of bacterial cell (Akhavan & Vijhani, 2019). It was found that *Klebsiella pneumonia* and *E. coli* are sensitive to chloramphenicol which inhibit protein synthesis in bacterial species and acts by binding reversibly to 50S subunits of the bacterial ribosome, this study is reported by Mahato et al. (2019). Many studies reported that there are many reasons for less ability of the essential oils like (eucalyptus and garlic) to inhibit the growth of fungal species, these reasons include: volatilization overtime of the more volatile essential oils affecting the growth inhibition of fungi , in addition to that the agar itself may affect the effectivity of the essential oils in their ability to inhibit growth, similarly the effectivity of the treatments may be from the fungi becoming less sensitive to the active compounds or due to the nutrient rich agar diluting the concentration of essential oil (Padhye et al., 2013). According to the study of Shishodia et al. (2019) that reported Asperigillus species are resistant to fluconazole due to presence of certain factors including (enzyme from cell wall remodeling, oxidative stress response and energy metabolism) and also due to production of the enzymes by alterations in promoters and transcription factors in the fungal species (Macedo et al., 2018).

Conclusion

In our study it was concluded that *Staphylococcus aureus* was the most common isolated bacteria which is susceptible to the both of natural plant extracts and synthetic antimicrobials. While it was found that from all fungal isolates *Asperigillus niger* was the most common isolated fungi followed by *Penicillium* species. Amphotricine-B is considered the only effective drug when tested against most of fungal species while both of natural extracts have no effect on the isolated fungi. Future study will

be carried out to determine MIC concentration of these natural plant extracts as antimicrobial agents and a good choice for antibiotic discovery and infection control, especially against pathogenic bacterial and fungal isolates.

References

Akhavan, B.J., Vijhani, P. (2019) "*Amoxicillin*", Stat Pearls [Internet]. StatPearls Publishing (Treasure Island Book). StatPearls Publishing.

Akinsulire, O.R., Aibinu, I.E., Adenipekun, T., Adelowotan, T., Odugbemi, T. (2007) *In vitro* antimicrobial activity of crude extracts from plants *Bryophyllum pinnatum* and *Kalanchoe crenata. African journal of Traditional, Complementary and Alternative Medicines,* **4**(3), 338-344.

Alghamdi, A.I., Ababutain, I.M. (2019) Research article phytochemical screening and antibacterial activity of *Eucalyptus camaldulensis'*s leaves and bark extracts. *Asian journal of Scientific Research,* **12**(2),202-210.

Amanah, A., Apriyanto, D.R., Fitriani, H. (2019) Isolation of surveillance pathogenic fungal microbial contaminant on mobile phone. *Open Access Macedonian Journal of Medical Sciences*, **7**(20), 3493-3496.

Amini, N., Rezaei, K., Yazdannik, A. (2016) Effect of nebulized eucalyptus on contamination of microbial plaque of endotracheal tube in ventilated patients. *Iranian Journal of Nursing and Midwifery Research*, **21**(2), 165.

Ammer, M.R., Zaman, S., Khalid, M., Bilal, M., Erum, S., et al. (2016) Optimization of antibacterial activity of Eucalyptus tereticornis leaf extracts against Escherichia coli through response surface methodology. *Journal of Radiation Research and Applied Sciences,* **9**(4), 376-385.

Atata, R., Sani, A., Ajewole, S.M. (2003) Effect of stem back extracts of Enantiachloranta on some clinical isolates. *Biokemistri*, **15**(2), 84-92.

Bachir, R.G., Benali, M. (2012) Antibacterial activity of the essential oils from the leaves of Eucalyptus globulus against Escherichia coli and Staphylococcus aureus. *Asian Pacific Journal of Tropical Biomedicine*, **2**(9), 739

Bereket, W., Hemalatha, K., Getenet, B., Wondwossen, T., Solomon, A., et al. (2012) Update on bacterial nosocomial infections. *European Review for Medical and Pharmacological Sciences,* **16**(8), 1039-1044.

Bouza, E., Alonso, S., Asensio, A., De Juan, G., Lucio, C.G., Larrosa, C., De la Serna, J.L. (2019) Information on nosocomial infections in the mainstream media: an opinion document. *Revista Española de Quimioterapia*, **32**(2), 165.

Chand, B. (2019) Antibacterial effect of garlic (*Allium sativum*) and ginger (*Zingiber officinale*) against *Staphylococcus aureus*, *Salmonella typhi, Escherichia coli* and *Bacillus cereus*. *Journal of Microbiology, Biotechnology and Food Sciences*, **2**, 2481-2491.

Cruickshank, R., Dguid, J.P., Marmion, B.P., Swain, R.H.A. (1975) "*Medical Microbiology*", Vol. II, 12th ed., Crurchill Living stone, New York, pp. 31-57, pp. 96-218.

Cui, Z.-H., He, H.-L., Wu, S.-B., Dong, C.-L., Lu, S.-Y., Shan, T.-J., Fang, L.-X., et al. (2021) Rapid screening of essential oils as substances which enhance antibiotic activity using a modified well diffusion method. *Antibiotics*, **10**(4), 463. https://doi.org/10.3390/antibiotics10040463

Elsonbaty, S.M., Eltahawy, N., Al-dmour, S.M., Easa, S., Qaralleh, H. (2020) Ionizing radiation effect on *Teucrium polium:* Phytochemical contents antioxidant and antibacterial activity. *Arab Journal of Nuclear Sciences and Applications,* **53**(2), 98-110.

Disha, B., Mrinalini, M., Dimple, R. (2018) *In vitro* comparison of the antibacterial activities of 2% chlorhexidine and Hiora solution on *Enterococcus faecalis*. *International Journal of Applied Dental Sciences,* **4**(4), 174-177.

Easa, Saadia M. (2002) The effect of Garlic (*Allium sativum*) on lipid pattern of selected dermatophytes. *An International Journal. Al-Azhar, J.Microbial. Cairo, Egypt,* **56**(4), 194-217.ISSN:1110-5879.

Easa, Saadia M., El-Beih, F.M. (2002) The Antibacterial effect of some medicinal plants on certain human pathogenic bacteria. *An International Journal, Al Azhar Journal of Microbiology. Cairo, Egypt*, **58**(10), 218-233.

Easa, Saadia M., Hamdy, A-H.A., Mekawey, Amal A., Wadee, Ereny A. (2018) Antifungal activities of some plant extracts against pathogenic fungi. *Egyptian Academic Journal of Biological Sciences* **10**(1), 1-19.

Easa, Saadia M., Ibrahim, M.F., Abdel-Shafi, Seham, Osman, A., Abdel-Monaem, Al-Shaymaa (2019) The inhibition of *Staphylococcus pasteuri* using *Moringa oliferea* leaves extract. *Global Scientific Journals*, 7(9), ISSN: 2320-9186.

Eliakim-Raz, N., Lador, A., Leibovici-Weissman, Y., Elbaz, M., Paul, M., Leibovici, L. (2015) Efficacy and safety of chloramphenicol: Joining the revival of old antibiotics? Systematic review and meta-analysis of randomized controlled trials. *Journal of Antimicrobial Chemotherapy*, **70**(4), 979-996.

Gaddeyya, G., Niharika, P.S., Bharathi, P., Kumar, P.K.R. (2012) Isolation and identification of soil mycoflora in different crop fields at Salur Mandal. *Advances in Applied Science Research,* **3**, 2020-2026.

Holt, J.G., Krieg, N.R. (1984) "*Bergey's Manual of Systematic Bacteriology*", Vol. 1. The Williams and Wilkins Co., Baltimore, 1388p.

Kaur, S., Preet, R.R., Nanda, S.A.N.J.U. (2011) Amoxicillin: A broad spectrum antibiotic. *International Journal of Pharmacy and Pharmaceutical Sciences* **3**(3), 30-7.

Khan, H.A., Ahmad, A., Mehboob, R. (2015) Nosocomial infections and their control strategies. *Asian Pacific Journal of Tropical Biomedicine,* **5**(7), 509-514.

Khan, H.A., Baig, Fatima K., Mehboob, R. (2017) Nosocomial infections: Epidemiology, prevention, control and surveillance. *Asian Pacific Journal of Tropical Biomedicine,* **7**(5), 478-482.

Krzywinski, M., Altman, N. (2013) Points of significance: Significance, P values and t-tests. *Nature Methods*, **10**(11), 1041-1042.

Lakens, D. (2017) Equivalence tests: a practical primer for t tests, correlations, and meta-analyses. *Social Psychological and Personality Science*, **8**(4), 355-362.

Macedo, D., Leonardelli, F., Dudiuk, C., Theill, L., Cabeza, M.S., Gamarra, S., Garcia-Effron, G. (2018) Molecular confirmation of the linkage between the *Rhizopus oryzae* CYP51A gene coding region and its intrinsic voriconazole and fluconazole resistance. *Antimicrobial Agents and Chemotherapy*, **62**(8), e00224-18.

Mahato, S., Mahato, A., Pokharel, E., Tamrakar, A. (2019) Detection of extended-spectrum beta-lactamase-producing *E. coli* and *Klebsiella* spp. in effluents of different hospitals sewage in Biratnagar, Nepal. *BMC Research Notes*, **12**(1), 1-6.

Muhammad, U.K., Isa, M.A., Aliyu, Z. (2017) Antimicrobial resistance pattern of pathogenic bacteria isolated from four hospital environment in Sokoto Metropolis, Northwestern, Nigeria. *Journal of Microbiology and Biotechnology Research,* **3**(1), 120-124.

Noor, A., Preuss, C.V. (2018) "*Amphotericin B*". Stat Pearls. Stat Pearls Publishing, Treasure Island Book (FL).

Osman, A.E.M.A.E., Hashim, S.O., Musa, M.A., Tahir, O.M. (2018) Bacterial hospital acquired infection in Port Sudan Teaching Hospital, Red Sea State, Sudan. *International Journal of Pharmacy and Chemistry*, **4**(1), 1-7.

Padhye, S., Rai, S., Narula, N., Dekate, S. (2013) Screening of antifungal activity of some essential oils and antifungals on clinical fungal isolates from diabetic patients. *Research Journal of Pharmaceutical, Biological and Chemical Sciences,* **4**(2), 16-21.

Pesarico, A.P., Sartori, G., dos Santos, C.F., Neto, J.S., Bortolotto, V., Santos, R.C., Nogueira, C.W., Prigol, M. (2013) 2, 2′-Dithienyl diselenide pro-oxidant activity accounts for antibacterial and antifungal activities. *Microbiological Research*, **168**(9), 563-8.

Pincus, D. H. (2006) Microbial identification using the bioMérieuxVitek® 2 system. In" "*Encyclopedia of Rapid Microbiological Methods*". Bethesda, MD:

Rad, H.I., Arzanlou, M., Omid, M.R., Ravaji, S. (2017) Effect of culture media on chemical stability and antibacterial activity of allicin. *Journal of Functional Foods,* **28**, 321-325.

Roux, D., Aubier, B., Cochard, H., Quentin, R., Van

Der Mee-Marquet, N. (2013) Contaminated sinks in intensive care units: an underestimated source of extended-spectrum beta-lactamase-producing Enterobacteriaceae in the patient environment. *Journal of Hospital Infection*, **85**(2), 106-111.

Sanders, E.R. (2012) Aseptic laboratory techniques: Plating methods. *Journal of Visualized Experiments*, **63**, 3064. doi: 10.3791/3064

Scardovi, V., Sneath, P.H.A., Mair, N.S., Sharpe, M.E. (1986) "*Bergey's Manual of Systematic Bacteriology*", Baltimore (Ed.), Sneath.

Seyyednejad, S.M., Motamedi, H., Najvani, F.D., Hassannejad, Z. (2014) Antibacterial effect of *Eucalyptus microtheca*. *International Journal of Enteric Pathogens,* **2**(2), e16515.

ShahidiBonjar, G.H. (2004) Evaluation of Antibacterial properties of Iranian Medicinal plants against *Micrococcus aureus*, *Serratia marcescens*, *Klebsiella pneunomiae* and Bordellabronchoseptica. *Asian Journal of Sciences,* **3**(1), 82-86.

Shishodia, S.K., Tiwari, S., Shankar, J. (2019) Resistance mechanism and proteins in *Aspergillus* species against antifungal agents. *Mycology*, **10**(3), 151-165.

Tiwari, S., Gupta, N., Malairaman, U., Shankar, J. (2017) Anti-aspergillus properties of phytochemicals against aflatoxin producing *Aspergillus flavus* and *Aspergillus parasiticus*. *National Academy Science Letters*, **40**(4), 267-71.

Vazquez, L. (2016) Antifungal prophylaxis in immunocompromised patients. *Mediterranean Journal of Hematology and Infectious Diseases,* **8**(1), e2016040.

Viegas, C., Faria, T., Meneses, M., Carolino, E., Viegas, S., Gomes, A.Q., Sabino, R. (2016) Analysis of surfaces for characterization of fungal burden. *International Journal of Occupational Medicine and Environmental Health*, **29**(4), 623-632.

Yazdani, D., Rezazadeh, S., Amin, G., Zainal Abidin, M.A., et al. (2009) Antifungal activity of dried extracts of anise (*Pimpinella anisum* L.) and star anise (*Illicium verum* Hook. f.) against dermatophyte and saprophyte fungi. *Journal of Medicinal Plants*, **8**(29), 24-29.

دراسة الكائنات الممرضة والمسببة للعدوى المكتسبة فى بعض المستشفيات الحكومية بمصر وكيفية التحكم فيها

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تعد العدوى المكتسبة من المستشفيات أحد العدوى الهامة التى تواجه الصحة العامة، حيث إنها تسبب مشاكل مالية وأحيانا تصل إلى أنها تسبب الوفاه فى بعض المستشفيات خاصة فى الدول النامية.

فى هذه الدراسة تم تجميع 350 عينة من مختلف الأماكن بالمستشفيات الحكومية الثلاثة بمحافظة القاهرة، وقد وُجِد أن الحوض الخاص بغسيل الأيدى من أكثر الأماكن تلوثاً بالبكتيريا والفطريات، ويتبعها لوحة المفاتيح الخاصة بأجهزة الحاسب الآلي والأجهزة الإشعاعية، وطبقاً لجهاز "vitek" تم تعريف الكائنات البكتيرية المعزولة كالتالى:

Acnietobacter baumanii, Bacillus cereus, Bacillus subtilis, Escherichia coli, Klebsiella pneumonia,Pseudomonas auerignosa, Staphylococcus aureus and *Staphylococcus saprophyticus.*

تم تعريف الفطريات المعزولة (عن طريق دراسة خصائصها من حيث الشكل الظاهري وخصائصها تحت الميكروسكوب) إلى:

Asperigillus niger, Asperigillus fumigatus, Asperigillus flavus, Asperigillus terrus, Rhizopus stolonifer, Fusarium oxysporum, Fusarium proliferatum, Fusarium nivale, Fusarium dimerum, Cladosporium herbarum, Penicillium melanoconidium, Penicillium carneum, Penicillium sclerotigenum, Penicillium viridiactum and *flavigenum. Penicillium*

يُعتبر كلاً من *S.aures* and *Klebsiella pneumonia* من أكثر البكتيريا المعزولة فى هذه الدراسة، بينما يُعتبر *A.niger* من أكثر الفطريات التي تم عزلها من الأماكن المختلفة بالمستشفيات.

تعتبر المواد المضادة للبكتيريا والفطريات هي الأكثر شيوعاً فى استخدامها لمحاربة العدوى الناتجة عن الميكروبات، ولهذا ظهرت أبحاث كثيرة تشير عن مدى أهمية استخدام المواد الطبيعية المستخلصة من النباتات كمضادات لهذه العدوى.

فى هذه الدراسة تم اختبار تأثير كلاً من المستخلصَين الطبيعيين لنبات الكافور وبودرة الثوم، كما أنه تم خلطهما ببعض، بالإضافة إلى اختبار كل من المواد المصنعة المضادة للبكتيريا (الاموكسيللين والكلورامافينيكول) وخليطهم ببعض باستخدام طريقة agar well diffusion.

يُعتبر *B.cereus* من أكثر الفصائل البكتيرية حساسية تجاه بودرة الثوم، بينما يُعتبر كلاً من *S.aureus* and *B.cereus* من أكثر الفصائل البكتيرية حساسية تجاه مستخلص نبات الكافور.

عند خلط كل من المواد المصنعة والطبيعية المضادة للبكتيريا، وُجد أن لها تأثير علاجي على الفصائل البكتيرية، بينما عند خلط كل من المستخلصَين الطبيعيين ببعضهما كان له تأثير عكسي على الفصائل البكتيرية، وعلى الجانب الآخر وُجد أن للمواد المستخلصة الطبيعية تأثير ضعيف على الفطريات المسببة للعدوى، ووُجد أيضاً أن خلط المواد المستخلصة الطبيعية بالمواد المصنعة ضد الفطريات له تأثير عكسي على الفطريات المعزولة.