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Microorganisms isolated from surgical wounds infection and treatment with different natural products and antibiotics

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Surgical site infections (SSIs) are common nosocomial infections in surgical patients resulting in significant increases in postoperative morbidity and mortality. This study aimed to isolate and identify bacteria that cause SSIs in Medical Research Institute, Alexandria University, Egypt, and compare their sensitivities to selected group of antibiotics and natural products. Isolates from 20 patients with SSIs were identified by culturing on blood and MacConkey agars, Gram staining and biochemical reactions. Most Gram-negative isolates (*Pseudomonas aeruginosa, Klebsiella spp, Acinetobacter spp*) were sensitive to amikacin, imipenem. Gram-positive isolates were *Enterococcus faecalis* which was sensitive to chloramphenicol, vancomycin, and *Staphylococcus aureus* which was sensitive to amikacin, imipenem, chloramphenicol. Gram-negative isolates were more sensitive to olive oil at 70 and 100% concentrations, and *Acinetobacter spp* were resistant at 30% concentration. Gram-positive *E. faecalis* isolates were most sensitive at 30% concentration. Garlic and oregano oils were more effective against most Gram-positive and negative isolates. *E. faecalis* was resistant to garlic oil at 30% concentration. *Klebsiella spp* were resistant to *Nigella sativa* oil at all concentrations, *Acinetobacter spp* were resistant at 30% concentration, and *P. aeruginosa* was more sensitive at all concentrations than Gram-positive isolates. *P. aeruginosa* and *S. aureus* were most sensitive, while *Klebsiella spp* were resistant to commercial oils (olive, garlic, and chamomile). Olive leaf extract was more effective at four concentrations against all Gram-negative isolates. Gram-positive and -negative isolates were most sensitive to whey mixed with honey. This study refers to the possibility of using olive, garlic and oregano oils, olive leaf extract, and whey mixed with honey in treatment of SSIs.

Key words: Isolated bacteria, surgical wounds, natural herbal products.

INTRODUCTION

Surgical site infections (SSIs) are the most common nosocomial infection in surgical patients, accounting for 38% of all infections. They are a significant source of postoperative morbidity resulting in longer hospitalization, increased cost, and increased incidence of postoperative mortality (Malone et al., 2002). Most SSIs are contaminated by the patient's own endogenous flora which is present on the skin, mucous membranes, or hollow viscera. Usual pathogens on skin and mucosal surface are Gram-positive cocci, mainly *Staphylococcus aureus* (Rosenblueth et al., 2004). However, Gram-negative aerobic and anaerobic bacteria can contaminate skin wounds of the groin and perineal areas. The contaminating pathogens in gastrointestinal surgeries are the intrinsic bowel flora,

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which include Gram-negative bacilli and Gram-positive microbes, including enterococci and anaerobic organisms (Schaechter et al., 1993). The most common microbiological cause of nosocomial infection is Gram-negative bacteria, *Escherichia coli, Proteus mirabilis,* and other members of family known as Enterobacteriaceae (Podschun et al., 1998). They spread via fecal contamination of people, instruments or other surfaces. Other Gram- negative bacteria include members of the genera *Pseudomonas* and *Acinetobacter.*

According to the Center for Disease Control (CDC), 1990, nosocomial infection is defined as a localized infection or one that is widely spread throughout the body that results from an adverse reaction to an infectious microorganism or toxin that was not present at the time of admission to the hospital (Garner et al., 1996). On the other hand, National Nosocomial Infection Surveillance (NNIS) System defines SSI as either superficial incisional infection involving only skin and subcutaneous tissue or deep involving muscle layers and internal organs in the operation site which occurs within 30 days after the surgical procedure (Malone et al., 2002).

For most bacterial nosocomial infections, the infection usually becomes evident after 48 h (the typical incubation period) or more after admission. In general, nosocomial infections are more serious and dangerous than community-acquired infections. The most important factor responsible for the severity of nosocomial infections is that the causative bacteria are usually resistant to a number of antibiotics in common use in hospitals (Sutherland and Rolinson, 1964; Schaberg et al., 1991). In addition, poor health state which impairs the immune defenses, and the use of invasive devices increase the vulnerability to and severity of nosocomial infections (Schaberg et al., 1991; Schwarzkopf et al., 2011).

The search for new compounds which are effective for eradication of nosocomial infections is still going on. It was encouraging to investigate the effect of some natural products with known antibacterial activity (olive oil, garlic oil, oregano oil, *Nigella sativa* oil, olive leaf extract, and whey protein) for eradication of pathogenic bacteria responsible for nosocomial infections which were isolated from surgical wound infections in patients after different operations.

MATERIALS AND METHODS

Patients and collection of samples

Twenty patients from the Department of Surgery, Medical Research Institute, Alexandria University, Egypt, were included in the present study. Inclusion criteria are adults and middle-aged patients of either sex (males and non-pregnant females), without any other systemic disease or immune compromised conditions. The patients received antibiotic prophylaxis with a third-generation cephalosporin (ceftriaxone) before doing the surgery. The patients include nine females with breast cancer operation (age, 32-63 years), three females (age, 50-60 years) and three males (age, 42-47 years) with hernia operation, two females (age, 25-30 years) with cholecystectomy operation, one male (age, 20 years) and one female (age, 47 years) with laparotomy operation, and one female (age, 50 years) with spleenectomy operation. The written consent was obtained from all patients before starting the study. Twenty surgical wound swabs taken from postoperative contaminated wounds were collected from the patients before the use of postoperative antibiotics.

Characterization and identifications of the isolates

To identify the isolated pathogens, each swab was subjected to gram staining, and culturing on basal medium agar, blood agar and MacConkey agar. Also, a series of biochemical reactions was applied (catalase test, citrate test, indole test, coagulase test, urease test, motility testing and triple sugar iron agar).

Antibiotics sensitivity testing

The antibiotics used in this study were obtained from Sigma-Aldrich Co, USA. Mueller-Hinton agar was used for determination of antibiotic sensitivity patterns by applying Bauer-Kirby technique (Bauer et al., 1966). Sterile cotton tipped swab was dipped and drained in the test culture, and streaked evenly over the prepared Mueller-Hinton agar plates dried at 37°C for 30 min before use. The plates were allowed to dry for 5 min, then using a fine point forceps, the filter paper discs containing standard quantity of antibiotics to be tested were distributed on the plates, pressing each disc down firmly. The plates were immediately incubated at 37°C overnight, then the diameters of zones of growth inhibition around the antibiotic discs to which the organism being tested were measured in millimeter using metric rulers viewing from the back of the Petri-dish. Each isolate was kept in glycerol broth 10% at - 20°C until further processing with natural products.

For Gram-positive bacteria (*S. aureus*, and *Enterococcus faecalis*), the tested antibiotics were vancomycin (30 µg/disc), levofloxacin (5 µg/disc), ciprofloxacin (5 µg/disc), ofloxacin (5 µg/disc), penicillinG (10 µg/disc), imipenem (10 µg/disc), cefepime (30 µg/disc), meropenem (10 µg/disc), ceftriaxone (30 µg/disc), ceftazidime (30 µg/disc), aztreonam (30 µg/disc), piperacillin(100 µg/disc), cefuroxime sodium (30 µg/disc), piperacillin/tazoabctam (110 µg/disc), amoxicillin/clavulanic acid (30 µg/disc), amikacin (30 µg/disc), gentamicin (10 µg/disc), azithromycin (15 µg/disc), chloramphnicol (30 µg/disc), gatifloxcin-oxacillin (1 µg/disc), cefoperazone (75 µg/disc).

For Gram-negative bacteria (*Pseudomonas aeruginosa, Acinetobacter spp,* and *Klebsilla spp*), the tested antibiotics were penicillinG (10 µg/disc), imipenem (10 µg/disc), cefepime (30 µg/disc), meropenem (10 µg/disc), ceftriaxone (30 µg/disc), ceftazidime (30 µg/disc), aztreonam (30 µg/disc), piperacillin (100 µg/disc), cefuroxime (30 µg/disc), piperacillin/tazoabctam (110 µg/disc), amoxicillin/ clavulanic acid (30 µg/disc), amikacin (30 µg/disc), gentamicin (10 µg/disc), ciprofloxacin (5 µg/disc), ofloxacin (5 µg/disc), chloramphnicol (30 µg/disc).

Natural products and their processing

Processing of pure oils

Garlic, oregano, olive, and *Nigella sativa* pure oils were obtained from herbal drug shops. The oils were sterilized and diluted using ethylene glycol to the concentrations of 30 and 70% (v/v). Also, concentrations of 100% (without dilution) were tested. Disc susceptibility testing was carried out. Sterile filter paper discs of 6 mm diameter were immersed in solutions of different concentrations of the tested oils. Sterile filter paper discs were placed aseptically over the Mueller-Hinton agar of bacterial cultures and incubated at 37°C, and the diameters of inhibition zones were measured after 24

Table 1. Relationship between type of operation and the isolated pathogen.

Type of operation	Isolated pathogen				
	Pseudomonas aeruginosa	***Klebsiella spp***	***Enterococcus faecalis***	***Acinetobacter spp***	***Staphylococcus aureus***
Hernia, n = 6 (30% of cases)	1	2	-	1	2
Breast cancer, n = 9 (45% of cases)	4	-	2	3	-
Spleenectomy, n = 1 (5% of cases)	-	-	-	-	1
Laparotomy, n = 2 (10% of cases)	1	-	-	-	1
Cholecystectomy, n = 2 (10% of cases)	-	2	-	-	-

h, and 2, and 3 days. A disc soaked in ethylene glycol was used as negative control. Also, the sensitivities of isolated pathogens to the undiluted commercial oils (garlic, olive, and chamomile oils) obtained from herbal drug shops were tested.

Preparation of olive leaf extract

The olive leaves were obtained from herbal shop, and the extract was prepared as described previously (Yin and Tsao, 1999; Karaman et al., 2003). The olive leaves were weighed (100 g), and washed with distilled water. The leaves were extracted by using a mixture of distilled water and acetone 1:1 (v/v) at room temperature. The extract was taken after 4 h, and 1, 2 and 3 days using filtration, and then sterilized by autoclaving for 20 min. Sterile filter paper discs of 6 mm diameter were immersed in different concentrations of olive leaf extract. Sterile filter paper discs were placed aseptically over the Mueller-Hinton agar of bacterial cultures and incubated at 37°C, and the diameters of inhibition zones were measured after 24 h, and 2, and 3 days.

Whey protein processing

The cheese whey was obtained from Faculty of Agriculture, Alexandria University, Egypt, and was processed as described previously (Marshall, 2004). The whey was heated at 45°C to remove water and then filtrated. The whey was used alone, in combination with honey (1:1), and in combination with pure oregano oil (1:1). Sterile filter paper discs of 6 mm diameter were immersed in different types of whey preparations. Sterile filter paper discs were placed aseptically over the Mueller-Hinton agar of bacterial cultures and incubated at 37°C, and the diameters of inhibition zones were measured after 24 h, and 2, and 3 days.

RESULTS

Rate of operation wound infection and the isolated pathogens

Based on the type of surgical procedure, the pathogens that are isolated from surgical site infection are presented in Table 1. A total of 20 patients with surgical site infection were included in the study. The maximum infection rate was seen in mastectomies (45%) for breast cancer, and minimum infection rate was seen in spleenectomy (5%).

Identification of the isolated pathogens

In the present work, the bacterial species isolated from patients having surgical wound infections were Gram-negative bacilli (*P. aeruginosa, Klebsiella spp,* and *Acinetobacter spp*), and Gram-positive cocci (*S. aureus* and *E. faecalis*). The isolated pathogens collected from infected surgical wound were cultured on blood and MacConkey agar to identify Gram-positive and Gram-negative bacteria. MacConkey agar is selective and differential media for Gram negative bacteria because it contains crystal violet which is inhibitory to Gram positive. *Klebsiella spp* formed typical red colonies indicating fermentation of lactose and acid production on MacConkey agar, and on blood agar, medium-size, grey colonies. *P. aeruginosa* formed medium size grey or bluish colonies on blood agar. In area of confluent growth, the colonies and agar dark due to production of pigments pyoverdin and pyocyanin, and MacConkey agar showed non-lactose fermenting colonies with yellow-green pigment in medium. *Acinetobacter spp* formed small, grey, smooth colonies that caused no alternation of the blood which was observed when grown on blood agar; on MacConkey agar, the pale color, indicates the absence of lactose. *S. aureus* forms medium-sized, raised, glistening colonies. The colonies are pigmented and the color varied from grey-white to golden yellow. Yellow haloes surrounded colonies of pathogenic *S. aureus* due to acid formation. *E. faecalis* formed pinpoint small, smooth, round, white on the entire colonies on blood agar. Gram staining showed that the isolated pathogens include Gram-positive bacteria (*S. aureus* and *E. faecalis*) which appear purple or deep blue microscopically, and Gram-negative bacteria (*P. aeruginosa, Klebsiella spp,* and *Acinetobacter spp*) which appear red under the microscope.

Antibiotic sensitivity of isolated bacterial strains

The sensitivities of different types of bacteria isolated from the surgical wounds were tested to many antibiotics available in clinical use. Most isolated *P. aeruginosa* and *Klebsiella*, *S. aureus* strains were found to be multi-resistant to the examined antibiotics, three strains identified as

Table 2. Antibiotic sensitivity of isolated *Pseudomonads aeruginosa* and *Acinetobacter.*

Number of pathogen Isolate	Antibiotic sensitivity															
	Amikacin	Cefuroxime	Gentamicin	Pipracillin-Tazobactam	Amoxicillin-clavulanic acid	Imipenem	Ceftazidime	Ceftriaxone	Cefoperazone	Chloramphenicol	Penicillin G	Meropenem	Pipracillin	Ciprofloxacin	Aztreonam	Cefepime
Pseudomonads aeruginosa																
Isolate No 6	I	R	R	S	R	S	S	R	S	×	×	R	R	R	S	I
Isolate No 8	I	R	R	S	R	S	S	R	S	×	×	R	R	R	S	I
Isolate No 9	I	R	R	S	R	S	S	R	S	×	×	R	R	R	S	I
Isolate No 12	S	R	R	R	R	R	R	R	R	×	×	R	R	R	S	I
Isolate No 13	S	R	I	S	R	S	S	I	S	×	×	S	S	S	S	S
Isolate No 15	S	R	R	S	R	S	S	R	R	×	×	R	R	R	S	R
Acinetobacter spp																
Isolate No 1	S	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R
Isolate No 3	S	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R
Isolate No 4	S	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R
Isolate No 11	S	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R

R, Resistant; S, sensitive; I, intermediate; x, not done.

Acinetobacter spp were sensitive to two different antibiotics of the sixteen examined kinds. The results of the antibiotic sensitivity tests are presented in Tables 2 and 3.

Antibacterial activity of natural products against isolated pathogens

The *in vitro* antibacterial activities of olive oil, oregano oil, garlic oil, and *N. sativa* oil in different concentrations (30, 70 and 100%), and commercial oils (olive, garlic, and chamomile oils) olive leaf extract, whey protein, whey protein plus honey, and whey protein plus olive oil were tested against the Gram-positive and Gram-negative bacteria isolated from surgical wound infections. The results obtained are shown in Tables 4 and 5. The results show that *P. aeruginosa* was the most sensitive bacteria to all concentrations of olive and oregano, and *N. sativa* oils, while *Klebsiella spp* were the most sensitive bacteria to all concentrations of garlic oil. *S. aureus* and *P. aeruginosa* were the most sensitive to the commercial undiluted oils (olive, garlic, and chamomile oils), while *P. aeruginosa, Klebsiella spp,* and *E. faecalis* showed the highest sensitivity to olive leaf extract. All pathogenic isolated bacteria were highly sensitive to the mix of whey protein plus honey (v/v).

DISCUSSION

The rate of surgical site infections and the frequency of various pathogens causing surgical site infection with their antibiotic resistance pattern in clinic of general surgery in Medical Research Institute, Alexandria University, Egypt were studied. The surgical site infection rates reported by different workers were considerably different. The overall infection rate in the present study was 100% and compares favorably with other reported

Table 3. Antibiotic sensitivity of the isolated *Klebsiella spp, Staphylococcus aureus, Enterococcus faecalis*.

Number of pathogen Isolate	Antibiotic sensitivity															
	Amikacin	Cefuroxime	Gentamicin	Pipracillin-Tazobactam	Amoxicillin-clavulanic acid	Imipenem	Ceftazidime	Ceftriaxone	Cefoperazone	Chloramphenicol	Penicillin G	Meropenem	Pipracillin	Ciprofloxacin	Aztreonam	Cefepime
Klebsiella spp																
Isolate No 7	S	R	R	R	R	S	R	R	R	S	×	S	R	R	R	R
Isolate No 10	S	R	R	R	R	S	R	R	R	S	×	S	R	R	R	R
Isolate No 16	S	R	R	R	R	S	R	R	R	S	×	S	R	R	R	R
Isolate No 20	S	R	R	R	R	S	R	R	R	S	×	S	R	R	R	R
Staphylococcus aureus																
Isolate No 14	S	R	R	R	S	R	R	R	R	R	R	I	R	R	I	R
Isolate No 17	S	R	R	R	R	R	R	S	R	R	R	R	R	R	R	R
Isolate No 18	S	R	R	R	S	R	R	S	R	S	R	R	R	R	R	R
Isolate No 19	S	R	R	R	S	R	R	S	R	R	R	I	R	R	I	R
Enterococcus faecalis																
Isolate No 2	×	×	×	×	R	S	R	×	×	R	×	R	R	S	R	R
Isolate No 5	×	×	×	×	R	S	R	×	×	R	×	R	R	S	R	R

R, Resistant; S, sensitive; I, intermediate; x, not done.

rate 71% (Malone et al., 2002). In contrast with our results, Lilani et al. (2005) reported rate of 8.95% and other reported rates ranged from 2.5 to 41.9%. A number of studies was carried out in India indicating an overall infection rate of 30% for clean surgeries and 45% for clean-contaminated surgeries. Lilani et al. (2005) show that there is significant rise in infection rate with increased degree of operative contamination; rate of infection for clean surgeries was 3.03% while in clean-contaminated surgeries it was 22.41%. The present study showed that rate of infection for clean surgeries was 90%, while in clean-contaminated, it was 10%.

Several studies have reported *S. aureus* as the commonest isolate from the postoperative wound infection (Ramos et al., 2011; Harrop et al., 2012). In the present study, predominance of *S. aureus* in surgical site infection is consistent with reports from other studies. Among Gram-negative bacilli, *P. aeruginosa* was the commonest isolate in the present study. Based on the type of surgical procedure, the pathogens that are isolated from surgical site infection vary. Other studies reported that the clean-contaminated wound is the most infected wound in surgical site infection (Lilani et al., 2005). The present work revealed that the clean wound is the most infected wound in surgical site infection. Several studies in the literature indicate gradual increase in the emergence of antibiotic resistant microorganisms in surgical patients. Special interest in *S. aureus* surgical site infection is mainly due to its predominant role in hospital cross infection and emergence of virulent antibiotic resistant strains. All strains of *P. aeruginosa* were resistant to gentamicin which is one of the antibiotics used for antimicrobial prophylaxis (Lilani et al., 2005).

In accordance with the present results, previous studies showed that *S. aureus* strains were resistant to various antibacterials. Resistance was highest to amikacin, followed by tetracycline,

Table 4. Effect of different dilutions of olive oil, oregano oil, garlic oil, and *Nigella sativa* oil against pathogenic isolates of bacteria.

	Olive oil			Oregano oil			Garlic oil			*Nigella sativa* oil		
	30%	**70%**	**100%**	**30%**	**70%**	**100%**	**30%**	**70%**	**100%**	**30%**	**70%**	**100%**
Acinetobacter spp	Nil	38	46	40	30	26	37	27	43	Nil	44	32
Enterococcus faecalis	> 46	17	13	37	34	28	Nil	> 43	> 43	47	31	25
Staphylococcus aureus	27	34	25	27	29	32	29	32	25	27	20	26
Pseudomonas aeruginosa	> 46	> 46	> 46	> 40	> 40	> 40	> 43	> 43	> 43	> 47	> 47	> 47
Klebsiella spp	20	29	27	37	34	33	> 43	> 43	> 43	Nil	Nil	Nil

Data are the mean diameters of inhibition zones in mm around 6 mm discs impregnated with the oil diluted by ethylene glycol (v/v) in µL. A disc soaked in ethylene glycol was kept as negative control.

cotrimoxazole, ciprofloxacin, ampicillin, ceftriaxone, tobramycin, gentamicin, and erythromycin. Also, previous studies demonstrated that *P. aeruginosa* strains were resistant to ampicillin, gentamicin, amikacin, ceftazidime, cefotaxime, ofloxacin and ceftriaxone, while *Klebsiella pneumoniae* strains were resistant to ampicillin, gentamicin, ciprofloxacin, amikacin, and ceftraixone. Also, in agreement with the present work, *Acinetobacter spp* strains were found to be resistant to ampicillin, gentamicin, tetracycline, and ceftriaxone. *E. faecalis* strains tested were resistant to gentamicin, ciprofloxacin, amikacin, tobramycin, and amoxicillin (Salman et al., 2002).

Regarding the antimicrobial activity of natural products, *N. sativa* oil showed dose-dependent antibacterial activity which was more against Gram-positive than Gram-negative bacteria. This is in accordance with a previous study which showed that Gram-positive bacteria are more sensitive to the antibacterial action of *N. sativa* (El-Fatatry, 1975). Gram-negative bacteria have an effective permeability barrier, comprised of the outer membrane, which restricts the penetration of amphipathic compounds, and multi-drug resistance pumps that extrude toxins across this barrier. It is possible that the apparent ineffectiveness of plant antimicrobials is largely due to the permeability barrier. Among Gram-positive bacteria tested *S. aureus*, *Staphylococcus epidermidis* were sensitive to the oil while *E. faecalis* was resistant. Among Gram-negative bacteria tested, only *P. aeruginosa* was sensitive to oil, while *Acinetobacter baumanni*, and *K. pneumonia* were resistant (Salman et al., 2002). Black seed oil possesses significant antimicrobial activity against methicillin-resistant *S. aureus* (MRSA). MRSA showed large zone of inhibition for the three dilutions (100, 50 and 25% concentration) and showed resistance for two dilution (12.5 and 6.25%) (Alhaj et al., 2008). Hanafy and Hatem (1991) observed antimicrobial activity against *S. aureus* and *P. aeruginosa* at high concentration of *N. sativa,* while Mashhadian and Rakhshandeh (2005) demonstrated antimicrobial activity against *S. aureus* at low concentration.

There are *in vitro* studies documenting that *N. sativa* have antibacterial activity against pathogens such as *P. aeruginosa* and *E. coli* (Morsi, 2000; Ferdous et al., 1992; Rathee et al., 1982). The present work showed that among Gram-negative bacteria tested, *P. aeruginosa* was more sensitive to oil, *Acinetobacter* was resistant at low concentration (30%). *Klebsiella spp* was resistant (not inhibit by oil in any of the concentration tested). Among Gram-positive bacteria tested, *E. faecalis* was sensitive at low concentration (30%).

All bacteria (*E. faecalis*, *E. coli, Klebsiella spp*, *Salmonella choleraensius*, *P. aeruginosa*, and *S. aureus*) were sensitive to the inhibitory effect of oregano essential oil, except *P. aeruginosa,* which is in agreement with findings of Sivropoubu et al. (1996) in which work origanum essential oils did not exhibit antimicrobial activity against this bacteria; all microorganisms were susceptible to the action of oregano essential oil with different dilution. In contrast with our results, the antibacterial effect of the essential oil from *Oreganum compactumon* strains assayed (*S. aureus*, *P. aeruginosa, Bacillus subtilis*, *Proteus micrabilis,* and *Enterococcus faecium*) from the recorded diameters of inhibitory zone, it was observed that except for *P. aeruginosa* which showed obvious resistance, the oil was active against all other bacterial strain, However this activity varies between the test bacteria (Bouhdid et al., 2008). The generally varied greatest resistance of Gram-negative bacteria to essential oils has been attributed in part to the great complexity of the double membrane containing cell envelope of these microorganisms, in contrast with the single membrane structures of Gram-positive bacteria (Cassiano et al., 2007). In contrast with our results, oil of oregano completely inhibited growth of the Gram-negative pathogens tested. Oregano was less effective in inhibiting *P. aeruginosa* (Elgayyer et al., 2001). Our results showed that the Gram-negative were more sensitive than Gram-positive for essential oil of oregano as also reported in a previous study (Di Pasqua et al., 2005).

Medicinal use of garlic as antiseptics, antifungal and antimicrobial, both internally as well as externally. Some constituents of garlic possess broad-spectrum antibiotics effects (McCann, 2003; Ivanova et al., 2009; Lu et al., 2011). The present results showed that Gram-negative and Gram-positive bacteria were sensitive to different dilution of garlic oil except *E. faecalis* which was resistant

Table 5. Effects of commercial oils (olive, garlic, and chamomile), olive leaf extract, whey protein, whey protein plus honey, and whey protein plus olive oil (100%) against pathogenic isolates.

	Commercial oils			Olive leaf extract				Whey		
	Olive oil	Garlic oil	Chamomile oil	4 h	1st day	2nd day	3rd day	Whey alone	Whey + honey	Whey + olive oil
Acinetobacter spp	40	47	45	37	26	35	29	28	38	30
Enterococcus faecalis	18	15	12	> 49	> 49	Nil	> 49	Nil	48	Nil
Staphylococcus aureus	> 47	> 47	> 47	33	20	30	24	Nil	45	Nil
Pseudomonas aeruginosa	> 47	> 47	> 47	> 49	> 49	> 49	> 49	34	40	24
Klebsiella spp	Nil	Nil	Nil	> 49	> 49	> 49	> 49	31	37	26

Data are the mean diameters of inhibition zones in mm around 6 mm discs impregnated with commercial oils without dilution. A disc soaked in ethylene glycol was kept as negative control.

to oil at low concentration (30%). The bacteriostatic and bactericidal activites of olive oil has been studied *in vitro* against many pathogenic microorganisms (Medina et al., 2007; Cicerale et al., 2010). Olive oil is able to inhibit the development and production of enterotoxin B by *S. aureus* (Omar, 2010). Olive oil completely inhibited the development of *K. pneumoniae* and *E. coli* (Upadhyay et al., 2010). Olive oil has wide antimicrobial activity because they may cause surface activity that damages the membranes of bacterial cell (Juven et al., 1972). The antibacterial effect of olive oil has been observed on a wide range of bacteria, however, no effect had been observed on yeast (Beuchat and Golden, 1989). The present work showed that Gram-negative and Gram-positive bacteria were sensitive to different dilutions of olive oil except *Acinetobacter spp* which was resistant to oil at low concentration (30%). *E. faecalis* and *P. aeruginosa* were most sensitive to oil at low concentration (30%). Antimicrobial activity of Chamomile has been studied in *vitro* and the results have been encouraging. Two such studies demonstrated that Gram-positive bacteria were more susceptible than Gram-negative bacteria to chamomile oil. It was most effective against *S. aureus, Streptococus mutans* and *Streptococcus salivarius,* and also *Bacillus megatherium*. In the present work, *Acinetobacter spp*, *S. aureus* and *P. aeruginosa* were more sensitive to chamomile oil, while *Klebsiella spp* was resistant.

It was found that olive leaf extract was effective against *Pediococcus cerevisia, S. aureus, B. subtilis, E. coli, Salmonilla tyhimurium, Pseudomonas fluorescens,* and *Pseudomonas salanacearum* (Keskin et al., 2012). Acetone extraction gave the most effective results on all test bacteria (*E. faecalis*, *S. aureus*, *E. coli*, *P. aeruginosa, Klebsiella spp, Acinetobacter spp,* and *Salmonella enterilids)* because of their dicarboxylic phenolic contents. Our results showed that *Klebsiella spp* was sensitive to all olive leaf extracts as also reported by Korukluoglu et al. (2004). Previous studies evaluated the *in vitro* activity of olive leaf extract against several Gram-positive bacteria (*B. cereus*, *B. subtilis* and *S. aureus*), and Gram-negative bacteria (*P. aeruginosa*, *E. coli* and *K. pneumonia*) (Pereira et al., 2007). In the present work, among Gram-negative bacteria tested *P. aeruginosa* and *Klebsiella spp* were more sensitive to all extract concentrations than *Acinetobacter spp*. Among Gram-positive bacteria tested *E. faecalis* was more sensitive to all extract concentrations but was resistant at the 2nd day concentration.

In whey protein, lactoferrin is the component of whey. In a concise review, Shah (2000) discussed the bacteriostatic and bactericidal activity of lactoferrin which inhibits a number of organisms, including *E. coli, Salmonella typhimurium, Shigella dysenteria, B. subtilis,* and *Micrococcus luteus*. Lactoferrin when in combination with lysozyme, is a more potent bacteriostatic agent against *P. aeruginosa* and *E. coli.* Our results showed whey protein mixed with honey gave the most effective results on all tested bacteria. *E. faecalis* and *S. aureus* were resistant to whey protein and whey protein mix with undiluted olive oil.

The present work, in agreement with previous reports, revealed that the isolated bacteria were multi-resistant to most of the used antibiotics. This multi-drug resistance may be due to plasmid-mediated resistance or mutations in the causative bacteria (Gan et al., 2012; Leeds et al., 2012). The use of combinations of different antibacterial agents or a combination of the natural herbal products with known antibacterial activity may be a solution for this major clinical problem. However, this point needs to be clarified in a further study.

In conclusion, the pathogenic bacteria isolated from surgical wound infection were multi-resistant to the commonly used antibiotics, and these bacterial isolates were effectively inhibited by medicinal oils especially olive oil, at high concentration, garlic and oregano oils, all concentrations of olive leaf extract, and whey plus honey. Therefore, these natural products can be considered as potential

therapeutic agents for eradication of bacteria responsible for nosocomial infections.

REFERENCES

Alhaj NA, Shamsudin MN, Alipiah NM, Zamri HF, Abdullah R (2008). Extraction of essential oil from *Nigella sativa* using supercritical carbon dioxide: study of antibacterial activity. Am. J. Pharmacol. Toxicol. 3: 225-228.

Bauer AW, Kirby WMM, Sherris JC, Turck M (1966). Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 36: 493-496.

Beuchat LR, Golden DA (1989). Antimicrobials occurring naturally in foods. Food Technol. 43: 134-142.

Bouhdid S, Skali SN, Idaomar M, Zhiri A, Baudoux D, Amensour M, Abrini J (2008). Antibacterial and antioxidant activities of *Origanum compactum* essential oil. Afr. J. Biotechnol. 7: 1563-1570.

Cassiano B, Altemir JM, Maria RA, Cansian RL, de Oliveira JV (2007). Evaluation of *Origanum vulgare* essential oil as antimicrobial agent in sausage. Brazilian J. Microbiol. 38: 610-616.

Center for Disease Control (CDC) (1999). Guideline for prevention of surgical site infection. www.cdc.gov/hicpac/SSI/001_SSI.html.

Cicerale S, Lucas L, Keast R (2010). Biological activities of phenolic compounds present in virgin olive oil. Int. J. Mol. Sci. 11: 458-479.

Di Pasqua R, De Feo V, Villani F, Mauriello G (2005). *In vitro* antimicrobial activity of essential oils from Mediterranean apiaceae, Verbenaceae and Lamiaceae against food borne pathogens and spoilage bacteria. Ann. Microbiol. 55: 139-143.

El-Fatatry HM (1975). Isolation and structure assignment of an antimicrobial principle from the volatile oil of *Nigella sativa* L. seeds. Pharmazie 30: 109-111.

Elgayyar M, Draughon F, Golden D, Mount J (2001). Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms. J. Food Prot. 64: 1019-1024.

Ferdous AJ, Islam SN, Ahsan M, Hasan M, Ahmed ZU (1992). *In vitro* antibacterial activity of the volatile oil of *Nigella sativa* seeds against multiple drug resistance isolated of *Shigella spp*. and isolated of *Vibrio chloerae* and isolated of *Escherichia coli*. Phytother. Res. 6: 137-140.

Gan HM, Lean SS, Suhaili Z, Thong KL, Yeo CC (2012). Genome sequence of Acinetobacter baumannii AC12, a polymyxin-resistant strain isolated from Terengganu, Malaysia. J. Bacteriol. 194: 5979-5980.

Garner JS, Jarvis WR, Emori TG (1996). CDC definitions for nosocomial infections. In: Olmsted RN (ed.): APIC Infection Control and Applied Epidemiology: Principles and Practice. St. Louis, Mosby, pp. A1-A20.

Hanafy MS, Hatem ME (1991). Studies on the antimicrobial activity of *Nigella sativa* seed (black cumin). J. Ethnopharmacol. 34: 275-278.

Harrop JS, Styliaras JC, Ooi YC, Radcliff KE, Vaccaro AR, Wu C (2012). Contributing factors to surgical site infections. J. Am. Acad. Orthop. Surg. 20: 94-101.

Ivanova A, Mikhova B, Najdenski H, Tsvetkova I, Kostova I (2009). Chemical composition and antimicrobial activity of wild garlic *Allium ursinum* of Bulgarian origin. Nat. Prod. Commun. 4: 1059-1062.

Juven B, Henis Y, Jacoby B (1972). Studies on the mechanism of the antimicrobial action of oleuropein. J. Appl. Bacteriol. 35: 559-567.

Karaman I, Şahin F, Güllüce M, Öğütçü H, Şengül M, Adıgüzel A (2003). Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus L*., J. Ethnopharmacol. 85, 231-235.

Keskin D, Ceyhan N, Uğur A, Dbeys AD (2012). Antimicrobial activity and chemical constitutions of West Anatolian olive (*Olea europaea* L.) leaves. J. Food Agric. Environ. 10: 99-102.

Korukluoglu M, Sahan Y, Yigit A, Ozer ET, Gucer S (2004). *In vitro* antibacterial activity of olive leaf (*Olea Europea L.*) extracts and their chemical characterization. Adnan Menderes University, 4th AACD Congress, Kuþadasý-Aydin, Turkey, Proceedings Book 291, pp. 563-565.

Leeds JA, Sachdeva M, Mullin S, Dzink-Fox J, Lamarche MJ (2012). Mechanism of action of and mechanism of reduced susceptibility to the novel anti-Clostridium difficile compound LFF571. Antimicrob. Agents Chemother. 56: 4463-4465.

Lilani SP, Jangale N, Chowdhary A, Daver GB (2005). Surgical site infection in clean and clean-contaminated cases. Indian J. Med. Microbiol. 23: 249-252.

Lu X, Rasco BA, Jabal JM, Aston DE, Lin M, Konkel ME (2011). Investigating antibacterial effects of garlic (*Allium sativum*) concentrate and garlic-derived organosulfur compounds on *Campylobacter jejuni* by using Fourier transform infrared spectroscopy, Raman spectroscopy, and electron microscopy. Appl. Environ. Microbiol. 77: 5257-5269.

Malone DL, Genuit T, Tracy JK, Gannon C, Napolitano LM (2002). Surgical site infection: reanalysis of risk factors. J. Surg. Res. 103: 89-95.

Marshall K (2004). Therapeutic applications of whey protein. Alternative Med. Rev. 9: 136–156.

Mashhadian NV, Rakhshandeh H (2005). Antibacterial and antifungal effects of *Nigella sativa* extracts against *S. aureus*, *P. aeruginosa* and *C. albicans*. Pak. J. Med. Sci. 21: 47-52.

McCann J (2003). Herbal Medicine Handbook. second ed., Philadelphia, Lippincott.

Medina E, Romero C, Brenes M, De Castro A (2007). Antimicrobial activity of olive oil, vinegar, and various beverages against foodborne pathogens. J. Food Prot. 70: 1194-1199.

Morsi NM (2000). Antimicrobial effect of crude extracts of *Nigella sativa* on multiple antibiotics-resistant bacteria. Acta Microbiol. Pol. 49: 63-74.

Omar SH (2010). Oleuropein in olive and its pharmacological effects. Sci. Pharm. 78: 133-154.

Pereira AP, Ferreira I, Marcelino F, Valentão P, Andrade PB, Seabra R, Estevinho L, Bento A, Pereira JA (2007). Phenolic compounds and antimicrobial activity of olive (*Olea europaea* L. *Cv.* Cobrançosa) leaves. Molecules 12: 1153-1162.

Podschun R, Acktun H, Okpara J, Linderkamp O, Ullmann U, Borneff-Lipp M (1998). Isolation of *Klebsiella planticola* from newborns in a neonatal ward. J. Clin. Microbiol. 36: 2331-2332.

Ramos N, Skeete F, Haas JP, Hutzler L, Slover J, Phillips M, Bosco J (2011). Surgical site infection prevention initiative - patient attitude and compliance. Bull. N.Y.U. Hosp. Jt. Dis. 69: 312-315.

Rathee PS, Mishra SH, Kaushal R (1982). Antimicrobial activity of essential oil, fixed oil and unsaponified matter of *Nigella sativa* L. Ind. J. Pharmacol. 44: 8-10.

Rosenblueth M, Martinez L, Silva J, Martínez-Romero E (2004). *Klebsiella variicola*, a novel species with clinical and plant-associated isolates. Syst. Appl. Microbiol. 27: 27-35.

Salman MT, Rahat AK, Tndu S (2002). *In vitro* antimicrobial activity of *Nigella sativa* oil against multi-drug resistant bacteria. Jawaharlal Nehru Medical College, Aligarh Muslim University, India.

Schaberg DR, Culver DH, Gaynes RP (1991). Major trends in the microbial etiology of nosocomial infection. Am. J. Med. 91: 72S-75S.

Schaechter M, Medoff G, Eisenstein BI (1993). Mechanisms of Microbial Disease, second ed., Williams & Wilkins Co., Baltimore, Md.

Schwarzkopf R, Russell TA, Shea M, Slover JD (2011). Correlation between nutritional status and Staphylococcus colonization in hip and knee replacement patients. Bull. N.Y.U. Hosp. Jt. Dis. 69: 308-311.

Shah NP (2000). Effects of milk-derived bioactives: an overview. Br. J. Nutr. 84: S3-S10.

Sivropoulou A, Papanikolaou E, Nikolaou C, Kokkini S, Lanaras T, Arsenakis M (1996). Antimicrobial and cytotoxic activities of origanum essential oils. J. Agric. Food Chem. 44: 1202-1205.

Sutherland R, Rolinson GN (1964). Characteristics of methicillin-resistant *staphylococci.* J. Bacteriol. 87: 887-899.

Upadhyay RK, Dwivedi P, Ahmad S (2010). Screening of antibacterial activity of six plant essential oils against pathogenic bacterial strains. Asian J. Med. Sci. 2: 152-158.

Yin M, Tsao S (1999). Inhibitory effect of seven *Allium* plants upon three *Aspergillus* species. Int. J. Food Microbiol. 49:49-56.