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# Microorganisms Isolated from Surgical Wounds Infection and Treatment with Different Natural Products and Medications

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***Abstract*****—**Surgical site infections (SSIs) are the most common nosocomial infection in surgical patients resulting in significant increases in postoperative morbidity and mortality. The commonly causative bacteria developed resistance to virtually all antibiotics available. The aim of this study was to isolation and identification the most common bacteria that cause SSIs in Medical Research Institute, and to compare their sensitivity to selected group of antibiotics and natural products (garlic, oregano, olive, and *Nigella sativa* oils). The isolated pathogens collected from infected surgical wounds were identified, and their sensitivities to the antibiotics commonly available for clinical use, and also to the different concentrations of the used natural products were investigated. The results indicate to the potential therapeutic effect of the tested natural products in treatment of surgical wound infections.

***Keywords*****—**Surgical wounds, multi-resistant bacteria, bacterial sensitivity, natural oils.

## I. 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 [1]. 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* [2]. However, Gram-negative bacteria can contaminate skin wounds of the groin and perineal areas. The contaminating pathogens in gastrointestinal surgeries are the intrinsic bowel flora, which include Gram-negative bacilli and Gram-positive microbes, including enterococci and anaerobic organisms [3]. The most common microbiological cause of nosocomial infection is Gram-negative bacteria, *Escherichia coli*, *Proteus mirabilis*, and other members of family known as *Enterobacteriaceae* [4]. They spread via fecal contamination, instruments and other surfaces. Other Gram-negative bacteria include members of the genera *Pseudomonas* and *Acinetobacter* [5], [6]. 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 many antibiotics in common use [7]. 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 [8]. The search for new compounds which are effective for eradication of nosocomial infections is still going on. This was encouraging to investigate the effect of some natural products (garlic, oregano, olive, and *Nigella sativa* oils) with known antibacterial activity [9]-[12] for treatment of surgical wound infections caused by different types of bacteria commonly responsible for nosocomial infections.

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## II. Materials and Methods

### *A. Sample Collection and Preparation*

Surgical wound swabs were collected from postoperative contaminated wounds from twenty patients in the Department of Surgery, Medical Research Institute, Alexandria University, Egypt, before the use of postoperative antibiotics. The written consent was obtained from all patients before starting the study. 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).

### *B. Isolated Microorganisms*

In the present work, the bacterial species isolated from surgical wound infections were Gram-negative bacilli (*Pseudomonas aeruginosa*, *Klebsiella* spp., and *Acinetobacter* spp), and Gram-positive cocci (*Staphylococcus aureus* and *Enterococcus faecalis).*

### *C. Antibiotics Sensitivity Testing*

Mueller-Hinton agar was used for determination of antibiotic sensitivity patterns by applying Bauer-Kirby technique [13]. A sterile cotton tipped swab was dipped and drained in the test culture, and streaked evenly over the prepared Mueller-Hinton agar plates dried at 37ºC for 30min before use. The plates were allowed to dry for 5min, 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 37ºC 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 - 20ºC until further processing with natural products. For Gram-positive bacteria (*Staphylococcus aureus*, and *Enterococcus faecalis*), the tested antibiotics were vancomycin (30µg/disc), levofloxacin (5µg/disc), ciprofloxacin (5µg/disc), ofloxacin (5µg/disc), penicillin G (10µg/disc), imipenem (10µg/disc), cefepime (30µg/disc), meropenem (10µg/disc), ceftriaxone (30µg/disc), ceftazidime (30µg/disc), aztreonam (30µg/disc), piperacillin (100µg/disc), cefuroxime sodium (30µg/disc), piperacillin/tazoabctam (110µg/disc), amoxicillin/clavulanic acid (30µg/disc), amikacin (30µg/disc), gentamicin (10µg/disc), azithromycin (15µg/disc), chloramphnicol (30µg/disc), gatifloxcin-oxacillin (1µg/disc), cefoperazone (75µg/disc). For Gram-negative bacteria (*Pseudomonas aeruginosa*, *Acinetobacter* spp., and *Klebsilla* spp.), the tested antibiotics were penicillin G (10µg/disc), imipenem (10µg/disc), cefepime (30µg/disc), meropenem (10µg/disc), ceftriaxone (30µg/disc), ceftazidime (30µg/disc), aztreonam (30µg/disc), piperacillin (100µg/disc), cefuroxime (30µg/disc), piperacillin/tazoabctam (110µg/disc), amoxicillin/ clavulanic acid (30µg/disc), amikacin (30µg/disc), gentamicin (10µg/disc), ciprofloxacin (5µg/disc), ofloxacin (5µg/disc), chloramphenicol (30µg/disc).

### *D. Processing of Pure Oils*

Garlic, oregano, olive, and *Nigella sativa* pure oils were obtained from herbal drug shops. The oils were 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 6mm 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 37ºC, and the diameters of inhibition zones were measured after 24h, and 2, and 3 days. A disc soaked in ethylene glycol as negative control.

## III. Results

### *A. Identification of the Isolated Pathogens*

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 its contain 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. *Pseudomonas 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 was observed when grown on blood agar, on MacConkey agar the pale color, indicating the absence of lactose. *Staphylococcus aureus* forms medium-sized, raised, glistening colonies. The colonies are pigmented and the color varied from grey-white to golden yellow. Yellow haloes will surrounded colonies of pathogenic *Staphylococcus aureus* due to acid formation. *Enterococcus faecalis* formed pinpoint small, smooth, round, white, entire colonies on blood agar. Gram stain was also used for identification of the isolated bacteria. Microscopically, Gram-positive bacteria (*Enterococcus faecalis*, and *Staphylococcus aureus)* appear purple, while Gram-negative bacteria appear red (*Acinetobacter* spp, *Klebsiella* spp, and *Pseudomonas aeruginosa).*

### *B. Antibiotic Sensitivity of Isolated Bacterial Strains*

Most isolated *Pseudomonas aeruginosa* and *Klebsiella*, *Staphylococcus aureus* strains were found to be multi-resistant to the examined antibiotics (Tables I-V).

### *C. Antibacterial Activity of Natural Products against Isolated Pathogens*

The *in vitro* antibacterial activities of olive, oregano, garlic, and *Nigella sativa* oils in different concentrations (30, 70, and 100%), were tested against the Gram-positive and Gram-negative bacteria isolated from surgical wound infections. The results obtained are shown in Tables VI-IX.

TABLE I
ANTIBIOTIC SENSITIVITY OF ISOLATED *PSEUDOMONADS AERUGINOSA* (R = RESISTANT, S = SENSITIVE, I = INTERMEDIATE)

| No of Isolated Pathogen | Amikacin | Cefuroxime | Gentamicin | Pipracillin-Tazobactam | Amoxicillin-Clavulanic acid | Imipenem | Ceftazidime | Ceftriaxone | Cefoperazone | Meropenem | Pipracillin | Ciprofloxacin | Aztreonam | Cefepime |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No 6 | I | R | R | S | R | S | S | R | S | R | R | R | S | I |
| No 8 | I | R | R | S | R | S | S | R | S | R | R | R | S | I |
| No 9 | I | R | R | S | R | S | S | R | S | R | R | R | S | I |
| No 12 | S | R | R | R | R | R | R | R | R | R | R | R | S | I |
| No 13 | S | R | I | S | R | S | S | I | S | S | S | S | S | S |
| No 15 | S | R | R | S | R | S | S | R | R | R | R | R | S | R |

TABLE II
ANTIBIOTIC SENSITIVITY OF ISOLATED *ACINETOBACTER* SPP STRAINS (R = RESISTANT, S= SENSITIVE)

| No of Isolated Pathogen | Amikacin | Cefuroxime | Gentamicin | Pipracillin-Tazobactam | Amoxicillin-Clavulanic acid | Imipenem | Ceftazidime | Ceftriaxone | Cefoperazone | Chloramphenicol | Penicillin G | Meropenem | Pipracillin | Ciprofloxacin | Aztreonam | Cefepime |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No 3 | S | R | R | R | R | S | R | R | R | R | R | R | R | R | R | R |
| No 4 | S | R | R | R | R | S | R | R | R | R | R | R | R | R | R | R |
| No 11 | S | R | R | R | R | S | R | R | R | R | R | R | R | R | R | R |

TABLE III
ANTIBIOTIC SENSITIVITY OF THE ISOLATED *KLEBSIELLA* SPP (R = RESISTANT, S = SENSITIVE, × = NOT DONE)

| No of Isolated Pathogen | Amikacin | Cefuroxime | Gentamicin | Pipracillin-Tazobactam | Amoxicillin-Clavulanic acid | Imipenem | Ceftazidime | Ceftriaxone | Cefoperazone | Chloramphenicol | Penicillin G | Meropenem | Pipracillin | Ciprofloxacin | Aztreonam | Cefepime |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No 7 | S | R | R | R | R | S | R | R | R | S | × | S | R | R | R | R |
| No 10 | S | R | R | R | R | S | R | R | R | S | × | S | R | R | R | R |
| No 16 | S | R | R | R | R | S | R | R | R | S | × | S | R | R | R | R |
| No 20 | S | R | R | R | R | S | R | R | R | S | × | S | R | R | R | R |

TABLE IV
ANTIBIOTIC SENSITIVITY OF ISOLATED *STAPHYLOCOCCUS AUREUS* (R = RESISTANT, S = SENSITIVE, I = INTERMEDIATE)

| No of Isolated Pathogen | Amikacin | Cefuroxime | Pipracillin-Tazobactam | Amoxicillin-Clavulanic acid | Imipenem | Ceftriaxone | Cefoperazone | Chloramphenicol | Penicillin G | Meropenem | Pipracillin | Ciprofloxacin | Aztreonam | Cefepime | Oflloxacin | Oxacillin |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No 14 | S | R | R | R | S | R | R | R | R | R | R | I | R | R | I | R |
| No 17 | S | R | R | R | R | R | R | S | R | R | R | R | R | R | R | R |
| No 18 | S | R | R | R | S | R | R | S | R | S | R | R | R | R | R | R |
| No 19 | S | R | R | R | S | R | R | S | R | R | R | I | R | R | I | R |

TABLE V
ANTIBIOTIC SENSITIVITY OF ISOLATED *ENTEROCOCCUS FAECALIS* (R = RESISTANT, S = SENSITIVE, × = NOT DONE)

| No of Isolated Pathogen | Amikacin | Cefuroxime | Gentamicin | Pipracillin-Tazobactam | Imipenem | Chloramphenicol | Penicillin G | Meropenem | Pipracillin | Ciprofloxacin | Aztreonam | Cefepime | Levofloxacin | Vancomycin | Gatifloxacin | Azithromycin |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| No 3 | × | × | × | × | R | S | R | × | × | R | × | R | R | S | R | R |
| No 5 | × | × | × | × | R | S | R | × | × | R | × | R | R | S | R | R |

TABLE VI
EFFECTS OF DIFFERENT DILUTIONS OF OLIVE OIL AGAINST PATHOGENIC ISOLATES OF BACTERIA, DATA ARE THE MEAN DIAMETERS OF INHIBITION ZONES

| Concentration/disc / Pathogen Isolate | 30 % | 70% | 100% | Ethylene glycol |
|---|---|---|---|---|
| *Acinetobacter* spp | Nil | 38 | 46 | Nil |
| *Enterococcus faecalis* | > 46 | 17 | 13 | Nil |
| *Staphylococcus aureus* | 27 | 34 | 25 | Nil |
| *Pseudomonas aeruginosa* | > 46 | > 46 | > 46 | Nil |
| *Klebsiella* spp | 20 | 29 | 27 | Nil |

TABLE VII
EFFECTS OF DIFFERENT DILUTIONS OF OREGANO OIL AGAINST PATHOGENIC ISOLATES OF BACTERIA, DATA ARE THE MEAN DIAMETERS OF INHIBITION

| Concentration/disc / Pathogen Isolate | 30 % | 70% | 100% | Ethylene glycol |
|---|---|---|---|---|
| *Acinetobacter* spp | 40 | 30 | 26 | Nil |
| *Enterococcus faecalis* | 37 | 34 | 28 | Nil |
| *Staphylococcus aureus* | 27 | 29 | 32 | Nil |
| *Pseudomonas aeruginosa* | > 40 | > 40 | > 40 | Nil |
| *Klebsiella* spp | 37 | 34 | 33 | Nil |

TABLE VIII
EFFECTS OF DIFFERENT DILUTIONS OF GARLIC OIL AGAINST PATHOGENIC ISOLATES OF BACTERIA, DATA ARE THE MEAN DIAMETERS OF INHIBITION ZONES

| Concentration/disc / Pathogen Isolate | 30 % | 70% | 100% | Ethylene glycol |
|---|---|---|---|---|
| *Acinetobacter* spp | 37 | 27 | 43 | Nil |
| *Enterococcus faecalis* | Nil | > 43 | > 43 | Nil |
| *Staphylococcus aureus* | 29 | 32 | 25 | Nil |
| *Pseudomonas aeruginosa* | > 43 | > 43 | > 43 | Nil |
| *Klebsiella* spp | > 43 | > 43 | > 43 | Nil |

TABLE IX
EFFECTS OF DIFFERENT DILUTIONS OF *NIGELLA SATIVA* OIL AGAINST PATHOGENIC ISOLATES OF BACTERIA, DATA ARE THE MEAN DIAMETERS OF INHIBITION ZONES

| Concentration/disc / Pathogen Isolate | 30 % | 70% | 100% | Ethylene glycol |
|---|---|---|---|---|
| *Acinetobacter* spp | Nil | 44 | 32 | NIL |
| *Enterococcus faecalis* | 47 | 31 | 5 | Nil |
| *Staphylococcus aureus* | 27 | 20 | 26 | Nil |
| *Pseudomonas aeruginosa* | > 47 | > 47 | > 47 | Nil |
| *Klebsiella* spp | Nil | Nil | Nil | Nil |

## IV. CONCLUSION

The natural oils used in the present work (garlic, oregano, olive, and *Nigella sativa* oils) were effective against the pathogenic bacteria isolated from surgical wound infections.

Therefore, these oils represent feasible candidates to treat these infections which are usually multi-resistant to commonly used antibiotics.

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