Cihan University-Erbil Scientific Journal (CUESJ)

RESEARCH ARTICLE

The Prevalence of Microorganisms and Antibiotics Activity Tests of Natural and Industrial Products in Surgical Site Infections in Referral Hospitals in Erbil City, Kurdistan Region – Iraq

Raid D. Thanoon[1]*, Yara M. Mohammed[2]

[1]Department of Medical Biochemical Analysis, Cihan University-Erbil, Kurdistan Region, Iraq, [2]Department of General Biology, Cihan University-Erbil, Kurdistan Region, Iraq

ABSTRACT

Background and Objectives: To study the prevalence of microorganisms and antibiotics activity tests of natural and industrial products, surgical site infection was found in referral hospitals in Erbil, Iraq's Kurdistan Province. **Methods:** Pus samples were collected from 29 surgical patients using sterile swabs and primed for culture collection. Disk diffusion method was used to determine the bacterial activity of three types of plants (cinnamon, ginger, and radish) against three bacterial species including *Staphylococcus aureus*, *Proteus* spp., and *Staphylococcus epidermidis*. **Results:** Overall, 29 pus samples were processed for culture testing. One Gram-negative bacterium (7.14%) and 13 Gram-positive cocci (92.85%) were isolated from the 14 bacteria genera. *S. aureus*, *Proteus* spp., and *S. epidermidis* most potent isolates were found to be resistant to the variable of tested antibiotics, while they showed any sensitivity to the tested plant extracts. Cinnamon, ginger, and radish extracts were discovered to be among the few variable effective plant extracts against the tested pathogenic bacteria isolates. **Conclusion:** The data collected from this research showed a better way of explanation of the microorganism's etiology of SSIs in hospitals where they might have epidemiological and healing insinuations.

Keywords: Surgical site infections, bacteria isolated from wounds, antimicrobial resistance, pus, plant extract

INTRODUCTION

Surgical site infection occurs when surgical wounds get infected with germs from a previously diseased location. According to the report, it can result in illnesses, hospitalizations, and even death.[1] The third most often observed nosocomial infection is surgical site infections.[2] The most prevalent causes of these contagions are exogenous and endogenous germs that reach the operating wounds during surgery.[2] The occurrence of infected operational injuries might be affected by such factors as pre-operative therapy, theater, post-operative care, and surgery.[2] *Staphylococcusaureus* is a naturally occurring bacterium that colonizes about 30% of the human population's nares and a new study indicates that very diverse nasal microbiosis is required if colonizations of *S. aureus* are to be encouraged or prevented. It spreads easily from person to person, onto health care workers' hands and clothing, as well as onto surfaces and into the air.[2,3] *Staphylococcus epidermidis*,once considered a comparatively harmless pathogen, is the biggest and best investigated member of the CoNS group.[4,5] It is considered as an unsafe commensal of the human skin and is becoming known for its useful role in skin immunity and microbiota.[4] The active metabolites of cinnamon bark (*Cinnamomum verum*) belonging to the Lauraceae family were cinnamon aldehyde and trans-cinnamic acid. The nutritional content, phenolic profile, and antioxidant activities of cinnamon were studied. According to a compositional study, this species is a great source of protein and minerals. The nutritional content, phenolic profile, and antioxidant activities of cinnamon were studied. According to a compositional study, this species is a great source of protein and minerals.[6] Radish (*Raphanus sativus* L.), belonging to the Brassicaceae family, is an annual or biennial root crop globally of economic importance. There are many resources of radish germplasm in China, and for conservation and use of the radish resources, cultivars and genetic diversity studies are very important.[7] Since antiquity, ginger (*Zingiber officinale*) belonging to the Zingiberaceae

Corresponding Author:
Raid D. Thanoon, Department of Medical Biochemical Analysis, Cihan University-Erbil, Kurdistan Region, Iraq. E-mail: raid.duraid@cihanuniversity.edu.iq

Received: May 30, 2021
Accepted: July 18, 2021
Published: August 10, 2021

DOI: 10.24086/cuesj.v5n2y2021. pp 6-12

family for more than 200 years, traditional Chinese medicine has been utilized as a spice and medication. Ginger is a major herb with numerous nutritional and therapeutic properties that are utilized in traditional Asian and Chinese medicine.[8] Ginger and its general components, such as Fe, Ca, Vitamin C, flavonoids, phenolic compounds, paradols, and so on, are used for a wide variety of symptoms, including vomiting, pain, and other herbal medications.[8]

Objectives

1. Isolation of microorganisms from surgical wounds
2. Natural and industrial products were tested for antibiotic activity.

MATERIALS AND PROCEDURES

Population Research

The research was carried out at Cihan University-Erbil's Biology Department. Specimens were collected from four hospitals between November 2019 and March 2020.

Specimen Collection and Transport

Under aseptic conditions, two sterile swabs were used to extract pus samples from each patient. Swabs were taken to the Department of Biology, Cihan University-Erbil. The specimens can be stored in the sample tube at 4°C for 72 h.

Types of Antimicrobials

Identification

The bacterial growth of the incubated plates was identified on the basis of cultural, morphological, and biochemical characteristics (Table 1).[9]

Production of Specimens

Inoculated specimens of pus have been immediately inoculated on MacConkey, chocolate, and mannitol salt agar dishes. They were incubated aerobically at 37°C for 24–48 h with a CO_2 concentration of 7–10%. After this, the specimens was inoculated on the plates using four flame methods.[10,11]

Table 1: Antimicrobials and their concentrations

No.	Name	Dose	Manufactures
1	Amikacin	10 mcg	Amikin
2	Amoxicillin	25 mcg	Trimox
3	Ampicillin/cloxacillin	25/5 mcg	AMPOXIN
4	Ceftazidime	30 mcg	Ceptaz
5	Ceftriaxone	10 mcg	Rocephin
6	Chloramphenicol	10 mcg	Fenicol
7	Ciprofloxacin	10 mcg	Ciloxan
8	Gentamicin	10 mcg	Gentasol
9	Imipenem	10 mcg	Primaxin
10	Netilmicin	30 mcg	Netmicin
11	Oxacillin	10 mcg	Bactocill
12	Rifampin	5 mcg	Rifadin
13	Tetracycline	10 mcg	Declomycin

Procedure of catalase testing

Fill a slide with a small amount of bacterial growth. Drops of H_2O_2 were added to the smear and blended with a toothpick.[12]

Gram staining

Overflow slide with mineral violet – 60 s by Gram – 180 s, iodine floods. Prudently decolor with 95% ethanol until the thinnest part of the smear is colorless. Flood with safranin (pink color) (10% fuchsine) – 60 s (wash on the water). Air dehydrated or spot by permeable paper.[13]

Slide coagulase test

Identify where the test strain (T) and the control (C) are placed by marking the slide. Set up positive and negative control organisms on the same slide for simultaneous testing. Emulsified test strain to obtain a homogeneous thick suspension. Observed self-agglutination. Within 10 s, there will be a visible cluster. Negative result: No visible clustering within 10 s.[14]

Testing for Antimicrobial Sensitivity

Drugs have been chosen on the basis of their impact on a specific organism, as well as hospital antibiotic policies.[20] The diameter of the inhibitor was measured on a scale of 24 h after 18–24 h of incubation. Each antimicrobial disk's area size was used to determine if it was susceptible, intermediate, or immune (CLSI, 2006). Mueller-Hinton agar and antibiotic disks were used.[15]

Ginger Extraction

Ginger was bought from local marketplaces in Erbil City, using ginger for this investigation. In boiling water bath for 30 min, 10 g of ginger powder combined with 40 ml soy oil was heated. Before disk diffusion testing, extracts were kept at 4°C to retain their antibacterial activities. The plates were dried in the air flow chamber of the laminar for 15 min. Extracts of ginger (20 μl) have been applied individually for 18–24 h on each disk.[16]

Radish Extraction

A local supermarket provided *Raphanus sativus* (white radish variety). Radish was lyophilized and crushed into a fine powder before ethanol extraction. Powder root was extracted for 24 h with 300 ml of ethanol at room temperature and 150 rpm.A filter paper disk diffusion test was used to assess the extract's antibacterial function.[17] The research bacteria inoculums were prepared according to McFarland standard 0.5. Uniform bacterial lawns were developed, using 100L of inoculum on a Mueller-Hinton agar plate. The plates were incubated at 37°C for 24 h.[18]

Cinnamon Extraction

After cleaning the spices, they were dried in sunlight for 2 days. The dried material was pulverized by a grinder into a fine powdered substance. Twenty grams of cinnamon powder (weighted by electric balance) were transferred to 100 ml of conical flask where 40 ml of ethanol was added. The ethanol crude was filtered using Whatman No. 1 filter paper. Cinnamon bark extract was made using dimethyl sulfoxide as a solvent at concentrations of 2.5%, 5%, and 10%. With a sterile cotton bud, the bacterial solution was spread on the whole surface of Mueller-Hinton agar. At 37°C, the Petri dish was incubated overnight.[19]

RESULTS

Specimens of Surgical Wound Infection

A total of 29 pus specimens were processed for culture testing. The present study being conducted between November (2019) and March (2020). The overall percentage of infection was (48.27%) while the remaining (51.72%) was negative [Figure 1]. Two patients from the maternity hospital were cultivated with MacConkey agar and the mannitol salt agar had a negative result and a positive result of *S. aureus* bacteria and specimens were taken from females. Ten patients from the cardiac center were sampled, two males and eight females, and nine of the 10 were negative on MacConkey agar, with *Proteus* spp. bacteria being the only positive result. Eight patients from Sardam Hospital, two specimens from females and six specimens from males, five of whom were cultivated with mannitol salt agar, and all of whom had a positive result were *S. aureus* bacteria and three of them cultivated with chocolate agar, had a positive result, two of whom were *S. aureus* bacteria, and one of them was *S. epidermidis* bacteria. Nine patients from Jamhuri Hospital, specimens taken from males, cultivated mannitol salt agar, five of whom were negative, and four of whom were positive, were *S. aureus* and *S. epidermidis* bacteria [Table 2].

Out of the 14 isolated bacteria, 1 was Gram-negative bacteria (7.14%) and 13 were Gram-positive cocci (92.85%). *S. aureus* was the most common bacteria 10 isolates (71.42%). Other isolated bacteria were *Proteus* sp. (7.14%) and *S. epidermidis* (21.42%) [Figure 2].

Antimicrobial Activity of Plant Extracts andAntibacterial Susceptible of *S. aureus*

Sensitivity pattern for *S. aureus* to the following antibiotics; RF (20 mm), TE (16 mm), NET (27 mm), and the other antibiotics resistance for *S. aureus* such as C, OX, CRO, IPM, CAZ, APX, and CRO [Figure 3a and b]. The result presented in Figure 4 was *S. aureus* control bacteria. Each extract was tested against *S. aureus* isolated. The results showed cinnamon resistance to antibacterial activity against *S. aureus* [Figure 5].

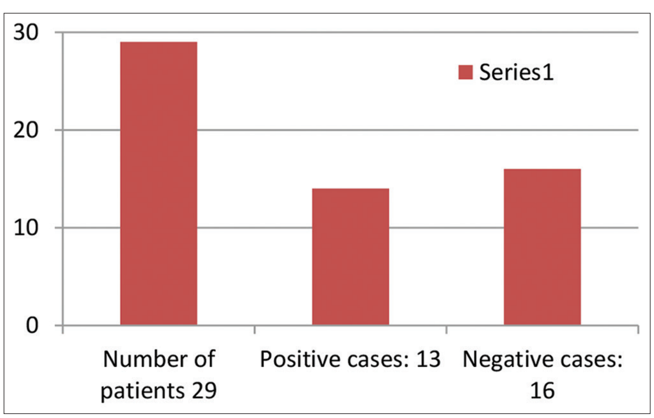

Figure 1: The prevalence rate of surgical site wound infections

Antimicrobial Activity of Plant Extracts and Antibacterial Susceptible of *S. epidermidis*

Antibacterial resistant and susceptible of *S. epidermidis* isolated from patients. The results show that *S. epidermidis* isolated from patients were susceptible to NET (34 mm) and APX (28 mm), and other antibiotic resistant for *S. epidermidis* such as TE, C, and CRO [Figure 6]. The result presented in Figure 7 exhibited *S. epidermidis* control bacteria. The results given in Figure 8 show that ginger, cinnamon, and radish root extracts are not effective against *S. epidermidis*.

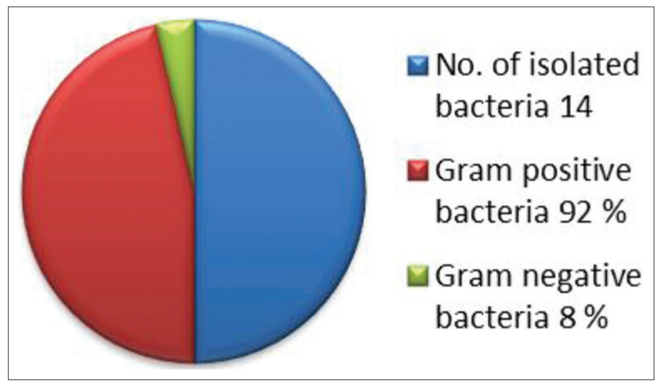

Figure 2: Number of isolated bacteria according to Gram-positive and Gram-negative bacteria

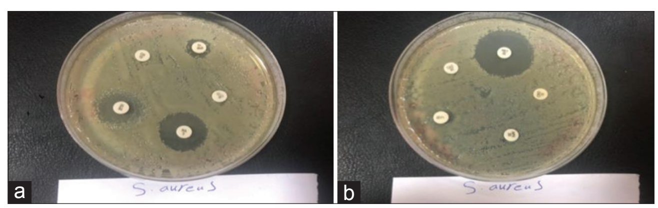

Figure 3: (a and b) Antibacterial inhibition zoneagainst *Staphylococcus aureus*

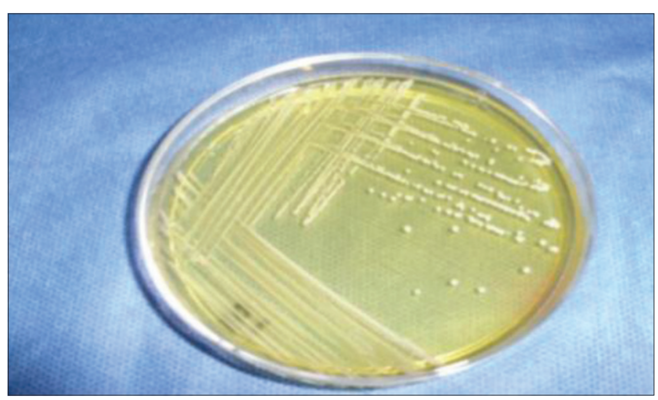

Figure 4: *Staphylococcus aureus* organism control

Table 2: The patients number, gender, isolated bacteria, media cultured from the surgical site of infection, and the name of the hospitals

Serial no. of patient	Media	Isolated bacteria	Hospitals	Sex
1	MacConkey agar	Negative (–)	Cardiac center	♀ Female
2	MacConkey agar	Negative (–)	Cardiac center	♀ Female
4	MacConkey agar	Negative (–)	Cardiac center	♂ Male
5	MacConkey agar	Positive (+) *Proteus* sp.	Cardiac center	♀ Female
6	MacConkey agar	Negative (–)	Maternity hospital	♀ Female
7	MacConkey agar	Negative (–)	Cardiac center	♀ Female
8	MacConkey agar	Negative (–)	Cardiac center	♀ Female
9	MacConkey agar	Negative (–)	Cardiac center	♀ Female
10	MacConkey agar	Negative (–)	Cardiac center	♀ Male
11	MacConkey agar	Negative (–)	Cardiac center	♀ Female
12	Mannitol salt agar	Positive (+) *S. aureus*	Maternity hospital	♀ Female
13	Mannitol salt agar	Positive (+) *S. aureus*	Sardam Hospital	♀ Female
14	Mannitol salt agar	Positive (+) *S. aureus*	Sardam Hospital	♂ Male
15	Mannitol salt agar	Positive (+) *S. aureus*	Sardam Hospital	♂ Male
16	Mannitol salt agar	Positive (+) *S. aureus*	Sardam Hospital	♂ Male
17	Mannitol salt agar	Positive (+) *S. aureus*	Sardam Hospital	♂ Male
18	Chocolate agar	Positive (+) *S. aureus*	Sardam Hospital	♂ Male
19	Chocolate agar	Positive (+) *S. aureus*	Sardam Hospital	♀ Female
20	Chocolate agar	Positive (+) *S. epidermidis*	Sardam Hospital	♂ Male
21	Mannitol salt agar	Positive (+) *S. aureus*	Jamhuri Hospital	♂ Male
22	Mannitol salt agar	Positive (+) *S. epidermidis*	Jamhuri Hospital	♂ Male
23	Mannitol salt agar	Negative (–)	Jamhuri Hospital	♂ Male
24	Mannitol salt agar	Negative (–)	Jamhuri Hospital	♂ Male
25	Mannitol salt agar	Positive (+) *S. aureus*	Jamhuri Hospital	♂ Male
26	Mannitol salt agar	Positive (+) *S. epidermidis*	Jamhuri Hospital	♂ Male
27	Mannitol salt agar	Negative (–)	Jamhuri Hospital	♂ Male
28	Mannitol salt agar	Negative (–)	Jamhuri Hospital	♂ Male
29	Mannitol salt agar	Negative (–)	Jamhuri Hospital	♂ Male

S. aureus: Staphylococcus aureus, S. epidermidis: Staphylococcus epidermidis

Antimicrobial Activity of Plant Extracts and Antimicrobial Susceptible of *Proteus* spp.

The results show that *Proteus* spp. isolated patients were susceptible to NET (17 mm), RF (19 mm), AK (25 mm), TE (26 mm), and CN (20 mm). Moreover, other antimicrobial is resistant to *Proteus* spp. OX, APX, CAZ, and AX [Figure 9a-c].

Results given in Figure 10 showed that ginger and radish root extract are not effective against *Proteus* species. The results showed that cinnamon had very little (5 mm) effect on *Proteus* species [Figure 10].

DISCUSSION

Surgical site infection is a prevalent kind of disease related to health-care research conducted by the way.[20-22] SSIs

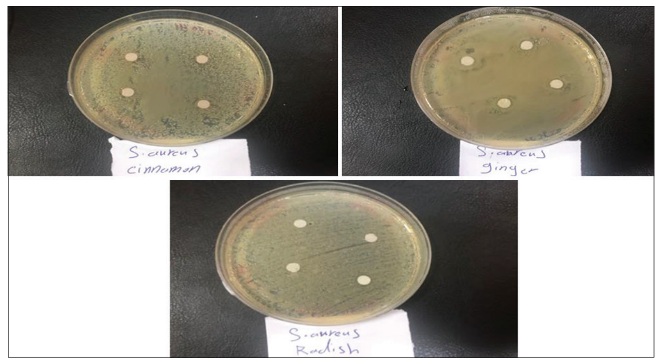

Figure 5: Inhibition zone of plants against *Staphylococcus aureus*

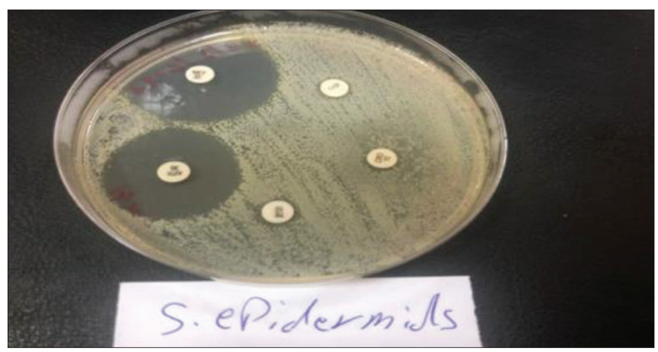

Figure 6: Antibacterial inhibition zone against *Staphylococcus epidermidis*

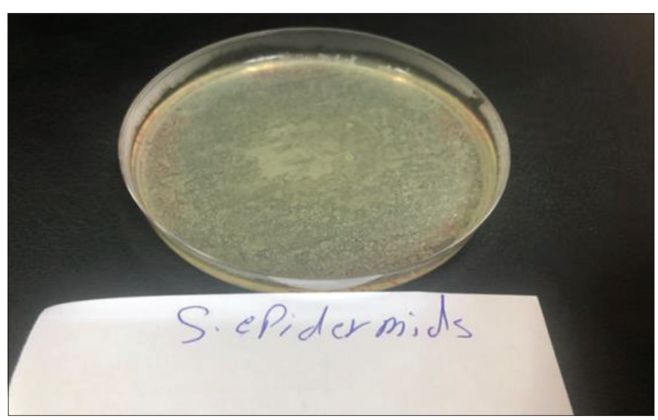

Figure 7: *Staphylococcus epidermidis* bacteria control

(infections at surgical site) were found to be 48.27 percent prevalent in the present specimens, with *S. aureus* and *S. epidermidis* being the most common etiological agents among Gram-positive bacteria. Gram-positive bacteria while the Gram-negative bacteria were Proteus spp. respectively.[23]

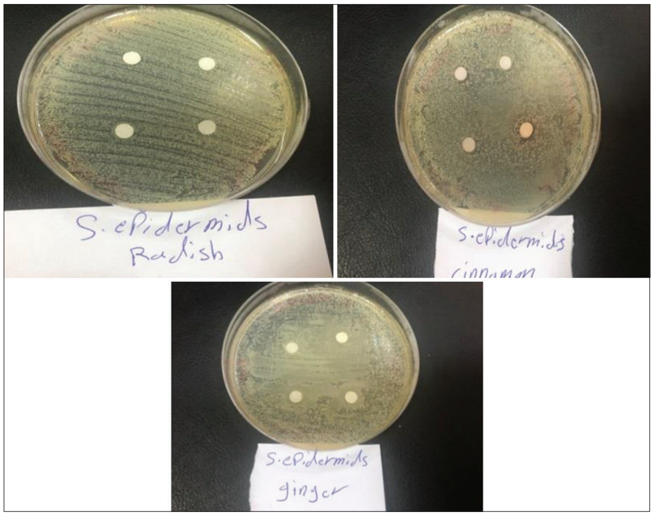

Figure 8: Antimicrobial disk diffusion test against *Staphylococcus epidermidis*

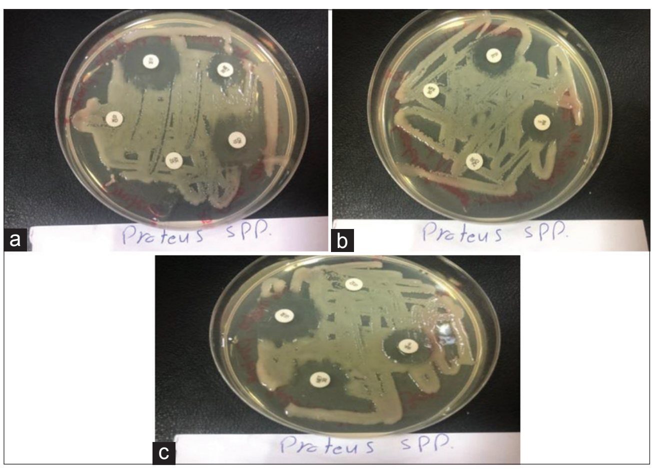

Figure 9: (a-c) Antimicrobial inhibition zone on *Proteus* spp.

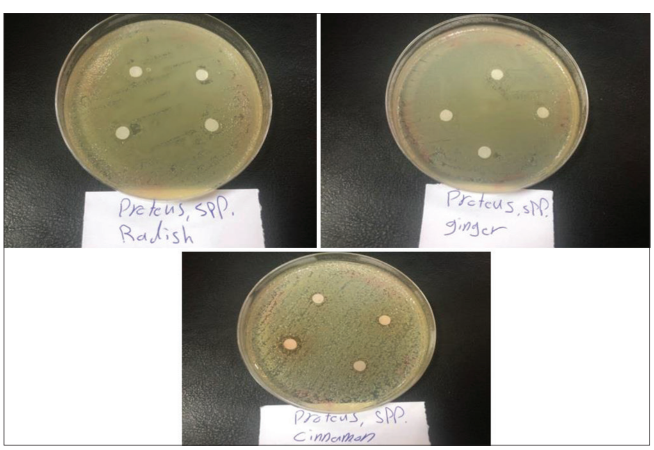

Figure 10: Antimicrobial disk diffusion test against *Proteus* spp.

These findings support that previous research *S. aureus* was the most common bacteria found in post-operative wound infections.[24,25] *S. aureus* was found in 71.42% of surgical site infections. The most common Gram-positive *S. aureus* isolate (71.42%) followed by *S. epidermidis* (21.42%) and *Proteus* spp. (7.14 percent of the total). In the current study, a shorter post-operative stay was seen in patients with acquired infection, who had a roughly triple longer patient without operational site infection. In hospitals, neonatal infections, operative care for the elderly and malnutrition, and individuals with diabetes and other chronic diseases, *S. aureus* is an important source of infection.[26] The minimal number of *S. aureus* sensitivity found in the results of this investigation against TE and C was compatible with studies published in Eritrea by certain Nigerian employees.[27,28] Sensitivity to RF, TE, NET, and the other antibiotics resistance for *S. aureus* such as C, OX, CRO, IPM, CAZ, APX, and CRO did not match the findings of Chowdhury *et al.*,[29] however, resistance to other antibiotics did. CAZ and CIP were consistent with the report published by the commission.[29] Moreover, *S. aureus* was sensitive to TE and resistance to CRO and C, which is not in agreement with the report published by the commission.[29] Pattern of antibiotic resistance of *S. epidermidis* isolated from hospital infectious specimens *S. epidermidis* resistance against: C, CRO, and TE were consistent with reports published by Chabi and Momtaz,[30] Wijesooriya *et al.*,[31] and *S. epidermidis* sensitive to APX and NET.[32] Results show that *Proteus* spp. isolated from patients were susceptible to NET, RF, AK, TE, and CN and resistant to *Proteus* spp.;OX, APX, CAZ, and AX.[33] Current results showed that cinnamon, radish, and ginger were resistant to *S. aureus*. When opposed to the previous findings,[18,34] the present findings show a higher level of activity. *Raphanus* extract had the best activity against a multiresistant strain of *P. aeruginosa,* while *P. mirabilis* was the most resistant to the extract's influence.[35] *Piper nigrum* and *Zingiber officinale* are active in *S. aureus* and *K. pneumoniae*. Cinnamon essential oil shows maximum activity against *E. coli* and *S. aureus.*[36] The results show that ginger extract and root radish extract are not effective against *S. epidermidis*.[37] The current results showed that cinnamon and ginger extract are not effective against *Proteus* species. The different concentrations of radish root extract had no effect on *Proteus* spp.[18] The antibacterial action of cinnamon was ascribed to the existence or long-term storage of specific active components which have been declined by melting and exposure to air.[38] The negative result of cultured pus specimens may not have been effective due to the transfer of the swabs, and the bacteria may have been dead. It could be the species of bacteria that may not grow in these media. They need special media or anaerobic bacteria could not grow aerobically in the 37°C incubator.

CONCLUSION

The findings of this research will aid in our understanding of the microbiological etiology of SSIs in our hospitals, which could have epidemiological and therapeutic implications. *S. aureus* was the most common surgical site infection we found (71.42%). This study demonstrates that RF, TE, and NET are sensitive to *S. aureus,* and NET and APX are sensitive to *S. epidermidis* and NET, RF, TE, AK, and CN are affective against *Proteus spp.* and C, OX, CIP, IPM, CAZ, APX, and CRO are resistant to *S. aureus*. TE, C, and CRO are resistant to *S. epidermidis*. OX, APX, CAZ, and AX are resistant against *Proteus* spp. Plant extracts have no antimicrobial activity against *S. aureus*, *S. epidermidis*, or *Proteus* ssp., which typically cause surgical wound infections, according to the findings of this report. The findings showed that cinnamon, radish root, and ginger are ineffective at inhibiting the growth of wound pathogenic bacteria.

RECOMMENDATION

The findings of this study can be used to launch an effort to strengthen hospital antimicrobial policy and antimicrobial prescribing guidelines. It is difficult to choose the best drug for any condition, particularly in infectious diseases. Since it is performed *in vitro*, the susceptibility pattern cannot be used as the sole criterion because it ignores the patient's immunological status and clinical condition. This research may be useful in determining which plant extracts to use for further isolation of constituents responsible for the studied species' behavior.

ACKNOWLEDGMENTS

I'd want to offer my heartfelt gratitude to Dr. Raid Duriad Thanoon, the research supervisor, for assisting me through each step of my research paper it would have been impossible to write such a significant and fascinating work without his help. And a special thanks to Ahmed Majeed Mohammed, who inspired us to achieve many goals during this research. Many thanks to biolaboratory for their help and guidance. Finally, many thanks to all the other people and teachers for their help and advice.

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