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Exploring the Impact of Antiseptics on Skin Microbiota in Surgical Settings

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Abstract: Antiseptics are commonly used in surgical settings to reduce the risk of infections. However, there is growing concern about the potential impact of antiseptics on the skin microbiota, which plays a crucial role in maintaining skin health and preventing infections. This study aims to explore the effects of antiseptics on the skin microbiota in surgical settings. Swabs were taken from 340 different sites of skin divided into four groups including; group 1 included160 swabs, group 2 included 50 swabs, group 3 included 100 swabs, group 4 included 30 swabs, taken from patients' skin before surgery at the site of cesarean incision from several positions before and after sterilization with 10% povidone-iodine and with 10% povidone-iodine mixed with 70% ethanol, and from infected surgical sites. The bacterial isolates were identified by phenotypic and biochemical tests, as well as VITEK-2 assay. *Staphylococcus epidermidis* was the prevalent bacteria isolated from skin sample sources in group 1, 2, and 3 with a total rate 81% followed by *Staphylococcus aureus* which was dominant in group 4 that included surgical site infection swabs. In addition, other bacteria species were isolated from different skin sites such as *Staphylococcus haemolyticus*, *Kocuria kristinae*, *Enterobacter cloacae*, *Aerococcus viridans*, *Pantoea*, and *Burkholderia cepacian*. Also, the study included the samples of skin microbiota will be collected from surgical staff and patients before and after exposure to antiseptics. Next-generation sequencing techniques will be employed to analyze changes in the composition and diversity of the skin microbiota. The findings of this study were provided valuable insights into the impact of antiseptics on the skin microbiota in surgical settings. This information can help healthcare professionals make informed decisions about the use of antiseptics and develop strategies to preserve the skin microbiota while effectively preventing infections during surgical procedures.

Keywords: Postoperative infections, Skin microbiota, Antiseptics.

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Introduction

The skin is considered as a first line of defense in human body and no microorganism is able to break through undamaged skin. Microflora near or at the surgical wound is the hidden reason of surgical site infection. Commensal skin microflora consists of many microbes with low pathogenicity such as coagulase negative *staphylococci* but also include sometimes pathogenic strains such as coagulase positive *Staphylococcus aureus*. The number of microorganisms on the skin may be decreased by using appropriate antiseptics limiting the risk of infection. However, using best antisepsis may fail to destroy the entire skin microflora as 20% of these microbes subsist underneath the surface, around pilous follicles or in sebaceous glands (1). Surgical infection occurs when a wound

is contaminated with a bacterium. The microorganism can be passed from nurses' hand or surgeons by contact, the bacterium could be airborne throughout surgery, and the patient may get the microorganism after surgery through contact with unclean beds, clothes or even contaminated dressing (2). Cesarean section is the frequent surgical procedure around the world that led to complications in some conditions.

Complications that may follow caesarean section differ depending on several circumstances such as age, obesity, and health status. Wound infection is one of the common complications after cesarean surgery(3). Proper preparation of skin before an operation is necessary to prevent surgical wound contamination. Several agents are obtainable, each that have a particular application guidance. The misuse of these agents effects the decrease of microbial load. Differential sterilization strategies in operating rooms to reduce surgical site infection among healthcare providers are typical and may highly affect the occurrence of these infections (4).

Post operative infections may take place as primary wound infections after a surgical operation from sources in the ward or as a secondary wound infection because of some other problems. Surgical site infection can be caused by endogenous or exogenous microbes; endogenous microorganisms cause most of the SSIs as they appear on the person's skin when the surgical incision is made. Gram-positive bacteria, as an example, *Staphylococcus aureus* is the frequent dominant causative skin-residence microbe (5).

Because of the importance of this topic, the current study came to reveal the extent of the best types of antiseptics used on skin before cesarean section to reduce bacterial load on skin that may somehow limit post operative infections. The findings of this study will provide valuable insights into the impact of antiseptics on the skin microbiota in surgical settings. This information can help healthcare professionals make informed decisions about the use of antiseptics and develop strategies to preserve the skin microbiota while effectively preventing infections during surgical procedures.

Material and methods Sample collection

This study included 100 woman who attended Al-Elwiya educational maternity hospital in Baghdad/Iraq during a period between August to November 2022, their ages ranged between (19) to (53) years, 340 swab samples were collected from different sites of patients' skin before and after sterilization during caesarean section, and surgical site infection. The individuals have been classified into four groups according to the swab sites that are taken from and type of sterilization as listed in Table (1). Amie's transport medium was used to transport the samples to laboratory for isolation and identification.

Isolation and Identification of bacteria

Bacteria were isolated and identified by using standard bacteriological techniques (6). Species were identified according to the morphological features on culture media, microscopic examination, and biochemical tests (7). VITEK-2 was used as a confirmed test for the automated identification of isolates.

Table (1): Types and number of swabs distributed according to the groups under study.

Tested groups	General Description	Patients No.	Type of sample source (No.)	No. of swabs for each group (Total No.)
G1*	Skin sterilization for patients under surgery with iodine 10% (Original method in hospital)	40	Skin after sterilization- skin before suture- first stitch- final stitch	160 (4 swabs for each patient)
G2⁂	Skin sterilization for patients under surgery with iodine 10% (Original method in hospital)	10	Skin before sterilization- skin after sterilization- skin before suture- first stitch- final stitch	50 (5 swabs for each patient)
G3	Skin sterilization for patients under surgery with iodine 10% + ethanol 70% (Modified method)	20	Skin before sterilization- skin after sterilization- skin before suture- first stitch- final stich	100 (5 swabs for each patient)
G4	Patients with postoperative infection	30	Infection site after cesarean surgery	30
Total		**100**		**340**

***The difference between G1 and G2 is in the sample sources type.⁂The difference between G2 and G3 is in the antimicrobial agent.**

Biofilm formation detection on Tissue Culture Plate (TCP)

Biofilm formation test was done by Tissue Culture Plate method (TCP) that designated by (8) as follows:

- Overnight Brain Heart Infusion (BHI) agar cultures were used to prepare bacterial suspensions.
- Bacterial suspension (20 µl) equivalent to 0.5 McFarland standard used as inoculation to inoculate 96-well flat-bottomed polystyrene tissue culture plate containing 180µl of BHI broth with 2% sucrose, triplicate was prepared for each species.
- The microtiter plate was covered with a lid and sealed with Para film during incubation at 37°C for 24 hrs.
- The content of each well was removed after incubation; each well was washed three times carefully with PBS (pH 7.2) and left to dry.
- After drying at room temperature for 15 min, adherent bacteria were fixed with 200µl of 99% ethanol per well for 15 min. The plates were decanted and allowed to dry.
- Crystal violate (1%) was added to the wells for 15 min. Then the solution was removed from wells, washed three times with PBS (pH 7.2) for removal of unbounded dye and allowed to dry at room temperature.
- An amount of 200µl ethanol was used to re-solubilize bounded dye to the adherent cells. The plates were decanted and allowed to dry.
- Finally, the absorbance (A^o) of each well was measured at 630nm using Enzyme linked immuno sorbent assay (ELISA) reader and the absorbance (A^o) value for control well was deducted.

The absorbance test value was performed in triplicate and repeated three times.

The tested species adherence capabilities were classified into four categories; above the mean A^o of the negative control which contained broth only was considered as the cut-off Ac^o.

For easier explanation of the results, the species were divided into the following categories:

1) Non biofilm producer $A^o \geq A^oc$

2) Weak biofilm producer $A^oc > A^o \geq 2 \times A^oc$

3) Moderate biofilm producer $2\times A^oc > A^o \geq 4 \times A^oc$

4) Strong biofilm producer $4 \times A^oc > A^o$

Antibiotics susceptibility test by disk diffusion method

The antibiotics susceptibility test was carried out by following Kirby Bauer method described by CLSI, 2021. Out of a pure and fresh culture, few colonies were transferred to a sterile test tube containing 5 ml of normal saline. Then it was compared with 0.5 McFarland standards (1.5x10 CFU/ml). A portion of bacterial suspension was carefully transferred by use of a sterile cotton swab and spread evenly on Mueller-Hinton agar medium. After that, plates were left to dry. Antibiotic discs were placed on the inoculated plate by use of a sterile forceps. The plates were inverted and incubated at 37 ºC for 18-24 hrs. After incubation, a metric ruler was used for measuring the inhibition zones around the discs. The results were estimated as Susceptible (S), Intermediate (I) and Resistant (R) by their relation to the universal antibiotics manual.

Macrolide phenotypic resistance was determined by the agar diffusion disk assay using antibiotic discs (erythromycin, clindamycin) on Mueller–Hinton agar medium according to the European Committee on Antimicrobial Susceptibility Testing recommendations. Inducible Clindamycin resistance was detected phenotypically by an inhibition zone between the erythromycin disks and clindamycin disks indicating a positive D-test (9).

Synergistic interaction between antibiotics was also detected on Muller Hinton agar, as one agent enhance the effect of the other by showing a combination zone between two antibiotics or more on the agar (10).

Antiseptic susceptibility test by agar well diffusion

Disinfectants are chemical products placed on the skin to decrease the bacterial load and the chance of postoperative infections. Each antiseptic has a spectrum of microbes targeted, a certain effective technique, and an undesirable effect incident that the surgeon should consider before choosing the agent for the operation. Antiseptics that cover most pathogens are broad-spectrum therefore; they are the most prevalent used in dermatologic surgery (11).

Agar well diffusion method was widely used to evaluate the antimicrobial activity of disinfectants as described by (12). A pilot study was conducted to select and compare the proper concentration of Povidone-Iodine in vitro. The experiment was conducted on 18 isolates chosen randomly from different isolation skin sites of the four groups.

The following steps were taken:

1. The inoculum used was prepared using a 24-hour bacterial culture. A suspension was made in a sterile saline solution (0.85%). The turbidity of the suspension was adjusted to match that of a 0.5 McFarland standard (1.5x10 CFU/ml).
2. A portion of bacterial suspension was carefully transferred by use of a sterile cotton swab and spread on 18-20 ml of Mueller-Hinton agar medium plates.
3. Then, 4 mm diameter wells were cut out of the agar surface and 50 µl of PVP-I in different concentration: 10%, 10.5%, 11%, 11.5%, and 12% were added to each well as a first step to detect the activity of different concentrations of the

antiseptic. It should be noted that the concentration of 10% is the concentration used in sterilization of surgical operations. Preparation of PVP-I concentrations are listed in Table (2).

4. Also, a well was filled with 50 µl normal saline as negative control and an antibiotic disk was applied as positive control.
5. After 24 hrs incubation at 37◦C, zones of inhibition were measured using a metric ruler and recorded. Three replicates were made for each isolate.

Table (2): Preparation of Povidone – Iodine concentrations.

Povidone-iodine (gram)	Distilled water (milliliter)	Final concentration of PVP-I (5ml)
0.5	4.5	10%
0.525	4.475	10.5%
0.55	4.45	11%
0.575	4.425	11.5%
0.6	4.4	12%

The main study

This step was done to detect the effect of 10% PVP-I mixed with 70% ethanol on bacterial species in vitro and comparing it with the in vivo study. Depending on the Pilot study, the concentration of PVP-I that was proven to be the optimal concentration for skin sterilization was chosen among the concentrations tested above to complete the sensitivity test of selected bacterial isolates, which included 25 isolates of Staphylococcus epidermidis and 25 isolates of *Staphylococcus aureus* (5 isolates of each species from 5 different isolation sources). The same procedure was done as mentioned in the pilot study but the prepared wells were filled with 50 µl of the optimal PVP-I concentration from the pilot study, 70% ethanol, 10% PVP-I mixed with 70% ethanol, and normal saline as negative control. Also, antibiotic disks were applied as positive control.

Statistical analysis

For the purpose of studying the significance level, or P value, between the different factors that were included in the study, the percentage and chi-square were calculated. Some obtained data were subjected to T test to compare various groups with each other. Results were expressed as mean ± standard deviation (SD) and values of $p>0.05$ were considered statically non-significant while $p\leq0.05$ considered significantly different the analysis of contingency tables, the statistical analysis was carried out by SPSS (v 20).

Results and discussion

Bacterial isolation and identification

The skin is considered a habitat for lots of microorganisms in which they vary at different skin sites, environmental factors, different ages, and gender. Any change in the microbiota of human skin stimulates the immune system therefore, it is important to know the occurrence and characteristics of skin microbes for appropriate treatment of skin disease (13). The total number of positive growths in all tested groups as a result in this study was 245 (72%) out of 340 from different samples taken from surgical sites of patient's skin. The current study showed a clear variation in the numbers of positive bacterial cultures with some mixed cultures, as

well as variation in bacterial species such as *Staphylococcus epidermidis*, *Staphylococcus lugdunensis*, *Staphylococcus haemolyticus*, *Kocuria kristinae*, and uncommon species such as *Aerococcus viridans*, *Pantoea spp*, and *Enterobacter cloacae* according to the different study groups, as well as the different isolation sites for each group, as follows: In G1/40 patients, 160 swabs, sterilization with 10% povidone-iodine; G2/10 patients, 50 swabs, sterilization with 10% povidone-iodine; G3/20 patient, 100 swabs, sterilization with 10% povidone-iodine with 70% ethanol; G4/30 patient, 30 swabs from surgical site infection, total positive growth was 100 out of 160, 48 out of 50, 70 out of 100, 27 out of 30 respectively. Taking into consideration, group 1 samples from Skin before sterilization were not taken in order to indicate the effect of antiseptic. The result of positive growth from skin samples taken after sterilization, skin edges before suture, first stitch, and final stitch were in G1; (17 (10.6%), 24 (15%), 30 (18.7%), 29 (18.2%), G2; (10 (20%), 10 (20%), 8 (16%), 10 (20%)), G3;(6 (6%), 16 (16%), 14 (14%), 14(14%)) respectively as listed in Table (3). In group 2 and 3, the result was 100% of bacterial growth from skin before sterilization due to the existence of skin microbiota or contamination with pathogenic microbes from the environment (14). Similar to the results of current study, a study by Cuchí, *et al*. (15) reported that 74 % of skin swabs were positive growth for bacteria while 26% were negative. Scharschmidt (16) mentioned that the number of bacteria reside mucosal and skin surfaces surpass the number of cells composing human body, and are able to stimulate adaptive and innate immunity.

Table (3): Numbers and percentages of positive and negative growth for all groups under study.

Sample source / Tested groups	Positive growth samples from Skin before sterilization No. (%)	Positive growth samples from Skin after sterilization No. (%)	Positive growth samples from Skin before suture No. (%)	Positive growth samples from First stitch No. (%)	Positive growth samples from Final stitch No. (%)	Positive growth samples from Infected surgical site No. (%)	Total No. of positive growth No. (%)	Total No. of Negative growth No. (%)
G1/40 patient (160 swab)	-	17 (10.6%)	24 (15%)	30 (18.7%)	29 (18.2%)	–	100 (62.5%)	60 (37.5%)
G2/10 Patient (50 swab)	10 (20%)	10 (20%)	10 (20%)	8 (16%)	10 (20%)	–	48 (96 %)	2 (4 %)
G3/20 patient (100 swab)	20 (20%)	6 (6%)	16 (16%)	14 (14%)	14 (14%)	–	70 (70 %)	30 (30 %)
G4/30 patient (30 swab)	–	–	–	–	–	27 (90%)	27 (90 %)	3 (10 %)
***Total and % within the group**	30 (12.2)	33 (13.5)	50 (20.4)	52 (21.2)	53 (21.6)	27 (11.1)	245 (72%)	95 (28 %)
Percentage between the groups 340 swabs	30 (8.8)	33 (9.7)	50 (14.7)	52 (15.3)	53 (15.6)	27 (7.9)	245 (72%)	95 (28 %)

***The percentage was calculated from total positive cases not from total sample number**
Note: all swabs taken from surgical instruments and medical supplies and 10% povidone- iodine opened bottles were negative for bacterial growth

In group 1 and 2, sterilization with 10% povidone-iodine showed little decrease in bacterial load with a total positive growth 100 (62.5%) and 48 (96%) respectively. While in group 3, sterilization that was used on skin before surgery in the operating room was modified from 10% PVP-I alone to 10% PVP-I mixed with 70% ethanol showing a better result in eradication of bacteria with a total positive growth 70 (70%) as compared with group 2. Despite that, positive culture became higher during wound closure at the wound edges and after suturing as shown in Figure (1). This result was supported by previous studies mentioning that at the end of surgery and following wound closure, the edges of the incision had a very high bacterial load compared to the skin area after sterilization (17). Furthermore, Surgical sutures may cause body response and form inflammation, also can play a role in SSI. It is recommended by CDC and WHO to use antimicrobial coated sutures (18, 19).

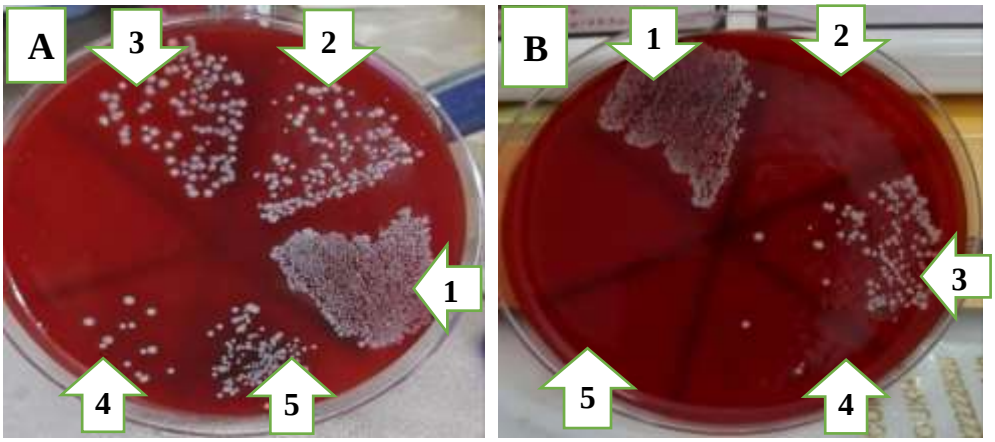

Figure (1): Positive growth on blood agar compering the use of two different types of antiseptics in vivo (A) group 2- 10% povidone-iodine (B) group 3- 10% povidone-iodine mixed with 70% ethanol. Skin sample sites in both groups; (1) skin before sterilization (2) skin after sterilization (3) skin edge before suture (4) first stitch (5) final stitch.

The use of a mixture of antiseptics usually gives more positive results in reducing microbes on the surface of the skin, and from studies close to the current study, it was indicated by Yoshii, *et al*. (20) that no different between applying povidone -iodine or chlorohexidine gluconate (CHG)-ethanol after skin pre-operation, also positive growth increased after surgical site closure from 4.2% to 8.4%. While Dörfel, *et al*. (21) revealed the reduction of aerobic and anaerobic skin flora by PVP-I-alcohol after 2-3min of application better than CHG-alcohol, mentioning that all coagulase-negative *Staphylococcus* bacteria (CoNS) were eliminated.

Techniques of application may influence antiseptic activity such as back and forth rubbing that has shown greater influence than concentric circle (22). Some antiseptics have a bactericidal activity, other are bacteriostatic and it is important to take into consideration bacterial antiseptic resistance that is a problem in sterilization leading to SSI (23).

Cesarean section may be accompanied with many postoperative complications, and SSI is one of them as a result of external of internal factors, and the detection of the causative pathogen in some cases is indistinct due to the type of pathogen or inappropriate sample taken from the infection site. Also, antibiotic prophylaxis plays a role in reducing the number of bacteria giving a false culture (24).

As revealed in the results, bacterial species isolated from surgical site infections somewhere differ from the species isolated from different skin sites, indicating that the infection may not be caused from skin microbiota in some cases otherwise, it may be a result of unappropriated disinfecting and dressing or unhygienic life style of the patient.

Results of bacterial biofilm formation

Bacterial biofilms are defined as communities of surface attached bacteria as a natural mode of microbial growth. Biofilm infections take part in up to 80% of human microbial infections and the diagnosis of infections caused by biofilms is determined by microbial composition in biofilms but still the prevention of a biofilm is a challenge(25). The results referred that each isolate showed a different potency to form biofilm under the same conditions of the test and most tested isolates were able to form biofilm on TCP, to some degree as shown in Figure (2).

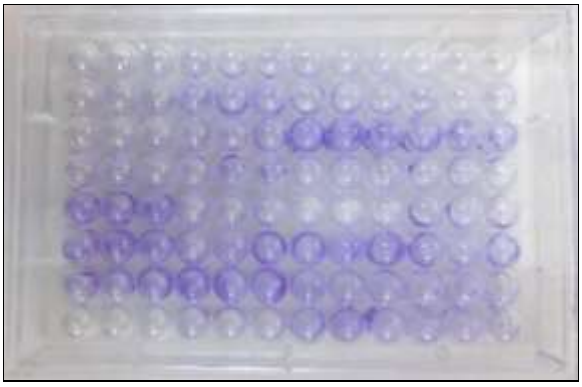

Figure (2): Biofilm formation on Tissue culture plate.

Results of bacterial biofilm formation in group 1

The current study showed that *S. epidermidis* in group 1 had the highest moderate biofilm production with 42 out of 87 isolates, mentioning that the highest moderate isolates were found in samples taken from skin before suture and first stitch, while 11, 17, and 17 isolates had non, weak, and strong production respectively, followed by *S. aureus* isolates expressing 8 out of 14 moderate producers with 2 weak and 4 strong biofilm producers, whereas the 5 isolates of *S. lugdunensis* had only 1 weak producer from skin before suture and 4 moderate biofilm producers isolated from first and final stitch samples, while the 3 isolates of *S. haemolyticus* showed moderate biofilm production. On the other hand, 1 isolate of *K. kristinae* showed non and 1 weak production. Results are listed in Table (4).

Table (4): Biofilm profile results of bacterial species isolated from study group 1.

Sample source / Bacterial isolate	Skin after sterilization *N (n-w-m-s)	Skin before suture N (n-w-m-s)	First stitch N (n-w-m-s)	Final stitch N (n-w-m-s)	Total N0. N (n-w-m-s)
Staphylococcus epidermidis	15 (1-3-9-2)	23 (3-4-12-4)	24 (3-5-13-3)	25 (4-5-8-8)	87 (11-17-42-17)
Staphylococcus lugdunensis	0	1 (0-1-0-0)	2 (0-0-2-0)	2 (0-0-2-0)	5 (0-1-4-0)
Staphylococcus haemolyticus	1 (0-0-1-0)	0	0	2 (0-0-2-0)	3 (0-0-3-0)
Staphylococcus aureus	2 (0-0-2-0)	4 (0-1-2-1)	4 (0-1-2-1)	4 (0-0-2-2)	14 (0-2-8-4)
Kocuria kristinae	0	0	2 (1-1-0-0)	0	2 (1-1-0-0)
Total	18 (1-3-12-2)	28 (3-6-14-5)	32 (4-7-17-4)	33 (4-5-14-10)	111 (12-21-57-21)

*N=Number / (n-w-m-s) =(non-weak-moderate-strong)

Results of bacterial biofilm formation in group 2

Results of group 2 as shown in Table (5) revealed that S. *epidermidis* had the highest moderate biofilm production with 25 out of 46 isolates, including 5 in each sample source, while 2, 11, and 8 isolates had non, weak, and strong production respectively, followed by *S. aureus* isolates expressing 3 out of 6 moderate producers with 2 weak and 1 non biofilm producer, whereas 1 non biofilm formatting isolate and 1weak were found in each of *K. kristinae* and *E. cloacae*.

Table (5): Biofilm profile results of bacterial species isolated from study group 2

Sample source / Bacterial isolate	Skin before sterilization *N (n-w-m-s)	Skin after sterilization N (n-w-m-s)	Skin before suture N (n-w-m-s)	First stitch N (n-w-m-s)	Final stitch N (n-w-m-s)	Total N0. N (n-w-m-s)
Staphylococcus epidermidis	10 (1-2-5-2)	10 (0-2-5-3)	10 (0-2-5-3)	8 (1-2-5-0)	8 (0-3-5-0)	46 (2-11-25-8)
Staphylococcus aureus	0	2 (0-1-1-0)	2 (1-0-1-0)	0	2 (0-1-1-0)	6 (1-2-3-0)
Kocuria kristinae	2 (1-1-0-0)	0	0	0	0	2 (1-1-0-0)
Enterobacter cloacae	2 (1-1-0-0)	0	0	0	0	2 (1-1-0-0)
Total	14 (3-4-5-2)	12 (0-3-6-3)	12 (1-2-6-3)	8 (1-2-5-0)	10 (0-4-6-0)	56 (5-15-28-8)

*N=Number / (n-w-m-s) =(non-weak-moderate-strong)

Results of bacterial biofilm formation in group 3

In this group, *S. epidermidis* had the highest moderate biofilm production with 25 out of 65 isolates, while 8, 16, and 16 isolates had non, weak, and strong production respectively, while the 4 isolates of *S. aureus* expressed 2 for each moderate and strong biofilm production, and the 4 isolates of *A. viridans* expressed 2 for each weak and moderate biofilm production, on the other hand, the 2 isolates of each *K. pneumonia* and *P. aeruginosa* showed moderate biofilm production while *K. kristinae* and *Pantoea spp.* showed weak biofilm formation as listed in Table (6).

Table (6): Biofilm profile results of bacterial species isolated from study group 3.

Sample source / Bacterial isolates	Skin before sterilization *N (n-w-m-s)	Skin after sterilization N (n-w-m-s)	Skin before suture N(n-w-m-s)	First stitch N (n-w-m-s)	Final stitch N (n-w-m-s)	Total N0. N(n-w-m-s)
Staphylococcus epidermidis	17 (2-5-7-3)	4 (0-0-2-2)	16(3-6-5-2)	14 (1-3-6-4)	14 (2-2-5-5)	65 (8-16-25-16)
Staphylococcus aureus	3 (0-1-2-0)	1 (0-0-1-0)	0	0	0	4 (0-1-3-0)
Aerococcus viridans	2 (0-1-1-0)	2 (0-1-1-0)	0	0	0	4 (0-2-2-0)
klebsiella pneumoniae	2 (0-0-2-0)	0	0	0	0	2(0-0-2-0)
Kocuria kristinae	1 (0-1-0-0)	0	0	0	0	1(0-1-0-0)
Pantoea spp	1 (0-1-0-0)	0	0	0	0	1(0-1-0-0)
Pseudomonas aeruginosa	2 (0-0-2-0)	0	0	0	0	2(0-0-2-0)
Total	28 (2-9-14-3)	7 (0-1-4-2)	16(3-6-5-2)	14 (1-3-6-4)	14 (2-2-5-5)	79 (8-21-34-16)

*N=Number / (n-w-m-s) =(non-weak-moderate-strong)

Results of bacterial biofilm formation in group 4

Regarding to group 4, the prevalent species was *S. aureus* with 23 isolates (as mentioned previously in Table 3-6), having 1 non, 4 weak, 13 moderate, and 5 strong biofilm producing isolates, moreover, *A. baumannii* and *S. haemolyticus* were moderate biofilm producers, while B. cepacia, E. coli, and kocuria species had weak biofilm production finally, *A. veronii* showed no formation of biofilm on TCP. Results are listed in Table (7).

Table (7): Biofilm profile results of bacterial species isolated from study group 4.

Sample source / Bacterial isolate	Surgical site infection N (n-w-m-s)
Staphylococcus aureus	23 (1-4-13-5)
Acinetobacter baumannii	3 (0-0-3-0)
Burkholderia cepacia	2 (0-2-0-0)
Escherichia coli	2 (0-2-0-0)
Kocuria kristinae	2 (0-2-0-0)
Kocuria rosea	2 (0-2-0-0)
Staphylococcus haemolyticus	2 (0-0-2-0)
Aeromonas veronii	1 (1-0-0-0)
Total	37 (2-12-18-5)

*N=Number - (n-w-m-s) =(non-weak-moderate-strong)

The current study observed high prevalence of *S. epidermidis* biofilm producing isolates in healthy skin from patients before and during surgery. Most isolates of *S. epidermidis* were moderate biofilm producers and that agreed with results of (26) in Iraq who found 42% moderate producing isolates. Also, a study in India by (27) reported 79% of moderate producing isolates.

While most studies such as a study done by (28) found no biofilm producing isolates of *S. epidermidis* from healthy skin. This may be due to environmental factors or climatic conditions that may have a role in the attitude of normal flora and biofilm production, conducting that it may be critical because wound healing can be reduced in the existence of *Staphylococci* biofilm (27). Most individuals lose 8 gram of skin daily by shedding with about 30 microbiota bacteria resulting in reduction to proliferate and form biofilm. Biofilm formation by the skin's innate microbes can be useful for the prevention of exogenous bacteria attaching and skin infection but on the other hand it can be critical to infections in another part of the body (29).

The results of the biofilm test showed that most of *S. aureus* isolates were moderate biofilm producers and these results agreed with (30), also (31) reported 56% moderate and 23% strong biofilm producing isolates of *S. aureus* from skin infections. While most isolates from post-surgical infections found by (32) in Iraq were strong biofilm producers. *S. aureus* has the ability to adhere and form biofilm on tissue as an opportunistic pathogen with the involvement of several surface proteins causing the delay of healing, also enhance antibiotic resistance (33).

(34) reported similar results of *S. lugdunensis* and *S. haemolyticus* biofilm producing isolates. A study by (35) showed somehow similar results as current study including *A. baumannii* with 33% strong and 6.6% moderate biofilm producers, *P. aeruginosa* with 15.4% moderate and 7.7% weak biofilm producers, *K. pneumonia* with 9.1% moderate and 18.2% weak biofilm producers, and non-biofilm producers by *B. cepacia*. Furthermore, a study in Iraq by (36) agreed with the results of *K. rosea* by producing biofilm but disagreed with *S. epidermidis*, *S. haemolyticus*, *S. lugdunensis*, and *A. viridans* by reporting them non biofilm producers. *Aeromonas* biofilm formation is related to modification to different environmental stress and pathogenesis (37). The variation in biofilm producing isolates may be influenced by temperature and seasons, presence of nutrient and oxygen gradients, antibiotic resistance, and also quorum sensing.

Results of Antibiotic resistance pattern

The problem of bacterial antibiotic resistance is increasing due to the misuse of appropriate antibiotics (38). *In vitro*, antibiotic susceptibility test was applied for all 283 isolates divided in their groups in the present study by using Kirby-Bauer method relied on measuring the diameter of the inhibition zone, and comparing it with Clinical and Laboratory Standards Institute (CLSI, 2021) as susceptible (S) and resistant (R), towards (13) antimicrobial agents that categorized into ten classes: Carbapenem (IMI), Glycylcycline (TGC), Glycopeptide (VA), Nitroimidazole (MTZ), Fluoroquinolone (CIP), Lincosamide (DA), Cephalosporins (CFM), (CRO), and (FOX), Penicillin (AM) and (AMC), Sulfonamide (SXT), and Macrolides (E). A variation was observed in resistance levels among isolates especially the same type species isolated from similar source samples in different groups.

Results of Antibiotic resistance pattern in group 1

In group (1) that included 111 isolates comprising *Staphylococcus epidermidis* as the prevalent species followed by *Staphylococcus aureus*, *Staphylococcus lugdunensis*, *Staphylococcus haemolyticus*, and *Kocuria kristinae*, isolated from 40 patients, 4 skin sample sites (surgical site of skin after sterilization with povidone-iodine 10%, skin before suture, first stitch, final stitch), and 160 swabs, All species in this group 100% showed resistance to Metronidazole followed by 87%, 81%, 79%, and 67% of species resistant to Cefixime, Ampicillin, Amoxicillin/Clavulanic acid, and Ceftriaxone respectively. While all species 100% showed sensitivity towered Imipenem and Tigecycline followed by 96%, 95%, and 90% of species sensitive towered Vancomycin, Ciprofloxacin, and cefoxitin. Results of antibiotic susceptibility pattern of group 1 are shown in Figure (3).

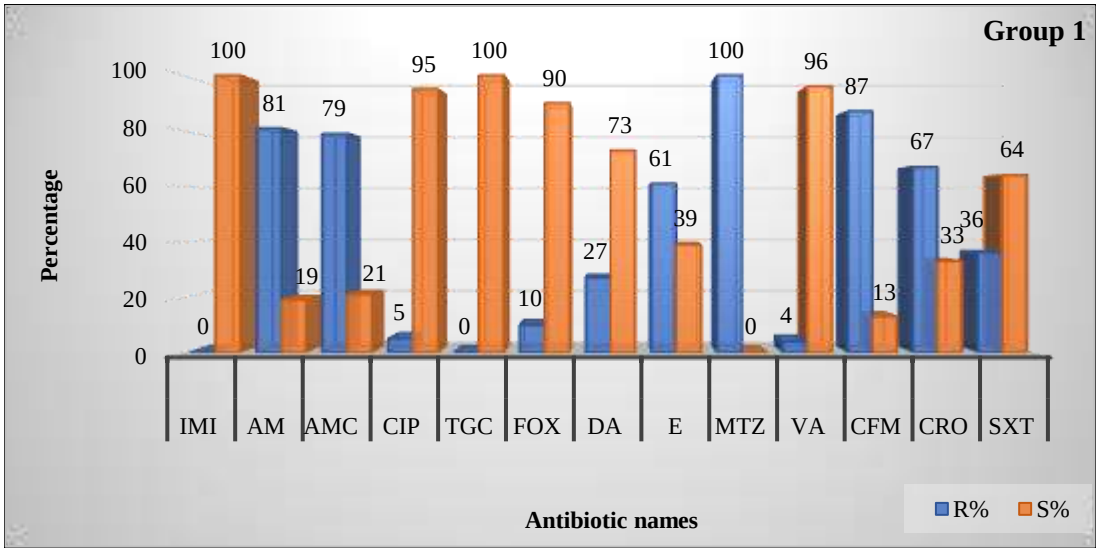

Figure (3): Antibiotic resistance pattern in group 1.

Results of Antibiotic resistance pattern in group 2

This group included 56 isolates comprising *Staphylococcus epidermidis* as the prevalent species followed by *Staphylococcus aureus*, *Enterobacter cloacae*, and *Kocuria kristinae*, isolated from 10 patients, 5 skin sample sites (surgical site of skin before sterilization, skin after sterilization with povidone-iodine 10%, skin before suture, first stitch, final stitch), and 50 swabs. Out of the total rate of species in this group, 96%, 96%, 93%, and 89% showed the highest resistance against Amoxicillin/ Clavulanic acid, Cefixime, Ampicillin, and Metronidazole respectively. While all species 100% showed sensitivity towered Imipenem and Tigecycline followed by 97%, 93%, and 89% of species sensitive towered Vancomycin, Ciprofloxacin, and cefoxitin respectively as shown in Figure (4).

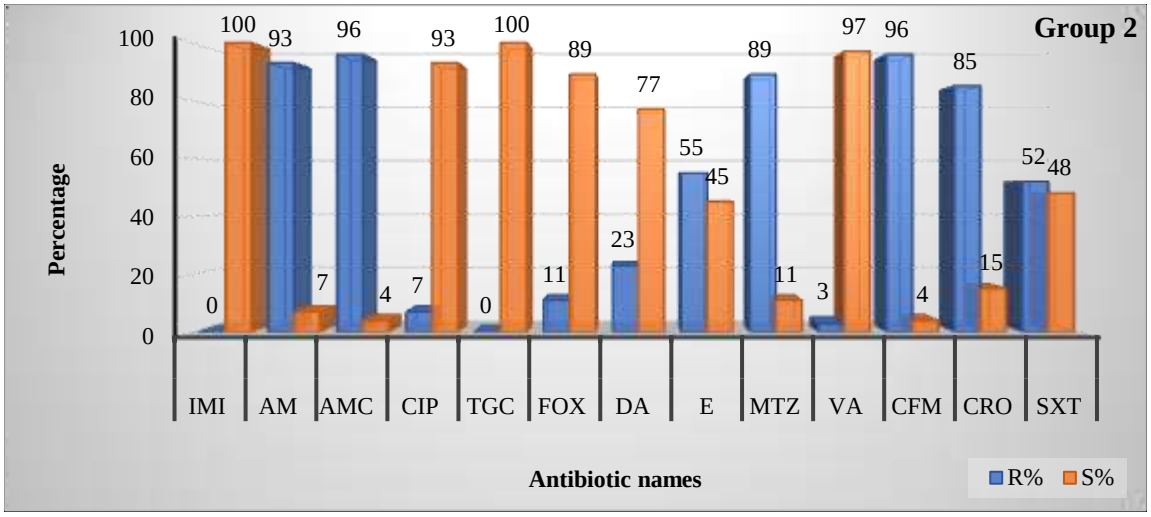

Figure (4): Antibiotic resistance pattern in group 2.

Results of Antibiotic resistance pattern in group 3

The third group in this study included 79 isolates comprising *Staphylococcus epidermidis* as the prevalent species followed by *Staphylococcus aureus*, *Aerococcus viridans Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Pantoea spp*., and *Kocuria kristinae*, isolated from 20 patients, 5 skin sample sites (surgical site of skin before sterilization, skin after sterilization with povidone-iodine 10% mixed with ethanol 70%, skin before suture, first stitch, final stitch), and 100 swabs. Out of the total rate of species in this group, 82%, 78%, and 73%, showed the highest resistance against Amoxicillin/Clavulanic acid, Metronidazole, and Ampicillin respectively. While all species 100% showed sensitivity towered Imipenem, Tigecycline, and Ciprofloxacin followed by 89% of species sensitive towered Vancomycin. Results of antibiotic susceptibility pattern of group 3 are shown in and Figure (5).

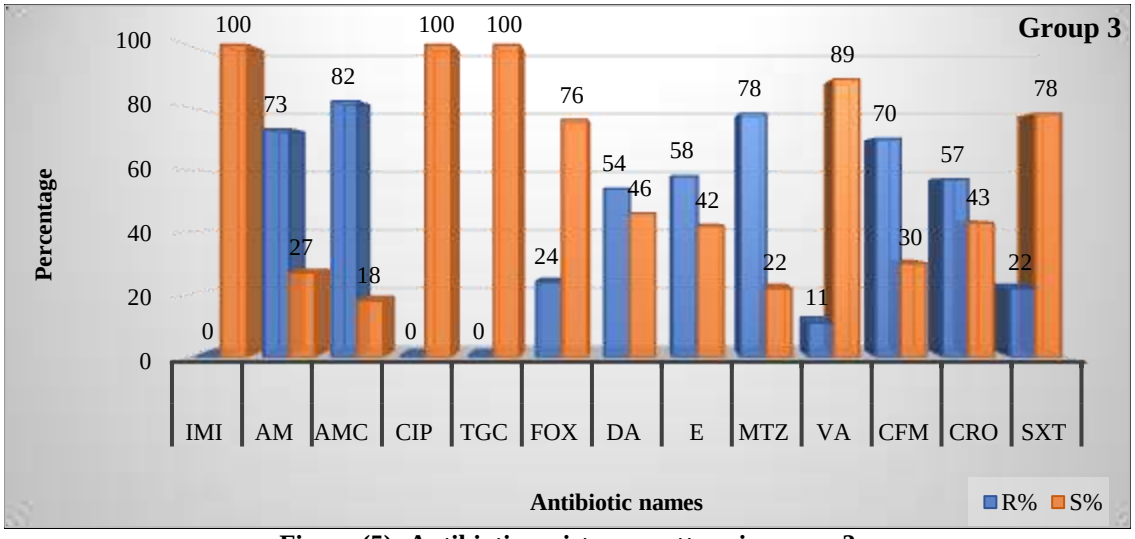

Figure (5): Antibiotic resistance pattern in group 3.

Results of Antibiotic resistance pattern in group 4

In group (4) that included 37 isolates comprising *Staphylococcus aureus* as the prevalent species followed by, *Acinetobacter baumannii*, *Escherichia coli*, *Burkholderia cepacia*, *Kocuria kristinae*, *Kocuria rosea*, and *Aeromonas veronii* isolated from 30 patients, and 30 swabs from cesarean surgical site infection, out of the total rate of species in this group, 89%, 86%, and 83%, showed the highest resistance against Metronidazole, Ampicillin, and Cefexime respectively. While all species 100% showed sensitivity towered Imipenem and Tigecycline followed by 81% and 78% of species sensitive towered Ciprofloxacin and Vancomycin respectively as shown in Figure (6).

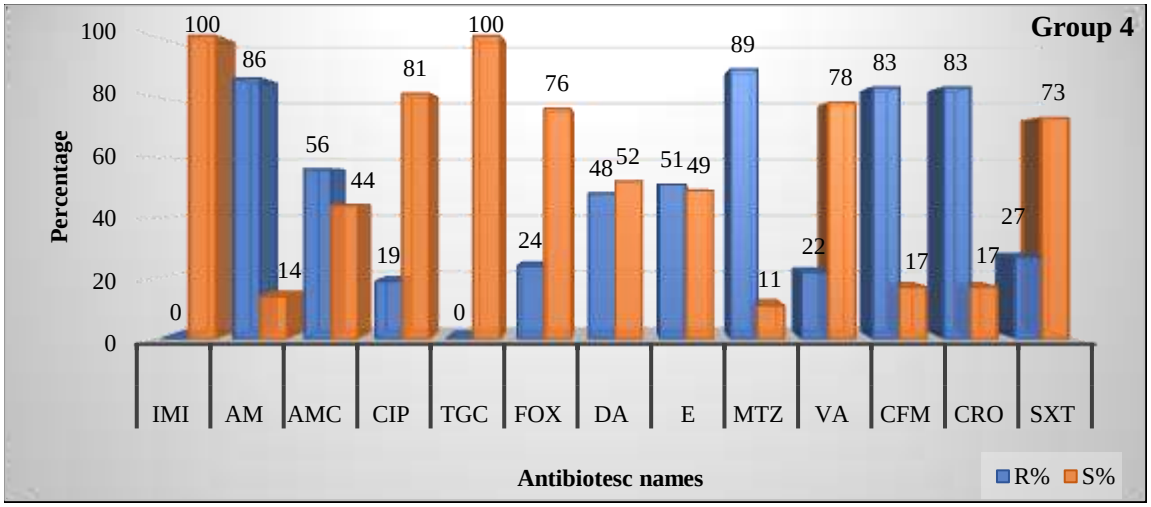

Figure (6): Antibiotic resistance pattern in group 4.

Some isolates in all tested groups showed inducible Clindamycin resistance (D-zone) as shown in Figure (7) especially *S. epidermidis* and *S. aureus* isolates. Resistance against macrolide and lincosamide antibiotics are either inducible or constitutive. The constitutive resistance mechanism is mediated through *msrA* genes, in which *S. aureus* strains are resistant to erythromycin and sensitive to clindamycin, in both *in vivo* and *in vitro*. The constitutively resistant strains do not develop clindamycin resistance during treatment. The inducible resistant isolates show resistance against erythromycin but are susceptible to clindamycin. Inducible resistance develops in the presence of a powerful methylase enzyme inducer like erythromycin. Unlike constitutive resistance, inducible resistance cannot be detected by standard susceptibility testing. Otherwise, can be detected by the D-zone test, due to a D-shaped inhibition zone around clindamycin under the in-vitro effect of erythromycin. It is very important to identify inducible Clindamycin resistance for accurate handling of *S. aureus*. Otherwise, clindamycin application can cause treatment impairment by the development of constitutive resistance (39).

Combination of certain antibiotics gives a greater therapeutic effect than the sum of each drug as it can help in the reduction of resistance also it will provide a wide coverage of bacterial invasion. Furthermore, several combinations are able to decrease the interval of therapy (40). The present study observed synergism between some antibiotics in all tested groups such as in group 1 between Imipenem with several antibiotics including Amoxicillin/ Clavulanic acid, Ceftriaxone, Cefoxitin, Ampicillin, and Trimethoprim/ Sulphamethoxazole. In group 2 between Imipenem with Ciprofloxacin and Ciprofloxacin with Cefoxitin. In group3 between Imipenem with Ceftriaxone. In group 4 between Imipenem with Ciprofloxacin as shown in Figure (8).

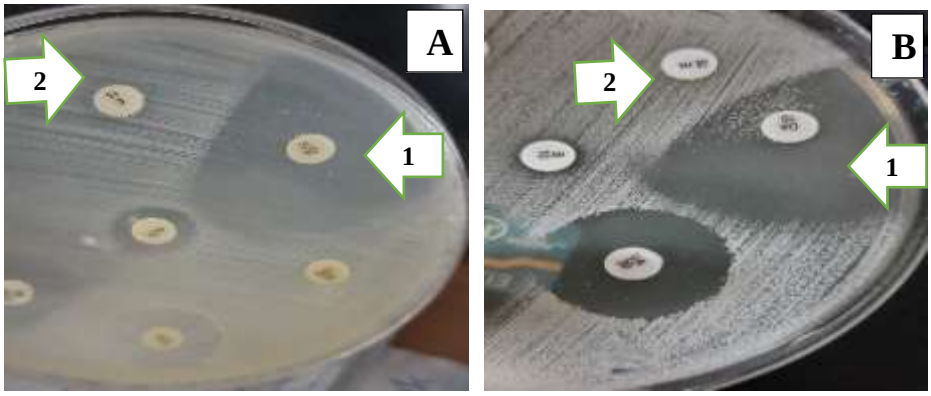

Figure (7): Inducible Clindamycin resistance (D-zone) on Muller Hinton agar. (A) *Staphylococcus aureus* (B) *Staphylococcus epidermidis* (1) Clindamycin (2) Erythromycin.

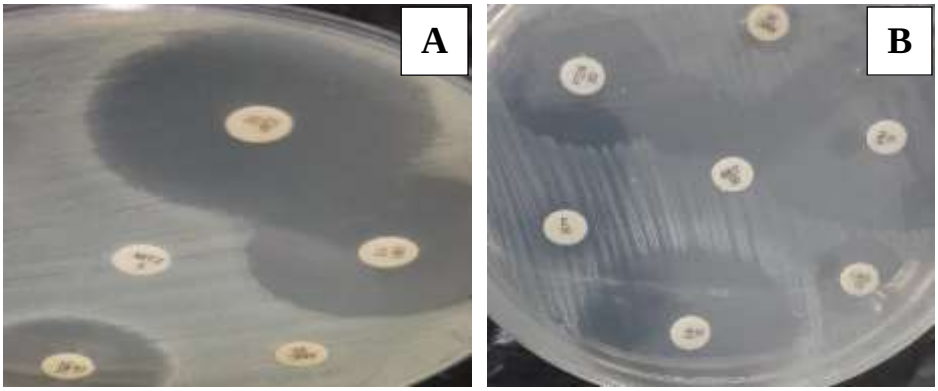

Figure (8): Antibiotic synergism on Muller Hinton agar. (A) *Staphylococcus aureus* from surgical site infection, synergism between Ciprofloxacin and Imipenem (B) Staphylococcus epidermidis from first stitch, synergism between Cefoxitin and Imipenem.

The results correspond with the study of (41) who found that 85.7% of *S. epidermidis* isolates were resistant to Amoxicillin/Clavulanic acid, while all isolates were susceptible to Tigecycline.

(42) found that isolates had high resistant rates against beta-lactam antibiotics, but low rates toward non beta-lactam antibiotics because of inactivation mechanisms of *Staphylococcus epidermidis* including permeability modification, bacterial beta lactamase enzymes and/or change of main target of antibiotics. While in

Basrah (43) had different results in his study with *S. epidermidis* that revealed high resistance to cefoxitin. The results were close to study by (44) showing that most *S. aureus* isolates were resistant to Ampicillin and Erythromycin, while only 12% of isolates were resistant to vancomycin. A study done by (45) observed that 45% of isolates were resistant to Erythromycin and this is due the change in the target site as a result of mutation. Also, in his study he indicated 3% of isolates resistant to Imipenem despite the current study. Glycopeptides are given to patients with intense infections caused by multidrug CoNS. They act by inhibiting bacterial cell wall synthesis but bacterial resistance towered glycopeptides are expanding due to the uncontrolled use. (46) disagreed in their study with current study as he found that *S. haemolyticus* gave a high level of resistance against Erythromycin (95%), fewer isolates were resistant to Clindamycin (77%), Trimethoprim/ Sulfamethoxazole (73%), Ciprofloxacin (58%). The resistance of *S. haemolyticus* is helped by rearrangement of its chromosome and recombination processes that assist evaluation and adaptation leading in pathogenesis and survival in hospitals (47).Resistance to most β-lactam antibiotics is allowed by the *mecA* gene, which is obtained by horizontal transfer of mobile genetic elements causing many hospitals acquired infections, *mecA* encodes penicillin binding protein that helps form the bacterial cell wall with low affinity for beta-lactam antibiotics inhibiting there binding and activity. Most resistant genes are acquired and not intrinsic and they are prevalent in *S. epidermidis* and *S. aureus* (48). Results of *K. kristinae* correspond with the study of (49) who found that there was 40% resistance to Erythromycin and 100% sensitive to Imipenem, Ciprofloxacin, and Trimethoprim. *A. baumannii* can naturally transfer to uptake naked DNA, while *P. aeruginosa* exchanges AMR genes by conjugation. Both of them have different mechanisms for antimicrobial resistance, including efflux pumps that allow resistance to Tigecycline and Ciprofloxacin, alteration of target sites, enzymes, permeability defects, and modification of aminoglycosides (50). Antimicrobial resistance continues to be an issue particularly in hospital acquired infections due to evaluation of bacteria, misuse and misunderstanding of antibiotics mechanism and dosage.

Results of antiseptic susceptibility test by agar well diffusion method (*in vitro*).

Povidone-iodine has been considered the antiseptic of choice due its tolerability and favorable efficacy, broad spectrum of activity, ability to penetrate biofilms, low cytotoxicity, and anti-inflammatory properties. The active part is iodine in which oxidizes proteins, fatty acids, and nucleic acid of the pathogen leading to death. Povidone iodine is destined as a preoperative skin preparation, not a solution to be poured into an open surgical wound. Pouring PVP-I onto the surgical site from a bottle that has been opened multiple times is not recommended, as any nonsterile solution introduced into a wound creates a risk of contamination by nonresident microbes (51).

Results of pilot study

Antimicrobial susceptibility test can be used for drug discovery, epidemiology and prediction of therapeutic outcome. This part of the study focused on the use of antimicrobial testing methods for *in vitro* investigation of PVP-I in different extracts as potential antiseptic agents. A pilot study was designed on 18 bacterial isolates chosen randomly from different skin sites to detect the best concentration of PVP-I that can be used for skin disinfection before and after surgery.

In the current study, both the Gram-positive and Gram-negative bacteria were evaluated for antibacterial susceptibility. The current results obtained from the agar well diffusion method had shown that the different concentrations of PVP-I displayed great activity against all the tested bacteria.

All isolates had a response against the antiseptic in different degrees as shown in Table (8) and Figure (9). Concentration of PVP-I at 10.5%, 11%, and 11.5% showed lower activity against tested isolates depending on the statistical analysis that showed significantly decreasing of inhibition zone diameter with increasing concentration, probably because of their lower oxidation effect. Polyvinylpyrrolidone has an affinity to cell membranes as it delivers free iodine to bacterial cell surface. Iodine's targets exist in bacterial cytoplasm causing the oxidation of peptides, enzymes, lipides, and cytosine resulting in inactivation of essential molecules for biological viability. A chemical equilibrium develops with only about one-thousandth part of the iodine being released and available as free molecular iodine, which is responsible for the germicidal activity (52).

The best concentration applied was 10% and 12% with few variations. However, no significant differences between the two tested concentration while high significant differences between all test concentration in general according to the statistical analysis. Therefore, the current study continued depending on the concentration of 10% PVP-I as it is a universal concentration and nontoxic despite 12% PVP-I that may be toxic to the body and/or may cause irritation to the skin, especially that there was no significant difference in the inhibition zone value for both concentrations. The 10% PVP-I solution generally contains 90% water, 8.5% polyvinylpyrrolidone, 1% available iodine, and iodide (53).

The number of experimental studies testing different concentrations of PVP-I from 0.1% to 10% for inactivating efficacy against gram-positive and gram-negative bacteria is extensive. As far as we know, we have not found any studies testing efficacy at concentrations higher than 10%. The inactivating effect depends on the concentration of free iodine, which decreases with the increasing concentration of PVP-I, resulting primarily in longer exposure times, and, especially at concentrations of 9%–10%. The best bactericidal effect found by Sauerbrei (23) of PVP-I was at the range 0.08% - 0.9% with 5 min exposure, while 6%-10% had moderate microbicidal activity with higher exposure time. Despite that, Gnanasekaran, *et al*. (54) found that concentration less than 5% had no efficacy at reducing bacterial growth.

Table (8): The effect on PVP-I concentrations on different isolates from different sample sources.

Bacterial spp.	Sample sources	PVP-I concentration					*P Value
		10%	10.5%	11%	11.5%	12%	
Aerococcus viridans	skin before sterilization	22	15	16	17	24	0.05
Aeromonas veronii	infection	14	13	13	14	15	0.09
Burkholderia cepacia	infection	14	13	12	14	14	0.09
Enterobacter cloacae	skin before sterilization	14	13	14	15	16	0.09
Escherichia coli	infection	13	12	13	15	15	0.09
Kocuria kristinae	first stitch	20	19	20	22	24	0.09
Kocuria kristinae	infection	16	16	16	17	18	0.09
Kocuria kristinae	skin before sterilization	23	18	20	25	26	0.09
Kocuria kristinae	skin before sterilization	19	17	17	19	20	0.09
Pseudomonas aeruginosa	skin before sterilization	11	10	10	11	12	0.09
Staphylococcus aureus	skin after sterilization	22	15	15	15	26	0.05
Staphylococcus aureus	skin after sterilization	20	17	19	24	26	0.05
Staphylococcus aureus	skin before sterilization	28	18	17	19	28	0.05
Staphylococcus epidermides	first stitch	17	15	15	16	20	0.08
Staphylococcus epidermides	skin before sterilization	22	15	16	17	24	0.05
Staphylococcus epidermides	skin before suture	21	15	16	17	22	0.05
Staphylococcus haemolyticus	infection	16	15	15	16	18	0.09
Staphylococcus haemolyticus	skin after sterilization	22	15	15	18	23	0.05
P value		0.01	0.09	0.08	0.09	0.01	

***P VALUE *between all tested concentration* and no significant differences between 10% and 12%/ p≤0.05 considered significantly different**

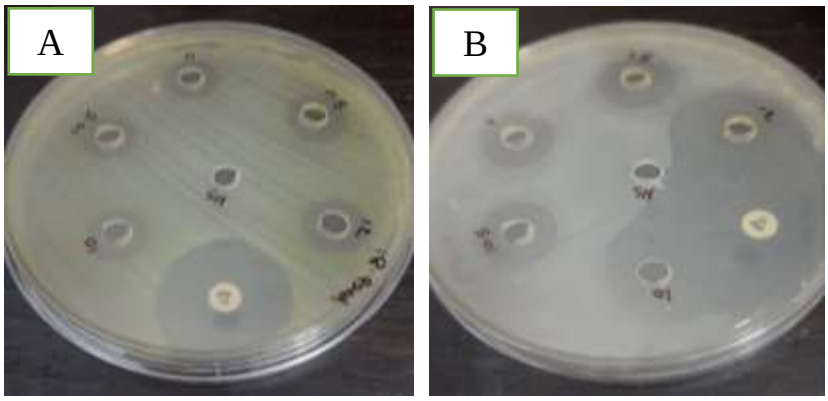

Figure (9): Effect of povidone iodine 10%, 10.5%, 11%, 11.5%, 12% on Muller Hinton agar (A) *Pseudomonas aeruginosa* (B) *Staphylococcus aureus*.

Results of mixed disinfectants

This experiment was designed to resemble the classification of tested groups under study in order to find out the nature and effectiveness of 10% PVP-I as well as 70% ethanol, together or alone. The prevalent bacteria in the present study from skin and infection samples were *S. epidermidis* and *S. aureus* therefore they were applied in this stage of the experiment to determine the activity of 10% PVP-I against 70% ethanol, and 10% PVP-I mixed with 70% ethanol by well diffusion assay in vitro, also antibiotics Imipenem and Cefixime were applied as control.

At first, it must be mentioned that all isolates showed no effectiveness towered 70% ethanol that was applied in the agar well, and that might be due to the fast evaporation of the antiseptic before bacterial growth. A safe bactericidal effect of ethanol can be expected at concentrations between 60% and 85%, and the exposure times vary between ≤0.5 and ≥5 min (55).

Table (9): The effect of disinfectants on different isolates from different sample sources.

Bacterial species	Sample sources /5 isolates for each source	10%Povidone iodine Mean ± SD	10%Povidone iodine + 70% Ethanol Mean ± SD	P VALUE
Staphylococcus epidermidis	skin before sterilization	20±0.5	19.6±0.7	0.09
	skin after sterilization	19.2±0.7	20±0.8	0.09
	skin before suture	19±0.5	18.6±0.5	0.09
	first stitch	19.6±0.9	19±0.5	0.09
	final stitch	20.2±0.4	21±0.5	0.09
P value		0.09	0.09	
Staphylococcus aureus	Sample sources /5 isolates for each source	10%Povidone iodine Mean ± SD	10%Povidone iodine + 70% Ethanol Mean ± SD	P VALUE
	skin before sterilization	19±0.5	19.6±0.5	0.09
	skin after sterilization	22±0.6	21.4±0.5	0.09
	first stitch	21±0.5	20±0.5	0.09
	final stitch	22±0.9	21.5±0.7	0.09
	infection	21±0.2	22±0.5	0.09
P value		0.08	0.08	
P value between the two *spp*.		0.09	0.08	
Antibiotics: Imipenem (sensitivity <18 mm) – Cefixime (sensitivity <15 mm) – Cefoxitin (sensitivity <17) p≤0.05 considered significantly different				

There were no significant differences between 10% PVP -I and 10% PVP-I mixed with 70% ethanol *in vitro* as shown in Table (9) and Figure (10), despite *in vivo*, that mixed disinfectant showed better effect on skin against bacterial species. This was proven in this study, specifically in the first two groups using 10% PVP-I and the third group using a mixture of 10% PVP-I and 70% ethanol as it was shown that a decline in the number of bacteria were observed in the case of using mixture antiseptics. Antiseptic agents in combination with alcohol were found to be the most effective in reducing skin contaminants as alcohol is fast-acting.

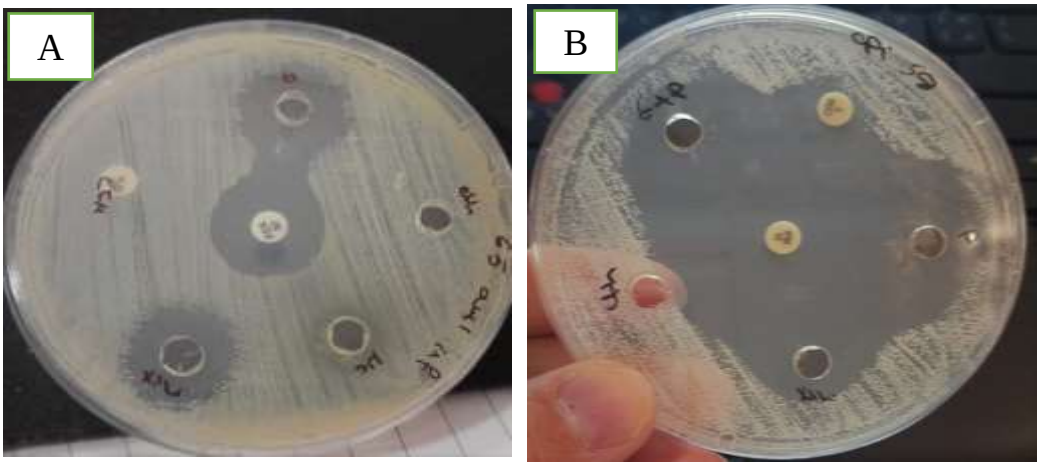

Figure (10): Effect of PVP-I 10% against ethanol 70%, and PVP-I 10% mixed with ethanol 70% by well diffusion assay on Muller Hinton agar (A) *Staphylococcus aureus* (B) *Staphylococcus epidermidis.* Antibiotics: Imipenem (sensitivity <18 mm) – Cefixime (sensitivity <15 mm) – Cefoxitin (sensitivity <17).

No current studies were found in which povidone-iodine and alcohol were used as dual agents *in vitro*. The combination of antiseptic agents and alcohol may be important for skin antisepsis as it increases alcohol efficacy, acting as an additional active treatment component and affecting the objective comparison of both active ingredients (19, 56). Moreover, Combination of povidone iodine and alcohol is often used for blood donor skin disinfection to limit contamination of blood therefore it is the best assay for disinfection (57).

Conclusion

This study finding need more concern about surgical contamination with different resistance bacterial species and also more attention from patients whose came from abroad may carry infection which may cause transferring this infection. Therefore, we recommend using combination of 10% povidone-iodine and 70% ethanol for disinfection of the skin before surgery, applying antiseptics by back and forth rubbing on skin to influence, applying UV lights in operating rooms as a type of sterilization, and increasing health awareness about self-hygiene especially before and after surgery.

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