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Investigation the Role of Various Antiseptics on the Prevalence of Skin Microbiota and Post Cesarean Surgery Infections

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ABSTRACT

During this study, 690 swabs were taken from different sites divided as the following: First, 350 swabs from surgical instruments, doctor gloves, and antiseptics before beginning cesarean surgery to ensure their sterilization. Second, 310 swabs from 70 skins (out of 100) of female patients attending Al-Elwiya Educational Maternity Hospital were taken at the site of cesarean surgery incision before and after sterilization with 10% povidone-iodine and 10% povidone-iodine mixed with 70% ethanol to detect the efficiency of antiseptics and any bacterial invasion might cause post operative infections. Furthermore, 30 swabs from infected surgical sites were taken from 30 female patients with post operative cesarean infections to detect the causative bacterial pathogen. The result of 350 swabs that were all taken from different surgical instruments, doctor gloves, and antiseptics before surgery in all groups showed negative growth culture. The bacterial isolates were primary identified by phenotypic examinations and biochemical tests and final identification by VITEK-2 system. *Staphylococcus epidermidis* was revealed to be the prevalent bacterial species from all skin sample sources, while *Staphylococcus aureus* was dominant in surgical site infections. Sterilization with 10% povidone iodine mixed with 70% ethanol showed less bacterial load on skin with a significant decrease in the numbers of isolated bacteria in comparison to use 10% povidone iodine solution alone.

KEYWORDS: postoperative infection, antiseptics, skin microbiota, VITEK-2 system

الخلاصة

تم اخذ ٦٩٠ مسحة من أماكن مختلفة شملت: ٣٥٠ مسحه من أدوات جراحية استعملت داخل صالة العمليات، قفازات جراحيه، معقمات قبل البدء بالعملية القيصرية, ٣١٠ مسحه من أماكن مختلفة للجلد في منطقة اجراء الشق الجراحي ل ٧٠ مريضة داخل صالة العمليات قبل وبعد التعقيم بنوعين من المعقمات الأول استخدام بوفيدون ايودين ١٠٪ والثاني محلول بوفيدون ايودين ١٠٪ مع كحول ٧٠٪ كما اخذت ٣٠ مسحه من منطقه الالتهاب بعد العملية القيصرية لتشخيص البكتريا المسببة للالتهاب. حددت العزلات وشخصت الأنواع مظهريا وأيضا تم اجراء الاختبارات الكيموحيوية واختبار نظام الفايتك VITEK-2. أظهرت جميع العينات المأخوذة من أدوات العملية والقفازات الجراحية والمعقمات نتيجة سالبة للزرع البكتيري. من ناحية أخرى ساد النوع *Staphylococcus epidermidis* في عينات الجلد قبل واثناء العملية بينما ساد النوع *Staphylococcus aureus* في منطقة التهاب الجرح. اظهر محلول بوفيدون ايودين ١٠٪ مع كحول ٧٠٪ كفاءة الاستخدام قبل العملية على الجلد وذلك من خلال الانخفاض الملحوظ في اعداد البكتريا المعزوله على استخدام محلول بوفيدون ١٠٪ لوحده.

INTRODUCTION

Post operative infections are considered as one of the leading health problems after many surgical procedures especially after gynecological procedures, sometimes even causing insufficiency of the surgery leading to

irreparable damage to the patient [1]. Surgical Site Infection (SSI) is defined by The Center for Disease Control and Prevention (CDC) as an infection follows a surgery in a section of the body where the surgery took place. Surgical site infection constitutes 38% of the nosocomial infections [2]. In 1992, the term surgical site infection replaced the term surgical wound infection that was defined by CDC in 1988[3]. Postoperative infection refers to an infection that occurs within (30) days after surgery. Patients with SSI had (3) to (11) times a higher risk of death compared to those who having a surgery without SSI. Therefore, these infections are a major source of morbidity and mortality during the postoperative phase for patients and prevention has become essential need [4]. One of the most life threating origins of nosocomial infection for patients is the contamination of operating theatres through medical staffs by moving between parts of the hospital and going back to the operating theatre without replacing their gowns or slippers. On the other hand, patients hygiene before their operation plays a role in decreasing the risk of post operation infection [5]. Caesarean section (CS) delivery is a major obstetrical surgical operation that aimed to save the lives of mothers and fetuses. As a surgical operation, caesarean delivery may be accompanied by some complications and surgical site infection might be one of them [6]. The first barrier of defense against bacterial infestation is the skin and when a surgical incision makes this barrier breach, the surgical wound may be contaminated with pathogens from multiple sources [7]. The pathogenic microorganisms isolated from surgical infections include Gram-negative and Gram-positive bacteria depending on the substantial problem, kind of surgical procedure and its location [8]. Surgical wound infections could be influenced by four major factors including patient variables, preoperative preparation, operative proceedings and postoperative patronage [9]. Microflora near or at the surgical wound is the hidden reason of SSI. Thus, the main source of bacterial isolates associated with SSI are mainly those of the skin flora. Considerably, commensal skin microflora consists of many microbes with low pathogenicity such as coagulase negative staphylococci but also sometimes can be pathogenic strains such as *Staphylococcus aureus*. The number of microorganisms on the skin may be decreased by using appropriate antiseptics limiting the risk of infections. However, using optimum antisepsis may fail to kill the entire skin microflora as 20% of these microbes subsist underneath the surface, around pilous follicles or in sebaceous glands [10]. Proper preparation of skin before an operation is necessary to prevent surgical wound contamination. Several agents are obtainable, each has a particular application guidance [11]. The present study was aimed to assess the rate of infections as well as the common bacterial species related with this type of infections. In addition, we aimed to assess risk factors, observe the trend of acquisition of bacterial infections, study the outcome of the related contagions and try to find ways to reduce such types of infections.

MATERIALS AND METHODS

Sample collection

This study included 100 woman who attended Al-Elwiya Educational Maternity Hospital during a period between August to November 2022, their ages ranged from (19) to (53) years. 690 swab samples were collected from different sites and classified into four groups:

G1/40 patients; 160 swabs from skin (40/skin after sterilization, 40/skin before suture, 40/first stitch, and 40/final stitch) and 200 swabs from surgical instruments (40/tissue, scissors. 40/skin blade, 40/retractor, 40/surgical suture, and 40/doctor gloves), sterilization with povidone-iodine 10%; G2/10 patients, 50 swabs (10/skin before sterilization, 10/skin after sterilization, 10/skin before suture, 10/first stitch, and 10/final stitch), 50 swabs from surgical instruments (10/empty plate of povidone. 10/povidone from the plate, 10/povidone from its bottle, 10/gauze, and 10/gauze with povidone), sterilization with povidone-iodine 10%; G3/20 patient, 100 swabs (20/skin before sterilization, 20/skin after sterilization, 20/skin before suture, 20/first stitch, and 20/final stitch), 100 swabs from surgical instruments (20/empty

plate of povidone. 20/povidone with ethanol from the plate, 20/povidone with ethanol from its bottle, 20/gauze, and 20/gauze with povidone and ethanol), sterilization with povidone-iodine 10% mixed with ethanol 70%; G4/30 patient, 30 swabs from surgical site infection, as listed in Table 1. Amie's transport medium was used to transport the samples to laboratory and cultured.

Isolation of bacteria

Aerobic and facultative anaerobic bacteria were isolated and identified using standard bacteriological techniques. All the swabs were cultured on blood agar, MacConkey agar for detection of Gram-negative bacteria, lactose fermentation, mannitol salt agar for mannitol fermentation as a first step in the diagnosis of isolates. For the facultative and microaerophiles bacteria, anaerobic jar with a candle was used, then all plates were incubated at 37°C for 1-2 days.

Bacterial identification

Bacterial species were identified according to morphological features on culture media, microscopic examination, and biochemical tests. In addition, VITEK-2 compact test was conducted to confirm the identification of the isolates.

Table 1: Site 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)**	**360**
			Tissue scissors- skin blade-doctor gloves-retractor-surgical suture **(200)**	
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)**	**100**
			Empty plate of iodine-iodine in the plate-iodine from its bottle-gauze- gauze with iodine **(50)**	
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)**	**200**
			Empty plate of iodine-iodine and ethanol in the plate-iodine and ethanol from its bottle-gauze- gauze with iodine and ethanol **(100)**	
G4	Patients with postoperative infection	**30**	Infection site after cesarean surgery	**30**
Total		**100**		**690**

The difference between group1 and group2 is in the sample sources type. difference between group2 and group3 is in antimicrobial agent.

Statistical analysis

Statistical analyses were carried out by SPSS program (NY, USA). Chi-square were applied to differentiate between measures of categorical collections, the product of correlation test by *p* values a lesser amount of than 0.05 were estimated statistically significant.

RESULTS AND DISCUSSION

Isolation of bacteria

The current study involved 690 swab samples divided into four groups as mentioned previously. All swabs cultured on MacConkey agar, blood agar, selective and differential media for aerobic and microaerophilic conditions. First, it must be noted that out of 690 samples, the result of 350 swabs that were all taken from different surgical instruments, doctor gloves, and antiseptic solutions in all groups showed negative culture growth. Surgical instruments in the operation ward of the hospital that prepared to use before the surgery and involved in this study were sterilized according to instructions provided by WHO [12]. On the other hand, as listed in Table 2, out of 690 swabs, the result of 340 swabs that were taken from different sites of surgical patient's skin showed 245 positive growths in all tested groups divided into: group 1 (100 out of 160 swabs), group 2 (48 out of 50 swabs), group 3 (70 out of 100 swabs), and group 4 (27 out of 30 swabs). Taking into consideration, group 1 samples from skins before sterilization were not taken to consider because the effect of antiseptic. Swab samples from surgical gloves and sutures showed no bacterial growth as it was sterile and opened before beginning the surgery. Surgical instruments that are reusable, especially that contact sterile tissue provide a probable path for pathogenic microbe's transmission. This is associated with unsuccessful or inappropriate sterilization of these items [13]. Infections by exogenous transmission from sterilized surgical instruments may occur due to biofilm formation as a result of retained tissue that prevent adequate steam sterilization or due to viable but non-culturable (VBNC) microbes that exist in the biofilm matrix [14]. Contamination of antiseptics may occur by resistant bacteria, especially from the water used during their production. Thus, biofilm existence in water systems is difficult to destroy unless the product is sterilized after packaging [15]. Gloves may be contaminated during the surgery.[16] reported in his study a reduction in SSI after changing gloves during cesarean section [16].In group 2 and 3, 100% of bacterial growth was obtained from skin before sterilization due to the existence of skin microbiota or contamination with pathogenic microbes from the environment. The number of bacteria resides mucosal and skin surfaces surpass the number of cells composing human body, and are able to stimulate adaptive and innate immunity [17]. In group 1 and 2, sterilization with 10% povidone-iodine decreased the bacterial load, while in group 3, sterilization with 10% povidone-iodine and 70% ethanol showed a better result in elimination bacterial growth. Despite that, positive culture became higher during wound closure at the wound edges and after suturing.

This result is supported by previous studies that mentioned at the end of surgery and following wound closure, the edges of the incision revealed an enhancement in the bacterial load in comparison with the skin area after sterilization [18]. The use of a mixture of antiseptics usually gives more positive results in reducing microbes on the surface of the skin, the current study results agreed with [19] who reported the reduction of aerobic and anaerobic skin flora by povidone iodine -alcohol after 2-3min of sterilization better than chlorohexidine gluconate-alcohol, in elimination dramatically of all coagulase-negative *staphylococcus* bacteria (CoNS). Cesarean section may be accompanied with many postoperative complications, and SSI is one of them as a result of external of internal factors. The detection of the causative pathogens in some cases is indistinct due to the type of pathogen or inappropriate sample taken from the infection site. Moreover, antibiotic prophylaxis plays a role in reducing the number of bacteria giving a false culture [20].

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Table 2: Number and percentage of positive and negative growth from skin swabs for all groups under study

Sample source / Tested Groups No.	Positive growth samples (Skin before sterilization) NO.(%)	Positive growth samples (Skin after sterilization) NO.(%)	Positive growth samples (Skin before suture) NO. (%)	Positive growth samples (First stitch) NO.(%)	Positive growth samples (Final stitch) NO.(%)	Positive growth samples (Infected surgical site) NO.(%)	Total nb. of positive growth NO.(%)	Total nb. of Negative growth NO.(%)
G1/40 patients (160 swab)	–	17 (10.6%)	24 (15%)	30 (18.7%)	29 (18.2%)	–	100 (62.5 %)	60 (37.5 %)
G2/10 Patients (50 swab)	10 (20%)	10 (20%)	10 (20%)	8 (16%)	10 (20%)	–	48 (96 %)	2 (4 %)
G3/20 patients (100 swab)	20 (20%)	6 (6%)	16 (16%)	14 (14%)	14 (14%)	–	70 (70 %)	30 (30 %)
G4/30 patients (30 swab)	–	–	–	–	–	27 (90%)	27 (90 %)	3 (10 %)
*Total & % 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 swab	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 povidone- iodine 10% opened bottles were negative for bacterial growth.

Identification of bacterial isolates

Hundreds of diverse bacterial species habitat the human skin, some of them contribute in the protection of the host against infections [21]. The bacterial growth obtained were identified by classical biological methods, that involved Gram stain, cultural, morphological, and biochemical tests. The final identification was performed by VITEK 2 Compact automated system containing (GP-ID, GN-ID) cards. This technique is characterized one of the rapid tests exploited for detection of bacteria. Identification results of VITEK 2 were identical with previous tests and confirmed with ID message assurance level ranging acceptable to excellent (Probability percentage from 87% to 99 %) [22].

Distributions of bacterial isolates in all tested groups

Three species of coagulase negative staphylococci (CoNS) were isolated from group 1 as the following: *Staphylococcus epidermidis*, *S. lugdunensis*, and *S. haemolyticus*. There were no isolates of *S. lugdunensis* before beginning the surgery and that might be because of the sample site or the bacteria were transferred from the surgical staff. *S. epidermidis* was the prevalent species found in all sample sources as 87 (78.4%) of the isolates and it is considered a normal flora of the skin followed by *S. aureus* as 14 (12.6%) of the isolates, while only 2 (1.8%) of *Kocuria kristinae* were found in samples taken from first stitch. In group 2, the results also showed that *S. epidermidis* was the most species isolated as 46 (82.1%) followed by

S. aureus 6 (10.7%), with 2 (3.6%) isolates for each of *Kocuria kristinae* and Gram-negative *Enterobacter cloacae*. The results of group 2 were somehow similar to group 1, as the antiseptic that was used for sterilization in both groups was PVP-I. The results of group 3 showed that the higher rate of isolates 65 (82.6%) was *S. epidermidis* followed by 4 (5%) isolates for each of *S. aureus* and *Aerococcus viridans*. There were no isolates identified from samples during the surgery for the previous two species, this may be due to the site of sample or the decimation of isolates after sterilization with time. Moreover, there were three species of Gram-negative bacteria found including *klebsiella pneumoniae*, *Pantoea spp.*, and *Pseudomonas aeruginosa*. Gram negative isolates in addition to *K. kristinae* were all eliminated after sterilization with povidone iodine-ethanol in this group due to the effect of sterilization. *S. epidermidis* is the most coagulase negative staphylococci isolated from all types of skin such as moist, dry and sebaceous, this is because its genome consists 80% of core genes and 20% is variable, meaning that the 20% of the genome can be exchanged with available genes by horizontal gene transfer. Hence, this leads to it's adaptive to different skin environments [23]. Alcohol destroys *S. epidermidis* rapidly whereases PVP-I is less efficient and the bacteria becomes viable during time [24]. While our results disagreed with [25] who recorded no differences between the two antiseptics on the growth of bacteria and alcohol has fewer side effects on skin [5].

S. haemolyticus is a part of the skin flora and it is widespread in hospitals causing nosocomial infections. On the other hand, *S. epidermidis* and *S. haemolyticus* are considered as the most CoNS acquired by infants after 48 hours of birth [26]. *S. lugdunensis* is a CoNS species which, is a normal skin commensal, causes many infections especially skin and soft tissue infections and has a low presence in human clinical samples [27]. The results in group 4 revealed that the most isolated bacteria from surgical site infections after cesarean section was *S. aureus* 23 (62.2%) followed by *Acinetobacter baumannii* 3 (8.1%), while *Burkholderia cepacian*, *Escherichia coli*, *S. haemolyticus*, *K. kristinae*, and *Kocuria rosea* had the same result with 2 (5.4%) for each species, and only one (2.7%) isolate of *Aeromonas veronii*. Vaginal preparation with antiseptics immediately before cesarean delivery probably reduces the incidence of post-cesarean endometritis from 7.2% to 3.2% [28]. When the human body has a decreased ability of producing antimicrobial peptides that normally prevent infestation of *S. aureus*, infection by the organism will increase [29]. Antiseptics are used in hospitals worldwide to reduce, inactivate, or eliminate potentially pathogenic microorganisms. Current studies showed that widely used wound antiseptics show relevant cytotoxicity and cross-reactivity with wound dressings. Furthermore, methicillin-resistant *S. aureus* (MRSA) show a highly significant and elevated resistance toward local antiseptics [30]. Distribution of isolates are shown in Tables 3, 4, 5, and 6.

Table 3: distribution of bacterial species isolated in group 1

Sample source bacterial isolate	Skin after sterilization	Skin before suture	First stitch	Final stitch	Total N0. (%)
S. epidermidis	15(13.5)	23(20.7)	24(21.7)	25(22.5)	87 (78.4%)
S. lugdunensis	0	1(0.9)	2(1.8)	2(1.8)	5 (4.5%)
S. haemolyticus	1(0.9)	0	0	2(1.8)	3 (2.7%)
S. aureus	2(1.8)	4(3.6)	4(3.6)	4(3.6)	14 (12.6%)
K. kristinae	0	0	2(1.8)	0	2 (1.8%)
Total	18(16.2)	28(25.2)	32(28.8)	33(29.8)	111(100)
Chi-square statistic is 13.9365. The p-value is 0.001The result is significant at $p < .05$.					

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Table 4: distribution of bacterial species isolated in group 2

Sample source / Bacterial isolate	Skin before sterilization	Skin after sterilization	Skin before suture	First stitch	Final stitch	Total N0. (%)
S. epidermidis	10 (17.9)	10 (17.9)	10 (17.9)	8 (14.3)	8 (14.3)	46 (82.1)
S. aureus	0	2(3.6)	2(3.6)	0	2 (3.6)	6 (10.7%)
K. kristinae	2 (3.6)	0	0	0	0	2 (3.6%)
E. cloacae	2 (3.6)	0	0	0	0	2 (3.6%)
Total	14 (25.1)	12 (21.5)	12 (21.5)	8 (14.3)	10 (17.8)	56
Chi-square statistic is 7.9365. The p-value is 0.05The result is significant at p < .05.						

Table 5: distribution of bacterial species isolated in group 3

Sample source Bacterial isolate	Skin before sterilization	Skin after sterilization	Skin before suture	First stitch	Final stitch	Total N0. (%)
Staphylococcus epidermidis	17(21.6)	4 (5.1)	16 (20.3)	14 (17.8)	14 (17.8)	65 (82.6%)
S. aureus	3(3.8)	1 (1.2%)	0	0	0	4 (5%)
A. viridans	2 (2.5%)	2 (2.5%)	0	0	0	4 (5%)
k. pneumoniae	2 (2.5%)	0	0	0	0	2 (2.5%)
K. kristinae	1 (1.2%)	0	0	0	0	1 (1.2%)
Pantoea spp	1 (1.2%)	0	0	0	0	1 (1.2%)
P. aeruginosa	2 (2.5%)	0	0	0	0	2 (2.5%)
Total	28(35.4%)	7(8.9%)	16(20.3%)	14(17.8%)	14(17.8%)	79(100%)

Table 6:distribution of bacterial species isolated in group 4

Sample source / Bacterial isolate	Surgical site infection
A. baumannii	3 (8.1%)
A. veronii	1 (2.7%)
B. cepacia	2 (5.4%)
E. coli	2 (5.4%)
K. kristinae	2 (5.4%)
K. rosea	2 (5.4%)
S. aureus	23 (62.2%)
S. haemolyticus	2 (5.4%)
Total	37(100%)

CONCLUSION

Through the current study, medical materials and supplies used in cesarean surgeries showed efficiency and quality of sterilization by negative bacterial growth result. In addition, *S. epidermidis* was the prevalent bacteria from all skin sample sources, while *S. aureus* was dominant in surgical site infections. Significant decrease in the numbers of bacteria isolated from the skin from different sites and stages of caesarean section surgeries by using a mixture of Sterilization with povidone iodine 10%

mixed with ethanol 70% instead of povidone iodine 10% alone.

Informed consent: all patients submitted their Informed consent prior to the inclusion.

Ethical approval: Ref. of ethical approval: BCSMU/1221/0001M.

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