Al-Mustansiriyah Journal of Science
ISSN: 1814-635X (print), ISSN:2521-3520 (online) Volume 34, Issue 4, 2023 DOI: https://doi.org/10.23851/mjs.v34i4.1341

Research Article **Open Access**

Biofilm And Hemolysis Profile Index in Bacteria Isolated from Pre-Cesarean Surgery and Post Cesarean Infections

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Article Info

Received
11/03/2023

Revised
29/04/2023

Accepted
22/05/2023

Published
30/12/2023

ABSTRACT

Swabs were taken from 340 different sites of skin divided into four groups including; group 1/160 swabs, group 2/50 swabs, group 3/100 swabs, group 4/30 swabs, taken from 100 female 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 examination, 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*. Most isolates had hemolytic activity and all of them showed Beta hemolysis except *Aerococcus viridans* that expressed alpha hemolysis. Most isolates from numerous bacterial groups showed moderate biofilm production.

KEYWORDS: postoperative infection, bacterial hemolysis, biofilm formation, skin microbiota.

الخلاصة

جرى أخذ ٣٤٠ عينة من مواقع مختلفة من الجلد مقسمة الى أربع مجاميع تضم: المجموعة الاولى/١٦٠ مسحة، المجموعة الثانية/٥٠ مسحة، المجموعة الثالثة/١٠٠ مسحة والمجموعة الرابعة/٣٠ مسحة من ١٠٠ مريضه من موقع شق الجراحة القيصرية من عدة أماكن قبل وبعد التعقيم باستخدام ١٠٪ البوفيدون اليود ومع ١٠٪ البوفيدون اليود الممزوج ب ٧٠٪ الإيثانول، ومن المواقع الجراحية المصابة بالعدوى بعد العملية. جرى تحديد العزلات وتشخيصها عن طريق فحص النمط الظاهري والاختبارات الكيميائية الحيوية، وكذلك Vitek-٢. كانت *Staphylococcus epidermidis* هي البكتيريا السائدة المعزولة من مصادر عينات الجلد في المجاميع الأولى، الثانية والثالثة بنسبة ٨١٪ تليها المكورات العنقودية الذهبية *Staphylococcus aureus* التي كانت سائدة في المجموعة الرابعة التي ضمت مسحات التهابات موقع الجراحة. بالاضافة الى ذلك أنواع اخرى من البكتريا جرى عزلها من مصادر مختلفة من الجلد مثل *Staphylococcus haemolyticus*, *Kocuria kristinae*, *Enterobacter cloacae*, *Aerococcus viridans*, *Pantoea* و *Burkholderia cepacian*. كان لمعظم العزلات نشاط انحلالي وأظهرت جميعها انحلال دم بيتا باستثناء *Aerococcus viridans* سببت انحلال الدم من نوع ألفا، وأظهرت معظم العزلات من مختلف المجاميع الجرثومية إنتاجا معتدلا للغشاء الحيوي.

INTRODUCTION

The first barrier of defense against bacterial infection is the skin and when a surgical incision makes this barrier breach, the surgical wound may be contaminated with pathogens from multiple sources [1]. Microflora near or at the surgical wound is the hidden reason for surgical site infection. Commensal skin microflora consists of many microbes with low pathogenicity such as coagulase negative

staphylococci but sometimes also include pathogenic strains such as *Staphylococcus aureus*. The number of microorganisms on the skin may be decreased by using appropriate antiseptics limiting the risk of infection. However, using the best antiseptics may fail to destroy the entire skin microflora as 20% of these microbes subsist underneath the skin surface, around pilous follicles or in sebaceous glands [2]. 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 [3]. Studies have reported differences in virulence of bacteria between inpatient and outpatient settings. Inpatient groups had a higher rate of resistant microorganisms that cause SSI [4][5]. Biofilms are communities of microorganisms attached to surfaces through a matrix of extracellular polymeric substance (EPS) [6]. The first microbes that are attached to wounds are usually Gram-positive aerobic bacteria. The type of first microbes' colonization on a wound depends on the site of the wound and its closeness to an adequate number of microbes. When the first attached microbes grow and multiply, they start to change the wound environment and begin to induce a host immunological response resulting in what might be called a quiet inflammatory state but with no symptoms or clinical signs observed [7]. Different microcolonies are formed while the number of bacteria in the wound develop and form biofilms. A higher immunological host response is elicited as the bacteria continue to replicate resulting in inflammation. At this stage, the wound is critically colonized or in another word, biofilm is developed [8]. To decrease biofilm development, it is significant to control the probability of cross contamination from bacterial reservoirs. Sufficient aseptic techniques and antimicrobials must be utilized to reduce the formation of biofilm since microorganisms including *S. aureus* and *S. epidermidis* habitat around hair follicles [9]. Hemolysins are lipids or proteins that cause lysis of red blood cells (RBC) by disrupting the cell membrane. They are one of the most important virulence factors of bacteria and are involved in their pathogenesis. In addition, they are synthesized and directed by different genes. There are four types of hemolysins: alpha (α) hemolysin encoded by the *hla* gene, produces a greenish dark zone on the blood agar as a result of incomplete hemolysis and has dermonecrotic effect. It is generally caused by peroxides produced by the bacterium that oxidizes hemoglobin to meta hemoglobin (non-oxygen binding form). Beta (β) hemolysin, encoded by *hlb*, causes complete lysis of RBC with a lightened area under the colony and it has high affinity for the cell membrane of different types of cells causing membrane instability. Delta (δ) hemolysin encoded by *hld*, produces a narrow zone of incomplete hemolysis, and only at high concentrations, it forms a trans-membrane pore which lyses the cell membrane. Gamma (γ) hemolysis causes no hemolysis, and the agar under the colony is unchangeable but it can damage RBCs [10]. The present study was carried out to assess the rate of bacterial virulence factors that affect the skin and post cesarean infections that may delay therapy and try to find ways to reduce such types of infections.

MATERIALS AND METHODS

Samples Collection

This study included 100 women who attended Al-Elwiya educational maternity hospital during the period between August to November 2022, their ages ranged between 19 to 53 years. Totally, 340 swab samples were collected from different sites of patients' skin before and after sterilization during caesarean section, and from surgical site infections. The individuals were classified into four groups according to the swab sites and type of sterilization including: G1/40 patients, 160 swab from skin (one swab from 4 sites for each patient), sterilization with 10% povidone-iodine; G2/10 patients, 50 swabs (one swab from 5 sites for each patient), sterilization with 10% povidone-iodine; G3/20 patient, 100 swabs (one swab from 5 sites for each patient), sterilization with 10% povidone-iodine mixed with 70% ethanol; 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 the laboratory.

Isolation and Identification of Bacteria

Bacteria were isolated and identified by using standard bacteriological techniques [11]. All specimens were cultured on the blood agar for detecting hemolysis pattern, MacConkey agar for detection of Gram-negative bacteria and lactose fermentation, and mannitol salt agar for staphylococci isolation and detection of mannitol fermentation. Species were identified according to the morphological features on culture media, microscopic examination, and biochemical tests [12]. The VITEK-2 assay was also used as a confirmatory test for the automated identification of isolates.

Table 1. Type and number of swabs distributed according to the groups under study.

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

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

Biofilm Formation Detection on Tissue Culture Plate (TCP)

Biofilm formation test was done by using the Tissue Culture Plate method (TCP) designated by [13] as follows: The bacterial suspension (20 µl) equivalent to 0.5 McFarland standard tube, prepared from overnight agar culture was used as an inoculum to inoculate each well of the 96-well flat-bottomed polystyrene tissue culture plate, which contained 180µl of Brain heart infusion (BHI) broth with 2% sucrose. Each species was tested in triplicate with incubation at 37°C for 24 hrs. After incubation, the content of each well was removed and washed three times carefully with PBS (pH 7.2) and left to dry. Then, adherent bacteria were fixed with 200µl (99% ethanol) per well for 15 min. The plates were allowed to dry. Crystal violates (1%) was added to the wells for 15 min. Then the dye was removed from the wells, washed three times with PBS (pH 7.2) for removal of unbounded dye and allowed to dry at room temperature. 200µl ethanol was used to re-solubilize the dye bound to the adherent cells. The plates were decanted and allowed to dry. Finally, the absorbance (A^o) of each well was measured at 630 nm using ELISA reader and the value for the control well was deducted.

Statistical analysis

The analyses were completed by the SPSS program (NY, USA). Chi-square was applied to differentiate between measures of categorical collections, correlation was done by p values

test as lesser amounts of 0.05 were estimated statistically significant.

RESULTS AND DISCUSSION

Distribution of Bacterial Isolates

Three species of coagulase negative staphylococci (CoNS) were isolated from the patients of group 1 including: *Staphylococcus epidermidis*, *Staphylococcus lugdunensis*, and *Staphylococcus haemolyticus*.

S. epidermidis was the most prevalent species (78.4%) among all the other sample's accounting for 87 as it is considered a normal flora of the skin followed by *S. aureus* (14, 12.6%), while only (2, 1.8%) of *Kocuria kristinae* was isolated from the first stitch.

In group 2, the results also showed that *S. epidermidis* was the most species isolated (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 3 showed that the highest rate of isolates (65, 82.6%) belonged to *S. epidermidis* followed by (4, 5%) isolates for each of *S. aureus* and *Aerococcus viridans*. However, there were no isolates identified from samples during the surgery for the previous two species, this may be due to the site of the sample or the elimination of isolates after sterilization with the time. Gram-negative isolates in addition to *Kocuria kristinae* were all eliminated after sterilization with PVP-I-ethanol in this group due to the effect of sterilization. The results in group 4 revealed that the most isolated bacteria from the surgical site infection after cesarean section was *S. aureus* (23, 62.2%) followed by *Acinetobacter baumannii* (3, 8.1%), while *Burkholderia cepacian, Escherichia coli, S. haemolyticus, Kocuria kristinae, and Kocuria rosea* had the same result (2, 5.4%) for each species, and only one (2.7%) isolate of *Aeromonas veronii* was obtained. Mixed cultures were obtained in all tested groups.

Results of Bacterial Hemolysis Activity

The results of hemolytic activity of bacteria in group 1 as shown in Table 2, revealed that 10 out of 111 isolates in this group showed no hemolysis activity. In *addition, 73* out of 87 isolates of S. epidermidis had β- hemolytic activity, while K. kristinae did not show any hemolysis on blood agar plates. Furthermore, the rest of the species in this group had a β-hemolytic activity. In group 2, 10 out of 56 isolates had no hemolysis, S. epidermidis and S. aureus had 3 non hemolytic isolates for each species, as well as the two isolates of each K. kristinae and E. cloacae as mentioned in Table 3. Fourteen out of 61 had non hemolytic activity in group 3, including 9 of S. epidermidis, 2 of K. pneumoniae, and *one isolate* of each of S. aureus, Pantoea, and K. kristinae. The two isolates of P. aeruginosa *showed β-* hemolysis as mentioned in Table 4. Despite that, A. viridans demonstrated α-hemolytic activity on the blood agar plates. On the other hand, group 4 had 24 β-hemolytic isolates including 21 isolates of S. aureus, 2 S. haemolyticus, and one isolate of *B. cepacia* from surgical site infection (Table 5).

Table 2. Hemolysis profile results of bacterial species isolated from study group 1.

Sample source / Bacterial isolate	Skin after sterilization *N (+/-)	Skin before suture N (+/-)	First stitch N (+/-)	Final stitch N (+/-)	Total No. (+/-)
S.epidermidis	15 (13/2)	23 (21/2)	24 (22/2)	25 (23/2)	87 (79/8)
S.lugdunensis	0	1 (1/0)	2 (2/0)	2 (2/0)	5 (5/0)
S.haemolyticus	1 (1/0)	0	0	2 (2/0)	3 (3/0)
S. aureus	2 (2/0)	4 (4/0)	4 (4/0)	4 (4/0)	14 (14/0)
K. kristinae	0	0	2 (0/2)	0	2 (0/2)
Total	18 (16/2)	28 (26/2)	32 (28/4)	33 (31/2)	111 (101/10)

ISSN: 1814-635X (print), ISSN:2521-3520 (online) Volume 34, Issue 4, 2023 DOI: https://doi.org/10.23851/mjs.v34i4.1341

*N (+/-): N=Number / (+) beta hemolysis/ (-) no hemolysis

Table 3: Hemolysis profile results of bacterial species isolated from study group 2.

Sample source / Bacterial isolate	Skin before sterilization *N (+/-)	Skin after sterilization N (+/-)	Skin before suture N (+/-)	First stitch N (+/-)	Final stitch N (+/-)	Total No. (+/-)
S. epidermidis	10 (9/1)	10 (9/1)	10 (9/1)	8 (8/0)	8 (8/0)	46 (43/3)
S. aureus	0	2 (1/1)	2 (1/1)	0	2 (1/1)	6 (3/3)
K. kristinae	2 (0/2)	0	0	0	0	2 (0/2)
E. cloacae	2 (0/2)	0	0	0	0	2 (0/2)
Total	14 (9/5)	12 (10/2)	12 (10/2)	8 (8/0)	10 (9/1)	56 (46/10)

*N (+/-): N=Number / (+) beta hemolysis/ (-) no hemolysis

Table 4. Hemolysis profile results of bacterial species isolated from study group 3

Sample source / Bacterial isolate	Skin before sterilization *N (+/-)	Skin after sterilization N (+/-)	Skin before suture N (+/-)	First stitch N (+/-)	Final stitch N (+/-)	Total No. (+/-)
S. epidermidis	17 (15/2)	4 (3/1)	16 (14/2)	14 (12/2)	14 (12/2)	65 (56/9)
S. aureus	3 (2/1)	1 (1/0)	0	0	0	4 (3/1)
A. viridans	**2 (0/2)	2 (0/2)	0	0	0	4 (4/0)
k. pneumoniae	2 (0/2)	0	0	0	0	2 (0/2)
K. kristinae	1 (0/1)	0	0	0	0	1 (0/1)
Pantoea spp	1 (0/1)	0	0	0	0	1 (0/1)
P. aeruginosa	2 (2/0)	0	0	0	0	2 (2/0)
Total	28 (19/9)	7 (4/3)	16 (14/2)	14 (12/2)	14 (12/2)	79 (65/14)

*N (+/-): N=Number / (+) beta hemolysis (**alpha hemolysis)/ (-) no hemolysis

Table 5. Hemolysis profile results of bacterial species isolated from study group 4.

Sample source / Bacterial isolate	Surgical site infection *N (+/-)
A. baumannii	3 (0/3)
A. veronii	1 (0/1)
B. cepacia	2 (1/1)
E. coli	2 (0/2)
K. kristinae	2 (0/2)
K. rosea	2 (0/2)
S. aureus	23 (21/2)
S. haemolyticus	2 (2/0)
Total	37 (24/13)

*N (+/-): N=Number / (+) beta hemolysis/ (-) no hemolysis

In his study, [14] had similar results by reporting that *S. epidermidis* from hospital samples had higher virulence factors than community samples as 21 out of 30 were hemolytic, and most of them were samples from wounds and patients with catheters. Beta-toxin is important due to its ability to enhance the escape of bacteria from the host immune system adding to its participation in nutrient utilization allowing the pathogen survival. Beta-hemolysin was detected in 81% of *S. haemolyticus* isolates [15]. *S. haemolyticus* causes severe infections even though its virulence is less than *S. aureus* [16]. Moreover, [17] founds that *S. aureus* β-hemolysin has a significant role in skin colonization and keratinocyte damage. In Iraq, [18] founds 22 and 2 out of 24 isolates of *S. aureus* with β- hemolysis and α- hemolysis respectively, on blood agar plates. *S. lugdunensis* produces virulence factors but few are known. 95% of these bacteria secrete α-hemolysin called *S. lugdunensis* synergism hemolysin (SLUSH) that has similar phenotypic properties to *S. aureus* δ- hemolysin acting synergistically with beta-hemolysin by producing a zone of complete hemolysis with the zone of incomplete hemolysis produced by a beta-hemolysin producing strains [19]. This activity was observed by [20] who reported that 39.5% of isolates carrying SLUSH which encode for hemolytic peptides with delta toxin like activity. While [21] reported similar results in her study about *A. viridans*, which is a human pathogen capable of causing infections with α-hemolysin. However, [22] reported non hemolysin activity of *A. baumannii* isolates from hospital samples in Baghdad. These bacteria have many other factors that enable them to be a pathogenic bacteria and causative of nosocomial infections. Hemolysis activity is shown in Figure 1.

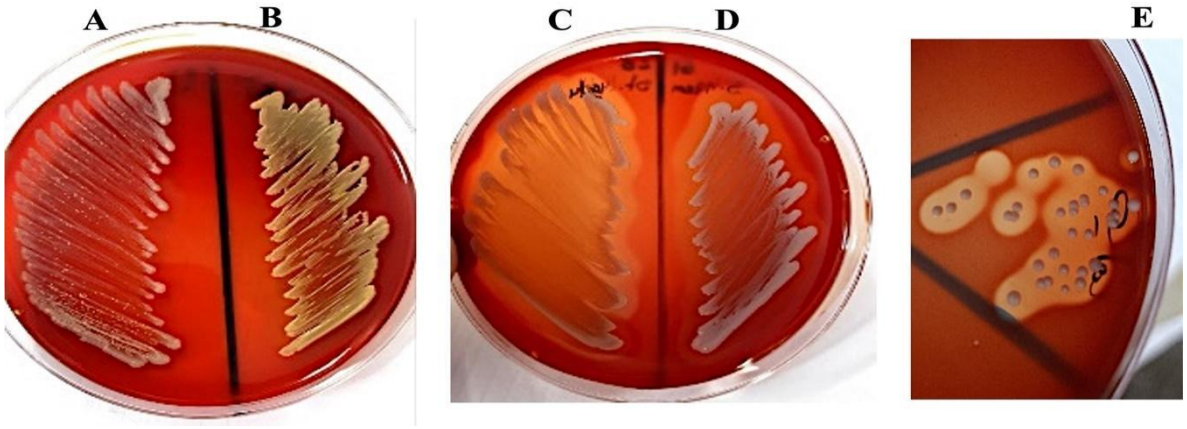

Figure 1. (A) *Kocuria Kristina* (B) *Kocuria rosea* – no hemolysis. (C) *Staphylococcus lugdunensis* (D) *Staphylococcus haemolyticus* (E) *Staphylococcus epidermidis* – Beta hemolysis.

Results of Bacterial Biofilm Formation

The results showed that each isolate had a different potency to form biofilm under the same conditions of the test. The biofilm producer isolates displayed three different categories as weak, moderate and strong biofilm former. The results of biofilm formation showed that most of

ISSN: 1814-635X (print), ISSN:2521-3520 (online) Volume 34, Issue 4, 2023 DOI: https://doi.org/10.23851/mjs.v34i4.1341

the tested isolates were able to form biofilm on Tissue culture plate TCP, to some degree. The current study shows that *S. epidermidis* in group 1 (42 out of 87 isolates) had the highest rate as moderate biofilm producer with 42 out of 87 isolates, and these isolates were found in samples taken from skin before suture and first stitch. In addition, 11, 18, and 17 isolates showed non weak, and strong production respectively, followed by *S. aureus* isolates (8 out of 14) were moderate producers with 2 weak and 4 strong biofilm producers. In contrast, only 1 isolate from skin before suture of the 5 isolates of *S. lugdunensis* was weak producer, and the other 4 were moderate biofilm producers, which isolated from first and final stitch samples. In group 2, also *S. epidermidis* (25 out of 46 isolates) had moderate biofilm production with 2 non, 11 weak, and 8 strong producer isolates. *S. aureus* isolates expressed 3 out of 6 moderate producers with 2 weak and 1 non biofilm producer, whereas *K. kristinae* and *E. cloacae* had 1 non biofilm producer isolate and 1weak producer in each species. The results of group 3 also showed that *S. epidermidis* (25 out of 65 isolates) had moderate biofilm production with 8 non biofilm producer isolates, 16 weak, and 16 strong producers. The 4 isolates of *S. aureus* expressed 2 moderate and 2 strong biofilm producers. Regarding group 4, the prevalent species was *S. aureus* (23 isolates) divided into: 1 non biofilm producer, 4 weak, 13 moderate, and 5 strong biofilm producers. Moreover, *A. baumannii* and *S. haemolyticus* were moderate biofilm producers. Finally, *A. veronii* isolates showed no ability to form biofilm formation. All these results are listed in Table 6 to 9 and shown in Figure 2.

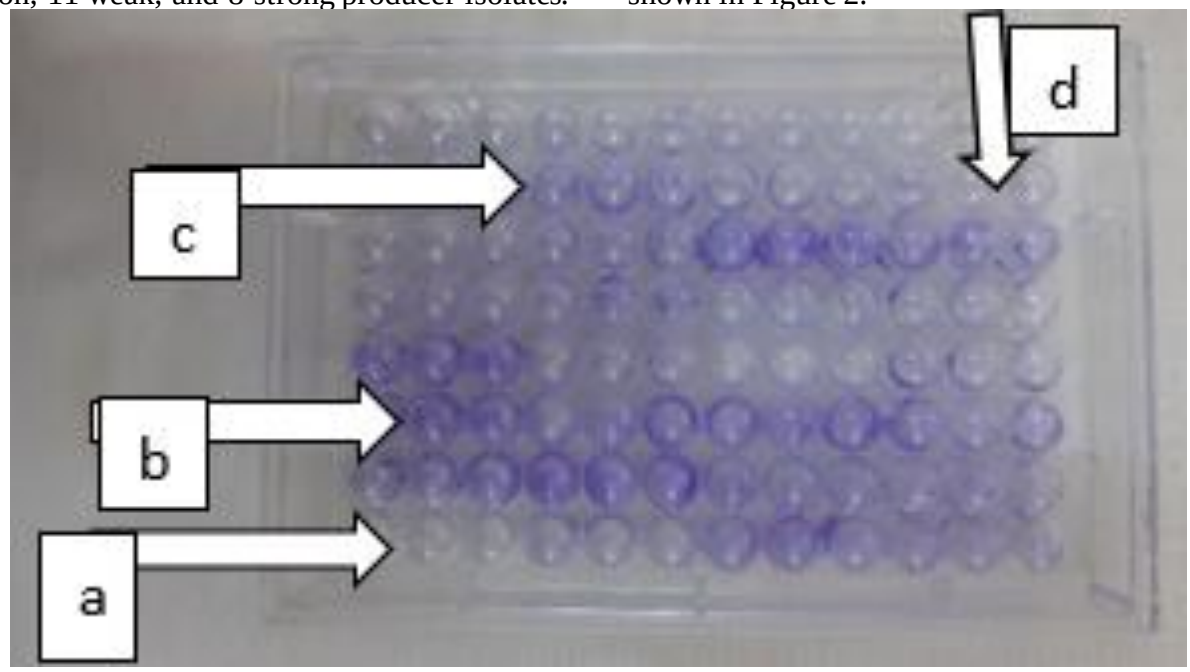

Figure 2. Biofilm production- a: non biofilm formation, b: strong, c: weak, d: moderate

Table 6. 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)
S. 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)
S. lugdunensis	0	1 (0-1-0-0)	2 (0-0-2-0)	2 (0-0-2-0)	5 (0-1-4-0)
S. haemolyticus	1 (0-0-1-0)	0	0	2 (0-0-2-0)	3 (0-0-3-0)

S. 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)
K. 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)

Table 7. 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)
S. 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)
S. aureus	0	2 (0-1-1-0)	2 (1-0-1-0)	0	2 (0-1-1-0)	6 (1-2-3-0)
K. kristinae	2 (1-1-0-0)	0	0	0	0	2 (1-1-0-0)
E. 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)

Table 8. Biofilm profile results of bacterial species isolated from study group 3.

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)
S. 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)
S. aureus	3 (0-1-2-0)	1 (0-0-1-0)	0	0	0	4 (0-1-3-0)
A. viridans	2 (0-1-1-0)	2 (0-1-1-0)	0	0	0	4 (0-2-2-0)
k. pneumoniae	2 (0-0-2-0)	0	0	0	0	2(0-0-2-0)
K. 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)
P. 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)

Table 9. Biofilm profile results of bacterial species isolated from study group 4.

Sample source / Bacterial isolate	Surgical site infection N (n-w-m-s)
A. baumannii	3 (0-0-3-0)
A. veronii	1 (1-0-0-0)
B. cepacia	2 (0-2-0-0)
E. coli	2 (0-2-0-0)
K. kristinae	2 (0-2-0-0)
K. rosea	2 (0-2-0-0)
S. aureus	23 (1-4-13-5)
S. haemolyticus	2 (0-0-2-0)
Total	37 (2-12-18-5)

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

The current study revealed a high prevalence of *S. epidermidis* biofilm producing isolates in skin from patients before and during surgery. Most isolates of *S. epidermidis* were moderate biofilm producers, this agrees with the results of [23] in Iraq who demonstrated the presence of 42% moderate producing isolates. While other studies, such as the study done by [24] who found no biofilm producing isolates among *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, conduct that may be critical because wound healing can be delayed in the existence of Staphylococci biofilm [25]. In the current study, the isolates of *S. aureus* were moderate biofilm producers and this agrees with [26], while most isolates from post-surgical infections found by [27] in Iraq were strong biofilm producers. *Staphylococcus 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 and enhancing antibiotic resistance [28]. Furthermore, [29] found in his study that 6 of 8 of *S. lugdunensis* and 9 of 10 of *S. haemolyticus* were biofilm producing isolates, indicating that biofilm producers were more resistant to antibiotics from non-producers. *S. lugdunensis* is a rapid biofilm producer and within six hours the biofilm formation is complete. Therefore, it will show resistance for antibiotic tretment leading to persistent infections [30]. The formation of biofilm by *S. haemolyticus* differs from *S. epidermidis* and *S. aureus*, as the biofilm of the last two is mediated by the *ica* operon, which codes for the enzymes that participates in the formation of the biofilm matrix. Nevertheless, the biofilm formation by *S. haemolyticus* is mainly *ica*-independent because no *ica* genes were observed in this bacterium. However, it is not clear [31]. A study by [32] showed somehow results similar to the current study, such as presence of 33% and 6.6% of *A. baumannii* with strong and moderate biofilm production respectively, along with 15.4% P. *aeruginosa* had moderate and 7.7% weak biofilm production. In addition, the same above research noticed that 9.1% and 18.2% of *K. pneumoniae* isolates were moderate and weak biofilm producers, 25% of *E. coli* showed a moderate and strong biofilm production, while *B. cepacia* was non-biofilm producers. On the other hand, [33] had different results in his study, reporting *A. baumannii* and *K. kristinae* with strong biofilm production. *A. baumannii* produce a biofilm-associated protein encoded by the gene *bap* that modulates the cell surface to facilitate adherence to epithelial cells [34]. 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 [35].

CONCLUSIONS

This study found that there was a great diversity in bacterial genera and species isolated from different sites and stages before and during caesarean section surgery, and these species showed a clear variation in the nature of hemolytic enzyme production as well as their ability to form a biofilm, which gives an idea of

their virulence and the difficulty of controlling their spread and causing infection as well as delay treatment**.**

Disclosure and Conflict of Interest: The authors declare that they have no conflicts of interest.

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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How to Cite

D. A. . Edwar, E. N. Naji, and A. . Maleki, " Biofilm and Hemolysis Profile Index in Bacteria Isolated from Pre-Cesarean Surgery and Post Cesarean Infections", *Al-Mustansiriyah Journal of Science*, vol. 34, no. 4, pp. 8–18, Dec. 2023.