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Risk factors and skin colonization/recolonization – a matter in the development of sternal wound infection after cardiac surgery

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ABSTRACT

Cardiac surgeries are classified as “clean procedures” as they do not involve access to any space populated by the patient's microbiota. Nevertheless, the development of sternal wound infection (SWI) can sometimes occur. The aim of the study was to analyze risk factors of SWI, efficacy of antiseptics procedures, skin microbiota colonization/recolonization, and their impact on infection development in patients undergoing cardiac surgery.

Of 500 patients who had undergone cardiac surgery through a median sternotomy, perioperative swabs of standardized surgical cut area were taken at three-time points: ‘1’-after admission to treatment rooms, ‘2’-before incision; ‘3’-before chest reconstruction. Material was cultured and grown bacterial colonies were counted.

Out of the total population, 23 patients developed SWI and 11 had suspicion of SWI while 466 were without SWI. Risk factors which had statistically significant impact of infection development were high body mass index (BMI) and use of cardiopulmonary bypass (CPB) during operation. The most common SWI-pathogens were coagulase-negative staphylococci (CoNS) and Gram-negative bacilli. From the perioperative period, CoNS and *Staphylococcus aureus* were isolated. When simultaneous positive cultures in 'swab-1 and -3' were observed (respectively, ≥ 87 and ≥ 17 bacterial colonies), the risk of complications was higher (patients with SWI – 69.57%; without SWI – 6.71%). Regarding point ‘3’, significant differences were noted in the general level of grown colonies. About 3 times more bacterial colonies were grown in the group with healing problems and SWI. SWI development is multifactorial. The rate of skin microbiota recolonization during surgery may suggest that being more colonized in the surgical incision area was more susceptible to local complications.

INTRODUCTION

The progress achieved in interventional medicine is undoubtedly immense, especially in cardiac surgery. Cardiothoracic surgeries sometimes represent the only form of treatment for patients with coronary disease, congenital defects, or heart valve issues. Although cardiac surgery is often called “clean surgery”, it is associated with a high risk of postoperative complications, including infections. These complications can increase morbidity, prolong hospitalization, raise associated costs, and occasionally lead to the patient's death. Following sternotomy, the risk

of developing both superficial and deep wound infections varies between 3.5% and 26.8%. Superficial Sternal Wound Infection (SSWI) affects only the skin and subcutaneous tissue. However, a deep sternal wound infection (DSWI) can lead to inflammation of the entire mediastinum, significantly increasing the patient's risk of death [1].

It is crucial, therefore, to take preventive measures by using sterile equipment and instruments, and ensuring proper care of patients during the perioperative period. Efforts are made to prepare patients as effectively as possible, including excluding carriers of *Staphylococcus aureus* in the nasopharynx, using antiseptic baths, appropriate patient transport to the operating room, hair removal

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at the incision site if necessary, decontamination, covering the surgical field, and implementing suitable perioperative antibiotic prophylaxis (OPA). It is also essential to monitor blood glucose levels continuously, optimize patient hydration, ensure adequate perfusion to maintain hemostasis, and maintain proper normothermia [2,3]. Administering antibiotics before the procedure aims to reduce contamination by environmental microorganisms and those constituting the patient's microbiota [4,5]. Sources indicate that endogenous biofilm, often composed of *S. aureus* or coagulase-negative staphylococci (CoNS), is the cause of surgical site infections (SSI)/sternal wound infection (SWI) in patients after sternotomy. Hence, early antibiotic therapy is crucial for reducing biofilm formation [6].

During cardiac surgeries, there can be a faster recolonization due to the invasiveness of the procedure, its duration, increased perfusion time (>100 min, especially in obese patients), intra-aortic counterpulsation, the need for perioperative red blood cell transfusion (>6 um) or plasma (>800 ml), and the use of cardiopulmonary bypass (CPB) with prolonged duration, leading to the induction of the inflammatory process in the body. Repeated reoperations and prolonged bleeding from the wound also affect the development of postoperative infections [6-9]. The aim of the study was to analyze frequency and etiological agents of SWI. Moreover, our intent was to assess the impact of potential colonization and recolonization of the surgical access site, as well as the effectiveness of the operative field antiseptic procedures, on the risk of surgical site infections in patients undergoing cardiac surgical procedures with a median sternotomy.

MATERIAL AND METHODS

Study group

The selected study group consisted of 500 patients: 349 males (69.80%) and 151 females (30.20%) undergoing elective cardiac procedures at the Department of Cardiac Surgery (Medical University of Lublin, Poland), within 18 months. The patients were in different age ranges: >60 years old – 356 (71.20%) individuals, between 50-60 years old – 111 (22.20%) and <50 years old – 33 (6.6%). Patients had a history of coronary artery disease (70.40%), hypertension (61.80%), diabetes (20.40%), hyperlipidemia (20.80%), asthma (4.00%), chronic obstructive pulmonary disease (COPD) (7.00%), recent heart attack (8.60%), and kidney failure (1.60%). The average BMI was 27.73, indicating overweight. All required cardiothoracic treatment with a median sternotomy. Patients operated on in the morning hours were selected. Most of the surgeries were performed using cardiopulmonary bypass (CPB) (97.40%, $p=0.001$). The average duration of the procedure was 221.87 minutes. Informed consent for the use of the results for scientific purposes was obtained from all participants.

Clinical material

The research materials consisted of swabs taken from the surgical field, which is the surgical incision area (median sternotomy) collected during the intraoperative period:

- ‘Swab 1’: taken upon admission to the operating room, before the use of antiseptic agents.

- ‘Swab 2’: taken after the use and drying of antiseptic agents, in the operating room, before draping the surgical field and making the surgical incision (preparation of Povidone-iodine/PVP-I; antiseptics with ethanol, isopropyl alcohol, benzyl alcohol).
- ‘Swab 3’: Checking the cleanliness of the field – the incision site before chest reconstruction - swab from the edges of the wound.

Swabs were collected using a sterile 0.9% NaCl-moistened swab from the standardized surface, a 3×15 cm area (by the “zigzag method”). After collection, the samples underwent microbiological analysis in the following order:

- a) Inoculation onto appropriate microbiological media (Columbia agar, Mannitol salt agar, MacConkey agar, Sabouraud agar).
- b) Incubation at 37°C for 24-48 hours under aerobic conditions.
- c) Counting of grown bacterial colonies and characterization of their growth on solid media (macroscopic colony evaluation).
- d) Preparation of a microscopic slide – staining with the Gram stain method.
- e) Biochemical identification and detection of resistance mechanisms.

Statistical analysis

The obtained data were subjected to statistical analysis using the Statistica v.10 software (StatSoft), while MS Excel 2010 (Microsoft) was used for data collection, entry, and support of the aforementioned analyses.

RESULTS

Among the study group, 23 patients developed SWI (SSWI – 14; DSWI – 9), which is 4.6%. The diagnosis was based on changes within the wound and microbiological test results. What is more, among patients with SWI, 18 had only wound infection, while the remaining 5 had SWI coexisting with another type of infection. In 2 individuals, endocarditis occurred ($p=0.043^*$). The surgery was mostly planned and performed with the use of cardiopulmonary bypass, CPB (86.96%; $p=0.001$), which had a statistically significant impact on infection development. The most common type of procedures was a single CABG (Coronary artery bypass graft surgery). We found that the type of operation did not have a significant influence on SSI. The average duration of cardiac surgery was 236.00 minutes. The average time of hospitalization was 38.65 days

In the group of “non-SWI” patients (477 individuals), there was a subgroup of patients with “suspected SWI” – 11 individuals. In these patients, postoperative clinical symptoms and delayed wound healing raised suspicion of infection. However, microbiological tests did not confirm this.

In the “non-SWI” group, the surgery was also planned and performed with the use of cardiopulmonary bypass – CPB (97.90%; $p=0.001$). The most common type of procedures was a single CABG procedure. The average duration of the surgery was 221.18 minutes and the average duration of hospitalization was 11.32 days.

Comparing the BMI values in the different groups (individuals with SWI – 30.17 vs. without SWI – 27.61), the significant influence of body mass index ($p=0.013^*$) on wound healing complications was demonstrated. In the respective groups, the duration of the operation did not have statistically significant importance in the occurrence of wound healing difficulties ($p=0.841$).

The most common infectious complication in the group without SWI, as well as in the group with SWI, was ventilator-associated pneumonia (VAP). Statistically significant differences between the groups were noted for the occurrence of both VAP and bloodstream infections ($p=0.000^*$)

Colonization and recolonization of the surgical area and the occurrence of sternal wound infection (SWI)

Cultures were performed on perioperative swabs collected at three time points from the surgical incision area of 500 patients. Only staphylococci were isolated from the obtained material. Based on the analysis of the tested material, it was found that the swabs contained strains of *S. aureus* and coagulase-negative staphylococci (CoNS, including *S. epidermidis*, *S. haemolyticus*, *S. hominis* and *S. capitis*). Methicillin sensitivity was determined for all isolated staphylococcal strains.

Positive culture results were obtained from swabs collected at the surgical incision site at three different time points, yielding various combinations of positive and negative results. The results are presented in Table 1, where the growth of microorganisms from a specific swab is marked as “+” for positive and “-“ for negative,

Table 1. Comparison of the growth of microorganisms from swabs (1, 2, 3) in different variants

*Swabs (1,2,3): culture results at different time points	Total number of patients		SWI group	Non-SWI group	
	n	%		Suspicion of SWI n	Without SWI complications n
1+ 2- 3-	224	44.8	1	0	223
1+ 2- 3+	239	47.8	22	11	206
1+ 2+ 3-	7	1.4	0	0	7
1+ 2+ 3+	30	6.0	0	0	30
total	500	100%	23	11	466

* (-/+) Results of negative/positive culture grow from a specific swab, regardless of the number of grown colonies (cfu)

On the incision surface (3×15 cm), a variable number of bacterial colonies grew from a single swab. With ‘swab 2’ – after the use of the antiseptic agent, it was observed that only in 37 cases, single bacterial colonies appeared – ranging from 1 to 5 colonies (with an average of 2.9 colonies). For ‘swab 1’ and ‘swab 3’, a different number of colonies was recorded, which depended on the patient group under investigation, i.e., those who later developed septic (SWI) or aseptic (suspected SWI) wound changes, or patients with a normal postoperative healing process at the surgical access site (without SWI).

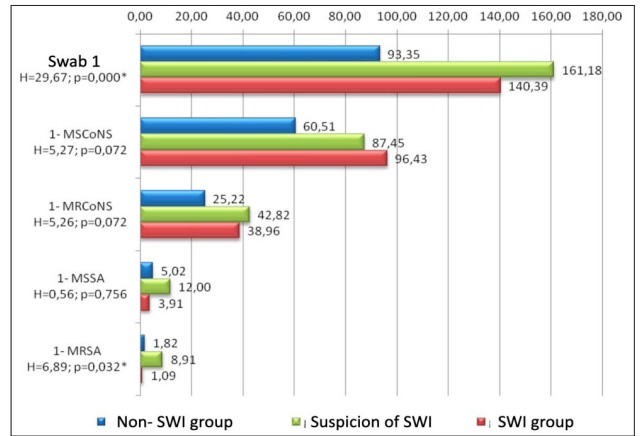
Table 2 presents the distribution and data regarding the number of grown colonies from ‘swab 1’ and ‘swab 3’ for each group: total.

Table 2. Summary of results regarding colonization and recolonization of the surgical incision area for each study group

Study group		Total number of bacterial colonies grown			
		‘Swab 1’		‘Swab 3’	
		n	SD	n	SD
Non-SWI	Without SWI complications	93.35	92.32	10.39	15.84
	Suspicion of SWI	161.18	53.79	32.00	12.58
SWI		140.39	85.16	31.52	18.16

SD – standard deviation

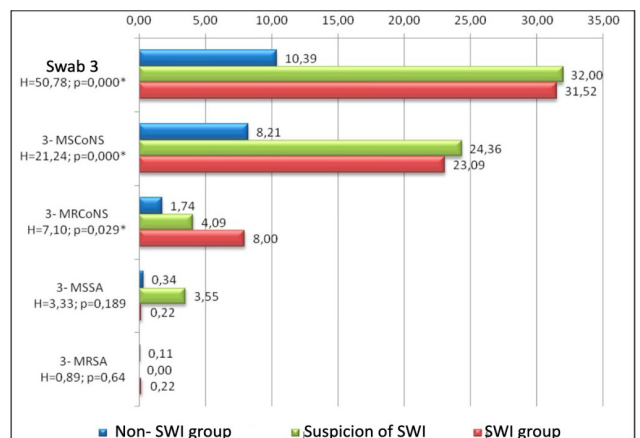
The results from individual groups were compiled and compared with a determination of statistical significance (Figs. 1 and 2).



MScoNS – methicillin-sensitive coagulase-negative staphylococci, MRCoNS – methicillin-resistant coagulase-negative staphylococci, MSSA – methicillin-sensitive *Staphylococcus aureus*, MRSA – methicillin-resistant *Staphylococcus aureus*; p – statistical significance

Figure 1. Analysis of the number of grown colonies from ‘swab 1’ in individual groups

Statistical analysis revealed statistically significant differences in the overall average level of ‘swab 1’ examination and with respect to MRSA strains. In both cases, the highest number of colonies occurred in the group with suspected SWI, followed by the SWI group. This suggests that patients who were more colonized on the standardized sampling surface were more susceptible to complications at the surgical access area.



MScoNS – methicillin-sensitive coagulase-negative staphylococci, MRCoNS – methicillin-resistant coagulase-negative staphylococci, MSSA – methicillin-sensitive *Staphylococcus aureus*, MRSA – methicillin-resistant *Staphylococcus aureus*

Figure 2. Analysis of the number of grown colonies from ‘swab 3’ in individual groups

Statistical analysis showed statistically significant differences in the overall level of ‘swab 3’ examination and with respect to individual groups of MSCoNS and MRCoNS microorganisms. Approximately three times more colonies grew in patients with wound healing complications and SWI.

The skin colonization at the surgical site in the SWI group – 23 patients – was analyzed for the occurrence of superficial infection (SSWI) in 14 individuals and deep infection (DSWI) in 9. No significant differences were found between the groups.

Colonization and recolonization of the surgical field and patient classification

An assessment of the risk of wound healing complications and surgical site infection was made depending on the number of bacterial colonies grown in the surgical field (3×15 cm). Based on the observations of the microbiological test results, the patients were categorized into risk groups (Table 3).

Table 3. Categorization of SWI risk based on colonization and recolonization of the surgical field in comparison with test results

Swab	Risk category of SWI	
	lower	higher
‘Swab 1’	<87 CFU	≥87 CFU
‘Swab 3’	<17 CFU	≥17 CFU
Group	Results of the study	
With SWI n=23	7 ppl (30.43%)	16 ppl (69.57%)
Without SWI n=477	445 ppl (93.29%)	32 ppl (6.71%)

ppl – people

Etiological agents of SWI and comparison with the results from the perioperative swabs

The most common bacteria isolated from SWI were *Staphylococci* CoNS (19 ppl/82.6%) – mostly *S. epidermidis*. What is more, there were Gram-negative bacilli isolated from 7 ppl/30.43% (*Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae*). We also found *S. aureus* (3 ppl/13.04%) and *Enterococcus faecalis* (4 ppl/17.39%). In 8 cases with SWI, we isolated several (2-4) types of bacteria during the infection period.

In 60.87% of all patients, the isolated agent from SWI were the same type of staphylococci with the same type of resistant to methicillin as that isolated from the swabs collected in the perioperative period. However, we did not undertake genetical analyzes of SWI agents and those from swabs, hence, we could only speculate that it was the same strain. It should also be noted that in several cases, the infection was of mixed etiology and it is possible that staphylococcal agent only colonized already infected wound surfaces. We are aware of all of this limitation of our work.

DISCUSSION

In publications, much attention is devoted to aseptic and antiseptic procedures, patient preparation for surgery, and draping of the surgical field. All of these efforts aim to improve the prevention system and, consequently, reduce infectious complications after surgery. There are studies

that demonstrate the effectiveness of preoperative antiseptic baths and the influence of patient colonization on the occurrence and speed of surgical site infections. This seems to be an important issue because the incision area, and later the postoperative wound, is exposed to external factors and can become quickly contaminated and colonized depending on how many microorganisms were isolated from a small surface – the incised body layers. Depending on the effectiveness of preoperative antiseptic baths, patients are more or less colonized by normal microbiota. However, as our research results from the first stage of collecting swabs from the surgical incision site (‘swab 1’) showed, methicillin-resistant staphylococcal strains were also isolated from patients. This may suggest colonization by hospital flora (possibly due to frequent previous hospital stays). It is emphasized that after a patient is admitted to the ward, before the procedure, colonization with hospital strains can occur as early as 24 hours after admission. Additionally, improper antiseptic measures can potentially cause the translocation of microorganisms to the incision site [10,11].

Scientific research analysis indicates that preoperative antiseptic bathing leads to the mechanical reduction of microorganisms residing on the skin. However, the effect of reducing local infections is not straightforward [12]. From our own research experience, it can be concluded that patients who underwent such preoperative bathing, with swabs (‘swab 1’) taken from the surgical incision site (3×15 cm), had staphylococci isolated with varying resistance and in varying numbers. Most frequently, MSCoNS staphylococci were obtained, followed by MRCoNS, and only occasionally was *S. aureus* (MRSA and MSSA) isolated. The type of antiseptic preparation plays a significant role in this regard, as reported in the literature. Agents containing chlorhexidine appear to be more effective in reducing MRSA strains from the skin, as compared to those containing triclosan. Furthermore, as for triclosan, it has been withdrawn from antiseptic products in both Europe and the United States due to research results that contradicted its bactericidal effectiveness and confirmed its carcinogenic properties [13]. On the other hand, there is little difference in reducing CFUs on the skin between chlorhexidine and octenidine dihydrochloride [12].

In our research, in the group that did not experience an infection, ‘swab 1’ taken before the procedure showed an average of 93.35 bacterial colonies in total. In contrast, in patients who experienced complications in terms of wound issues, an average of 140.39 colonies were cultured on the agar plates. The highest number was observed in the group of patients with suspected SWI – 161.18 colonies. This demonstrates significant variation and underscores the importance of performing antiseptic actions accurately before the procedure. Over all, our findings indicate that the greater the reduction in resident flora, the lower the risk of infectious complications at the surgical site.

Patient-dependent risk factors are another consideration. These factors appear to be indirectly related to the effectiveness of the antiseptic bathing process (among others, excessively developed subcutaneous tissue (high BMI), which may contribute to greater colonization, as well as thoroughness during the preoperative antiseptic process

performed by the patient). The condition of the patients' skin can also influence the number of isolated microorganisms in the perioperative period. Additionally, Kühme *et al.* suggest that gender is another important factor regarding colonization. They noted in their studies that the number of microorganisms on the skin surface was greater in men than in women during the perioperative period, with statistically significant differences found for CNS (Coagulase-Negative Staphylococci) and *Cutibacterium acnes* (*Propionibacterium acnes*) [14]. Moreover, as reported by Furui M. *et al.*, certain medical conditions significantly affect wound healing and the development of potential infections following sternotomy, such as diabetes and kidney failure [15].

As research results have shown, the use of the right antiseptic in the operating room is an effective method for eliminating microorganisms ('swab 2'). Complete sterilization of the skin is not possible because the resident flora resides in hair follicles, sweat glands, and sebaceous glands, and gradual recolonization occurs during the procedure [16,17]. Therefore, it is essential to determine whether and how many bacteria will be isolated during the closure of the surgical incision site. In cardiothoracic surgery, bacteria are almost always present in the wound at the end of the procedure. Therefore, self-adhesive drapes are routinely used, which are a recognized method to prevent infections. Their purpose is to protect the wound, isolate adjacent skin from the incision site, and protect against the invasion of microorganisms present on the skin during surgery. However, experimental studies do not necessarily confirm the effectiveness of such surgical draping in reducing surgical site infections [18]. Falk-Brynhildsen *et al.* examined the recolonization of the skin in 140 individuals during cardiac surgery (median sternotomy site). In their study, they observed an increase in *C. acnes* and CNS. Unfortunately, limitation of our study was using only aerobic culture, so the studies cannot be fully compared. What is more, Falk-Brynhildsen *et al.* when analyzing different groups: individuals who used self-adhesive surgical drapes and a group of patients who did not, they did not find significant effectiveness of the self-adhesive draping in preventing recolonization. In fact, there was even evidence that it accelerated recolonization [18]. Similar results were obtained by Rouquette *et al.*, who studied the effectiveness of drapes impregnated with antiseptic agents and those that were not. In both cases, the drapes did not prevent recolonization during the procedure. Sources suggest that these drapes may even accelerate the recolonization process of bacteria by tissue maceration [19]. In our study, recolonization (during the procedure) occurred in 53.8% of the patients ('swab 3'). It is worth noting that patients with later SWI (23 individuals) showed a positive bacterial growth in 95.65% of all cases (22 individuals) in the swab taken before closure ('swab 3'), and in the group with suspected SWI, positive results were observed in 100% of all cases (11 individuals). Thus, we can expect recolonization in patients who experience wound healing complications. However, this is not a prerequisite for the development of SSI, as research shows that recolonization also occurred in 50.64% of the group without complications.

As discussed earlier, staphylococci dominate the etiology of SSIs, but Gram-negative bacteria are also observed [20].

In the context of recolonization, these microorganisms are very rare and primarily result from hospital colonization during the patient's hospital stay. This is because they are not part of the normal flora of the anterior chest area [14]. In our own research, we did not observe an increase in these bacteria in recolonization; only staphylococci were isolated.

In the context of the discussed recolonization, it is essential to pay attention to the number of isolated bacteria from the edges of the wound at the end of the surgical procedure ('swab 3'). Our research results indicate that the resident flora remained below the initial values ('swab 1'). Recolonization occurred at a level about 5 times lower compared to the initial state for the group where later SWI was observed (140.39 vs. 31.52 colonies) or suspected SWI (161.18 vs. 32.00 colonies). In the group without complications, if microorganisms grew, which was observed in 50.64% of all patients, recolonization was about 9 times less compared to the initial state (93.35 vs. 10.39 colonies). Thus, there was differentiation between the analyzed groups regarding the total number of cultured colonies and individual bacteria, such as MSCoNS and MRCNS.

In summary, the number of bacteria at the incision site before the procedure (after the bath) influenced the number of bacteria involved in recolonization (during the closure of the body layers). It is important to emphasize that based on the number of cultured colonies in 'swab 1' and 'swab 3' in patients with SWI, we cannot determine whether it will be a superficial or deep infection. This is because the number of colonies cultured was similar.

Therefore, we can conclude that the patient's proper hygiene habits, a thorough and correct antiseptic bath, and the appropriate application of antiseptics determine the clinical effectiveness in recolonizing the closed wound, thereby minimizing the risk of infection.

Moreover, the likelihood of recolonization increases depending on the extent of the incision site and prolonged exposure to the operating room conditions, often exceeding 4 hours [21,22]. In our own study of a group of 500 patients in which surgical site colonization was examined, the average surgery time did not exceed 240 minutes (average 221.87 minutes), and the difference between groups was only 13 minutes (236 vs. 221.18 minutes). However, time did not play a significant role in recolonization here. In the Kühme *et al.* study, there was also no observed correlation between the length of cardiothoracic surgery and the number of bacteria in the wound [14]. Nevertheless, it should always be considered that a prolonged procedure significantly increases the likelihood of incision contamination or tissue desiccation [22].

The risk of infectious complications is a component of the number and virulence of microorganisms, as well as the local and systemic host response. According to the definition, an infection occurs when the number of pathogens reaches 10^5 CFU/g of tissue (colony forming unit), and in the case of opportunistic microorganisms, 10^3 CFU/g of tissue. For example, 10^4 CFU/g of tissue may not induce a response in a patient, whereas 10^5 of the same microorganism will cause an infection. Therefore, strains with greater virulence can cause changes with significantly fewer of them than strains with lower virulence [23]. It is worth noting

that in our study, out of 23 patients with SWI, a relationship between intraoperative colonization and the etiological agent of wound complications was observed in 15 of them. Among other issues, bacteria isolated during the intraoperative period were later isolated from complicated wounds.

So, could there be some cutoff point, a scale, that would help at least somewhat determine and classify patients into a group at risk of subsequent surgical wound contamination and infection development depending on the preoperative contamination of the incision site? Is there a predictive value for SSI in the quantity of microorganisms recolonizing the skin during surgery? Based on the results of our own research, we attempted to assess the risk of exposure to possible changes in the wound and classify patients. It can be assumed that, in order to assign a patient to the SWI risk group, microorganisms must simultaneously increase on the incision surface before the use of the proper antiseptic agent ('swab 1') and simultaneously at the end of the procedure ('swab 3'). The high-risk group would include those operated on in whom ≥ 87 bacterial colonies (grown from swabs collected from standardized surface and technic) increased before cardiac surgery, and at the same time, ≥ 17 colonies before closing the layers of the body. It should be noted that swab sampling helps us a little determine microorganisms in a qualitative and semi-quantitative way [24]. In light of the results obtained, considering that the skin is an endogenous source of infections, stratifying risk by assessing contamination may be helpful in further postoperative management. In that case, the risk of adverse infectious complications could be reduced.

We are aware of the significant limitations of the method used in our research of this problem; it is neither perfect nor quantitative. It, however, provides a general view of the potential strength of colonization. We recognize that the currently recommended method for determining wound colonization is CFU/g tissue or semi-quantitative analysis using the plait count-tact method.

CONCLUSIONS

A surgical site infection significantly prolongs the patient's hospitalization and increases treatment costs. Moreover, the patient becomes susceptible to additional types of infectious complications. The development of a surgical site infection is influenced by various risk factors related to the patient themselves, as well as the preparation of the patient for the procedure in terms of antiseptics and the recolonization of the surgical incision site during cardiothoracic surgery.

Analysis of the impact of risk factors identifies patients with a high body mass index and long CPB duration as those who should be given particular attention in predicting future infections. The profile of microorganisms involved in sternal wound infections is diverse, ranging from Gram-positive cocci to Gram-negative bacilli. Due to the wide spectrum of multidrug-resistant strains in the hospital environment, it is essential to determine their antibiotic susceptibility profile when initiating treatment.

Despite the limitations of our research about colonization/recolonization of incision area, the assessment of the

number of cultured bacterial colonies from a standardized surface can have some predictive value and can also aid in risk stratification and the classification of patients into low or high-risk groups for wound healing complications

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

INFORMED CONSENT STATEMENT

Informed consent was obtained from all subjects involved in the study (Bioethics Committee approval ref. KE-0254/185/2011). Informed consent has been obtained from the patient(s) for the use of results for scientific purposes.

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