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Study on the Identification of Aerobic Bacteria in the Occurrence of Burns and Determination of the Severity of Infection due to Burn

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ABSTRACT

Background: Although the advancement of treatment strategies improves outcomes for burn patients, they often develop infections that could be life-threatening. Multiple factors can lead to aerobic bacterial colonisation of the burn wound, including epithelial damage, immune suppression, and the use of invasive medical devices. It is necessary to determine the bacterial profile and understand antimicrobial resistance patterns to initiate effective treatment and prevention of septicemia in burn patients.

Methods: A prospective and cross-sectional study was conducted over one year at Thrissur Government Medical College. Sterilized Swab samples were collected from the burn wounds of 109 patients admitted to the burn ward and ICU at baseline, 5th and 10th days. The samples were cultured, and the bacterial isolates were identified.

Results: The study determined that 66% of the initial swabs were sterile, while bacterial isolates were observed in 34% of the patients. *Staphylococcus aureus* was the major pathogen, detected in 54.05% of positive cultures, followed by *Pseudomonas aeruginosa* (29.70%) and CONS (10.28%). We also found trace amounts of other bacteria (*E. coli*, skin flora, aerobic spore-forming bacteria, and CONS) in the wound infection. The study also indicates a higher incidence of severe burns in younger patients, with females being more affected than males. In the second swab, the sterility rate decreased sharply to 18%, while the percentage of isolates increased to 82%, with *P. aeruginosa* emerging as the dominant bacterium at 58.33%. In the third swab, the rate of *P. aeruginosa* also increased to 61.11%, while that of *S. aureus* decreased to 5.55%.

Conclusion: The study highlights the high incidence of severe burn injuries among females, predominantly from open flames, with significant bacterial pathogens like *S. aureus* and *P. aeruginosa* necessitating targeted infection control and early, precise antibiotic therapy.

Key-words: Burn wound, ICU, Antimicrobial therapy, Microbial isolates, Severity, Infection

INTRODUCTION

Despite recent improvements in burn wound treatment, infection continues to be a major cause of death for burn patients. The prevention of infection can be aided by early excision & grafting of the burn eschar, advanced dressings, topical antiseptics and antimicrobials, but systemic antimicrobial therapy is still often required when local defences are overwhelmed by infection [1].

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Sepsis is a serious risk for burn patients because of several factors: harm to the epithelium that allows pathogens to colonize the wound; localized loss of innate immune cells and proteins due to loss of fluid and protein; implantation of intrusive devices like respiratory tubing and catheters; and hyper-metabolic states that further reduce the number of amino acids that trigger natural immune responses as well as neutrophil dysfunction [2].

About 75% of burn patient fatalities still result from infection over the burned region, leading to septicemia, despite significant advancements in burn wound management. Another significant contributing factor to the length of hospital stays and postponement of skin transplantation is infection from burns [3]. In every burn

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unit, bacterial isolates from burn victims' wounds are known to change over time and across geographic regions. An increasing number of formerly uncommon pathogens, such as Candida and Enterobacter, are being identified in burn wounds [4]. The bacteria are further isolated from the host defense system and antibiotics are systemically delivered due to the avascularity of the charred region. Knowing the organism pattern that predominates on burn sites would therefore enable early systemic antibiotic administration in the event of an impending septic episode [5].

Both superficial swabs and tissue samples from burn sites are recovered, and the clinical microbiology laboratory identifies any potential bacteria. The most popular method for determining the degree of invasive wound colonisation is Gram staining; however, using a nucleic acid-selective acridine orange dye that resists alcohol-induced bacterial washing off the slide can yield more reliable results [6]. To distinguish between invasive infection and burn wound colonization, polymorphs must be present in the microscope image. Regular burn swab cultures provide valuable insights into potential microorganisms that cause colonization and infection, along with their corresponding antibiograms. A MacConkey agar plate and a Blood agar plate are inoculated using swabs obtained from the infected burn sites [7]. Plates are typically checked for growth after 24 hours of aerobic incubation at 37°C; however, anaerobic cultures are also performed, with plates incubated for 3 days in the event of a diagnosis of a deep, invasive infection. Rapid detection of infectious agents is crucial for guiding appropriate antimicrobial treatment and is associated with shorter hospital stays and lower medical expenses related to burn infections [8]. New techniques have been studied, including antigens for bacterial, fungal, or viral detection and matrix-assisted laser desorption/ionisation mass spectrometry (MALDI-TOF MS; Bruker, Australia). MALDI-ToF is already widely used in labs worldwide as a favored method for identifying bacteria and fungi [9]. It functions by measuring the mass-to-charge ratio of ions produced by a laser beam incident on a sample, then comparing the resulting spectra with previous spectra. Even though they yield immediate effects, clean cultures are necessary. Consequently, the time required for identification varies with the rate of growth of the target organism [10].

MATERIALS AND METHODS

Study design- This is a prospective and cross-sectional study among patients about the growth of aerobic bacteria in the case of burn wounds. The study was conducted among burn wound patients, including both male and female patients, admitted to the burn ward and the ICU department of Thrissur Government Medical College. The study was conducted over about 1 year, from 1.6.2011 to 31.5.2012. All patients admitted to the burn ward and the ICU at that government hospital were included in the study, and data were collected. A sample was collected from the burn wound area of the patients using a sterile swab, and further processing was performed. The number of swabs required for testing will be based on the number of patients in the ward. The study involved a detailed analysis of 109 burn cases, focusing on patient demographics, burn causes, burn severity, and the microbial profiles obtained from swab cultures. Initially, data on the age and sex of the patients were collected and categorized to determine the distribution across age groups and between genders. It was found that the majority of the patients were under 30 years old, with 38.5% falling into this category, and females represented 69% of the total cases, highlighting a higher prevalence of burn injuries among women.

The causes of burns were also documented, with flame burns being the most common, accounting for 90% of the cases, followed by scalds at 8%. Less common causes included chemical and electrical burns, each contributing to 1% of the cases. The study further assessed burn severity by calculating the total body surface area (TBSA) affected in each patient. The TBSA ranged from 5% to 100%, with a mean of 39.2%. Over half of the patients (53%) had burns covering more than 30% of their bodies, indicating a high prevalence of severe burns.

To monitor the microbial profile of the burn wounds, periodic swabs were collected on the 0th, 5th, and 10th days. The initial swab culture results showed that 66% of the swabs were sterile, while 34% exhibited bacterial growth. Subsequent swab cultures revealed a dynamic shift in the microbial environment, with significant numbers of bacterial isolates, particularly P. aeruginosa and S. aureus, becoming increasingly dominant in later cultures. The study also included a comparison of swab cultures and biopsies in 30 cases, finding that 60% of biopsy results were concordant with the swab cultures, while 20% showed discrepancies. This comprehensive

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approach provided valuable insights into the demographics, causes, severity, and microbial landscape of burn injuries, emphasising the importance of targeted infection control and appropriate treatment strategies.

Inclusion criteria

- Patients with only burn wounds will be considered for the study.
- Both male and female patients in the ward and ICU were included.
- Patients with proper self-consent and approval were included.

Exclusion criteria

- Patients moving out of the ward were excluded
- Patients who had died after being admitted to the hospital were excluded from the study.

Statistical Analysis- The study was analyzed using SPSS and MS Excel. The analysis examined patient demographics, burn causes and severity, and the microbial environment from swab cultures in 109 burn cases. Data on age, gender, and burn causes were categorised and analysed to identify trends. Burn severity was assessed using total body surface area (TBSA) measurements. Swab cultures were collected at intervals to monitor the presence and evolution of bacterial pathogens, and the results were compared with biopsy findings for concordance. The data were then analyzed to identify patterns in pathogen prevalence, burn severity, and the effectiveness of swab cultures in detecting infections.

RESULTS

Analysis of 109 burn cases provided crucial insights into patient demographics, burn causes, severity, and baseline swab culture results. Regarding age distribution, we found that the largest number of patients was under 30 years of age, accounting for 38.5% of cases. The next most represented age group was 30-39 years, accounting for 22.01% of the total. Older age groups, especially those aged 71-80 and older, were underrepresented, with the lowest representation (2.75%) (Table 1).

Table 1: Distribution of patients in relation to age

Age in years	No of cases	Percentage (%)
<30	42	38.5
30-39	24	22.01
40-49	18	16.51
50-59	12	11
60<	13	11.92
Total	109	100

The mean age of the patients was 38.88 years. The gender distribution shows that 69% of the patients were female. In comparison, 45% were male, resulting in a female-to-male ratio of approximately 1.4:1, indicating a higher prevalence of cases among females and significant differences in burn incidents between the sexes (Table 2).

Table 2: Distribution of patients in relation to sex

Sex	Number	Percentage (%)
Male	40	45
Female	69	69
Total	109	100

A large proportion of patients were from rural areas, and most injuries occurred at home rather than at work. Flame heat was the predominant cause of burns, accounting for 90% of the cases, while scalds or hot liquids were the second most common reason (8%). Chemical and electrical burns were less frequent, accounting for only 1% of all cases each. Especially, 80% of burns were caused by kerosene, indicating that open flames associated with cooking or other household activities are a critical risk factor for burns in this study population (Table 3).

Table 3: Causes of burn as found in this study

Cause of burns	No. of cases	Percentage (%)
Flame	98	90
Scald	9	8
Chemical	1	1
Electrical	1	1
Total	109	100

The extent of injury was determined by assessing burn severity in 109 cases. We found 53% of patients had burns covering more than 30% of their total body surface area (TBSA), indicating a high prevalence of severe burn injuries, with 47% of patients having minor burns, as they covered less than 30% of surface area. The TBSA ranges from 5-100% (lowest at 8.25% and highest at 3.66% of

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cases), with a mean of 39.2%. Overall, 79 cases (72.4%) had face, chest and abdomen injury and were confined to extremities among 30 (27.52%) patients (Table 4). This room indicates that a large proportion of patients suffered severe burns, thus requiring intensive care. The study also revealed that 67 cases had both superficial and deep burns. We also observed that 8 patients with diabetes had a prolonged hospital stay of more than 3 weeks.

Table 4: Distribution of patients in relation to TBSA

Severity of burns	No of cases	TBSA
<10	9	8.25
10-20	28	25.68
21-30	14	12.84
31-40	19	17.43
41-50	14	12.84
51-60	7	6.42
61-70	5	4.58
71-80	3	2.75
81-90	6	5.5
91-100	4	3.66
Total	109	100

Periodic swabs were collected from burn wounds at 0, 5th and 10th days. Initial swab culture results demonstrated that 66% of the swabs were sterile, indicating no bacterial growth. However, bacterial isolates were observed in 34% of the cases. The result also shows the second and third swab cultures, focusing on the presence of sterile samples and isolates. In the second culture swab, 13 samples were sterile, accounting for 18% of the total, while the majority, 60 samples (82%), showed the presence of isolates. In the third swab culture isolate, the situation was slightly different: 39 samples (80%) contained isolates, and 11 samples (20%) were sterile. This indicates that while the number of isolates decreased from the second to the third swab, a significant proportion of the samples still showed bacterial presence, demonstrating relatively stable persistence of isolates across the two culture swabs (Fig. 1).

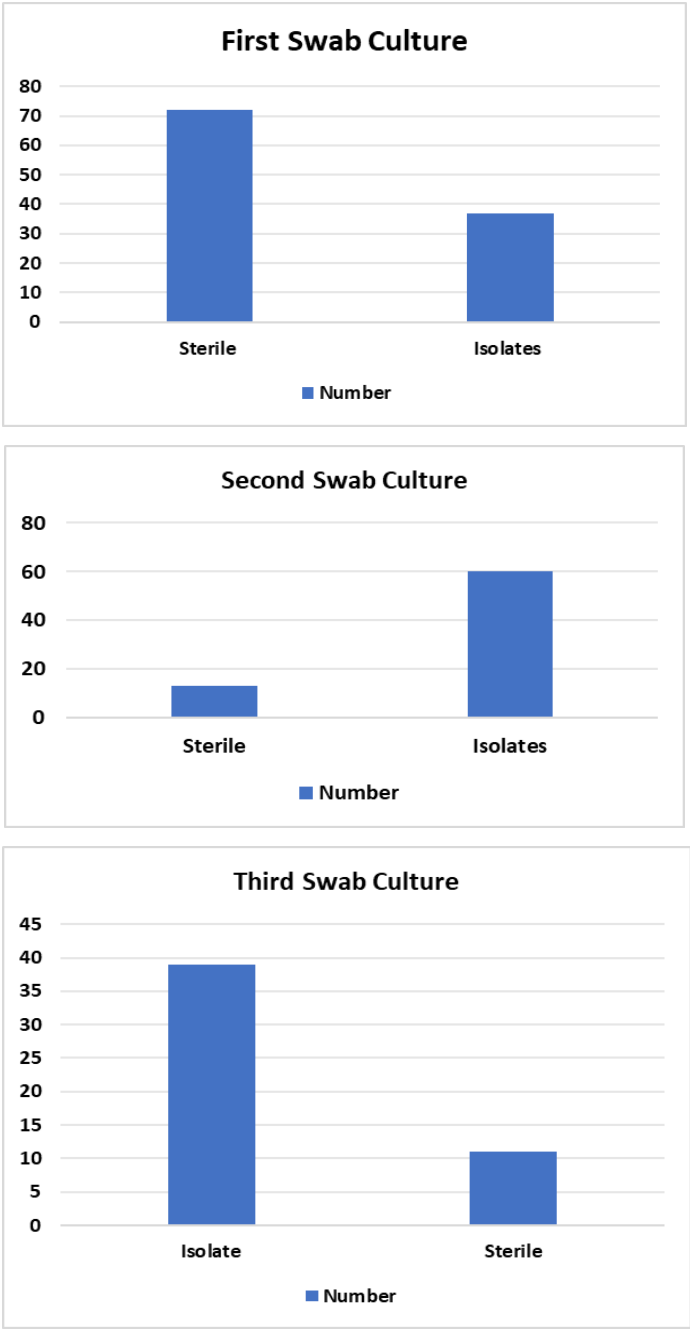

Fig. 1: Swab culture obtained from the burn wounds

The distribution of pathogens identified in three consecutive swab cultures. In the first swab culture, ***S. aureus*** was the most prevalent pathogen, identified in 20 cases (54.05% of the total isolates). *P. aeruginosa* followed this with 11 cases (29.70%). Other pathogens identified included coagulase-negative staphylococci (CONS) in 4 cases (10.28%), *E. coli* in 1 case (2.70%), *Corynebacterium* spp., and Environmental bacteria, each with 1 case (2.70%). In the second swab culture, *P. aeruginosa* became the most dominant pathogen, identified in 42 cases (58.33%), a significant increase from the first swab. *S. aureus* was identified in 18 cases

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(25.00%), a decrease compared to the first swab. Other pathogens detected included *E. coli* in 4 cases (5.50%), CONS and *A. baumannii* each in 3 cases (4.16%), and *K. pneumoniae* and *Proteus mirabilis* in 1 case each (1.50%). The third swab culture continued to show a high prevalence of *P. aeruginosa*, with 33 cases (61.11%) testing positive. There was a notable decrease in *S. aureus,* with only 3 cases (5.55%), and CONS**,** *E. coli***,** and *K. pneumoniae* were each identified in 4 cases (7.40%). Additionally, *Enterococci faecalis* was identified in 3 cases (5.55%), *A. baumannii* in 2 cases (3.70%), and Environmental bacteria in 1 case (1.85%).

Table 5 shows a shift in the predominant pathogens across the three swab cultures, with *P. aeruginosa* becoming increasingly dominant, while *S. aureus* decreased over time. This suggests a potential shift in the microbial environment or the effectiveness of treatment strategies as the cultures progressed.

Table 5: Identified pathogens with the number of cases for each

	No of cases	**Percentage (%)**
First swab culture isolates		
S. aureus	20	54.05
CONS	4	10.28
E. coli	1	2.70
Pseudomonas aeruginosa	11	29.70
Corynebacterium spp.	1	2.70
Environmental bacteria	1	2.70
Total	38	100
Second Swab Culture Isolates		
Pseudomonas aeruginosa	42	58.33
S. aureus	18	25
CONS	3	4.16
E. coli	4	5.50
Klebsiella pneumoniae	1	1.50
Proteus mirabilis	1	1.50
Acinetobacter baumannii	3	4.16
Total	72	100
Third Isolate Swab Culture		
Pseudomonas aeruginosa	33	61.11
S. aureus	3	5.55
CONS	4	7.40
E. coli	4	7.40
Klebsiella pneumoniae	4	7.40
Enterococci faecalis	3	5.55
Acinetobacter baumannii	2	3.70
Environmental bacteria	1	1.85
Total	54	100

Fig. 2 compares biopsy results with swab cultures in 30 cases. The findings indicate that in 20 of 30 cases (60%), the biopsy results were concordant with the swab culture results, meaning the same pathogens, or their absence, were identified by both methods. However, in 10 cases (20%), there was a discrepancy between the biopsy and swab results, with the pathogens identified or their presence not matching between the two methods.

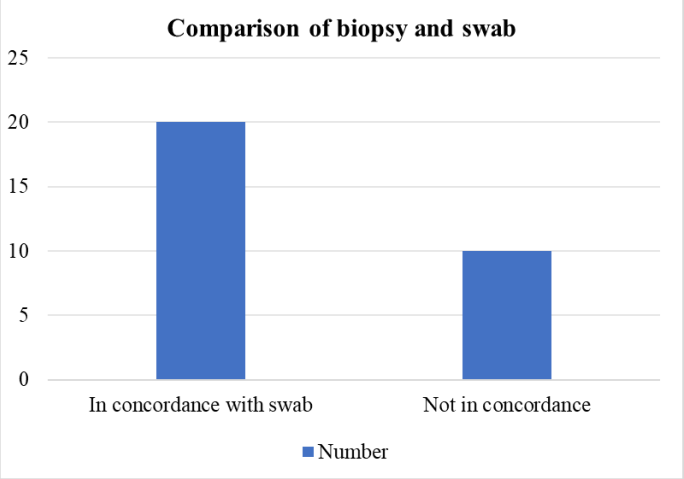

Fig. 2: Comparison of swab and biopsy

DISCUSSION

The current study provides an extensive overview of the demographic, clinical, and microbiological profiles of burn victims, with specific reference to injury and infection patterns. It was noted that there was an increased number of victims in the younger age groups, below 30 years, with females outnumbering males, and most of them from rural areas, with the majority of cases occurring in the home environment. Flame burns were the most common, and among these, 80% were associated with kerosene use. Also, it was noticed that several patients had suffered severe burns. In 53% of them, more than 30% of their total body surface area, including the face, chest, and abdomen, was affected. The microbiological results indicated a shift from a sterile medium to bacterial colonization by the 5th day, which

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persisted through the 10th day. It was also noted that there was a transition from *S. aureus* to *P. aeruginosa*, which could indicate changes in the nosocomial infection profile. It was also noted that there was 60% concordance between swab and biopsy results, suggesting limitations of surface cultures in diagnosing wound infections.

A study was conducted to ascertain the microbial profile in burn wound infections among burn patients. By the end of the fourth week of hospitalization, the research showed that at least one case of bacterial infection had reached 100% [11]. *K. pneumoniae*, and *S. aureus*, with a frequency of isolation of 20.2%, staphylococci were the most common species, followed by *E. coli* (10.1%) and *P. aeruginosa* (14.6%). It was discovered that fungi might invade burn wounds late in the second week following a burn, peaking in the fourth week and reaching 36% at the conclusion of the hospital stay [12]. Bacillus species demonstrated susceptibility patterns to 20 antimicrobial agents across a total of 745 identified microorganisms. Every strain was amenable to every antibiotic; however, several strains showed signs of resistance. This would reduce the total morbidity and mortality associated with infections by enabling the early use of suitable empirical systemic antibiotics to prevent septic episodes before they worsen, without waiting for culture results [13]. On the contrary, the results of the present study showed that bacterial colonization progressed much earlier, and the rate of increase in isolates was rapid, from 34% initially to 82% by the 5th day, then remaining high at 80% by the 10th day. Furthermore, although *S. aureus* (54.05%) was initially dominant, a significant shift to *P. aeruginosa* (58.33% and 61.11%) was observed in subsequent cultures, indicating that the shift to nosocomial gram-negative infections occurred earlier than the delayed infection observed in the previous study.

Over 5 years, a retrospective analysis was conducted to identify aerobic bacteria from pus/wound swabs collected from patients admitted to the Government burn unit. Medical College Hospital in Chandigarh, India [14]. The goal of the study was to describe trends throughout the study period and to assess the isolates' antibiotic susceptibility and bacterial composition. In all, 665 isolates had very high culture-positive rates (96%) from pus/wound swabs [15]. In terms of isolate frequency, *P. aeruginosa* accounted for 59%, with *S. aureus* (17.9%), *Acinetobacter* spp. (7.2%), *Klebsiella* spp. (3.9%), Enterobacter spp. (3.9%), Proteus spp. (3.3%), and others (4.8%), following closely behind. Over the five years, *P. aeruginosa* remained the most common isolate; however, the prevalence of Acinetobacter spp. showed a consistent and noteworthy rise [16]. The drug that worked best against gram-negative bacteria was amikacin; nevertheless, over 5 years, resistance to it grew dramatically. The most effective medications for both *S. aureus* and *P. aeruginosa* were netilmicin and piperacillin. High levels of resistance to antimicrobial drugs were seen in the majority of the isolates [17]. In comparison, in the present study, a similar trend of *P. aeruginosa* predominance was observed, rising from 29.70% in the initial swab culture to 58.33% and 61.11% in subsequent cultures, closely resembling the 59% prevalence. However, unlike the high culture positivity rate of 96% in the previous study, the present study showed low culture positivity in the initial cultures (34%), which increased to 82% by day 5. Moreover, unlike *P. aeruginosa*, which rose in prevalence in the present study, *S. aureus* showed a significant drop in prevalence from 54.05% to 5.55% in the previous study. In contrast, Acinetobacter spp. was detected at later stages (4.16% and 3.70%), consistent with the trend observed for gram-negative nosocomial pathogens.

For burn victims, infection is a prevalent cause of major morbidity and death. For several reasons, it is challenging to diagnose burn patients with bacteremia and/or sepsis clinically. It may be asymptomatic or symptomatic due to an immunological deficit brought on by heat damage. In burn victims, bacteremia was found in 42% of cases [18]. *S. aureus* (34.04%) and *P. aeruginosa* (31.8%) were the most often isolated pathogens. The percentage of isolates with multiple resistances was about 82.16%. Given the findings of a study, every patient must undergo a routine antibiotic resistance test to determine the best antimicrobial treatment [19]. In contrast, this study offers further insight into the progression of infection by demonstrating temporal changes in microbial flora and persistent isolates in sequential cultures, rather than evidence of bacteremia at a single time point. The fact that extensive burn areas were included in this study (mean TBSA 39.2%, with 53% being greater than 30%) and that injuries to critical areas were common also indirectly supports the potential for bacteremia. The discordance between swab and biopsy

results also points to potential limitations in routine diagnostic approaches.

Patients with burn injuries provide a unique set of challenges as they are susceptible to microbial colonisation. The infections that follow can cause major problems and, in many circumstances, result in the burn patient's death [20]. Optimizing the patient's care requires surgical intervention, wound dressings, and antibiotic therapy. It is critical for practitioners to appropriately choose, dose, and provide antibiotics to burn patients to determine the best course of care [21]. To prevent infections from spreading and ensure the right medication is selected, it is essential to monitor bacterial colonization. This will reduce the likelihood that bacteria will become multi-resistant [22].

CONCLUSIONS

It is concluded that the risk of severe burns from fire injury is more pronounced in females, as 53% of burn patients have burns above 30% TBSA. Microbiological studies showed a shift in the pathogenic spectrum of infections, with burn-site swabs initially sterile in 66% but later cultures showing an increased presence of opportunistic bacteria; for instance, *S. aureus* dominated the first swab (54.05%), while *P. aeruginosa* was the major organism in the second (58.33%) and third swabs (61.11%). The emergence of these new pathogens, alongside this change, underscores the need for proper surveillance and antibiotic treatment for the infection. Although the agreement between swab cultures and biopsies was 60%, it should not be forgotten that the disagreement rate was 20%. Moreover, the resistance patterns identified and the effects of underlying diseases, such as diabetes, which significantly increase hospitalization time, call for specific tests and care guidelines. It is clear, therefore, that appropriate surgical treatment and prevention strategies in high-risk groups, such as rural women, are crucial.

CONTRIBUTION OF AUTHORS

Research concept –Smina KI
Research design –Smina KI
Supervision – Girija KR, Smina KI
Materials –Smina KI
Data collection –Smina KI
Data analysis and interpretation – Girija KR
Literature search – Smina KI
Writing article – Girija KR
Critical review –Smina KI
Article editing –Smina KI
Final approval – Girija KR

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