*International Journal of Pathogen Research*

***Volume 13, Issue 6, Page 1-8, 2024; Article no.IJPR.124605***
***ISSN: 2582-3876***

# Etiological Agents of Superficial Surgical Site Infections among Post Operative Patients Attending a Teaching Hospital in Southeastern Nigeria

**Divine Jacob Ottong [a], Onyekachukwu Izuchukwu Udemezue [a*], Chidinma Princess Ezeamalu [a], Onyekachi Ijeoma Udemezue [b] and Prisca Amala Okoye [a]**

[a] *Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, P. M. B. 5025, Awka, Nigeria.*
[b] *Department of Obstetrics and Gynecology, Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Awka, Anambra State, Nigeria.*

***Authors' contributions***

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

***Article Information***

DOI: https://doi.org/10.9734/ijpr/2024/v13i6316

**Open Peer Review History:**
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/124605

***Original Research Article***

***Received: 03/08/2024***
***Accepted: 07/10/2024***
***Published: 09/10/2024***

**Corresponding author: Email: io.udemezue@unizik.edu.ng;*

***Cite as:*** *Ottong, Divine Jacob, Onyekachukwu Izuchukwu Udemezue, Chidinma Princess Ezeamalu, Onyekachi Ijeoma Udemezue, and Prisca Amala Okoye. 2024. "Etiological Agents of Superficial Surgical Site Infections Among Post Operative Patients Attending a Teaching Hospital in Southeastern Nigeria". International Journal of Pathogen Research 13 (6):1-8. https://doi.org/10.9734/ijpr/2024/v13i6316.*

## ABSTRACT

Surgical site infections (SSI) are infections at or around the site of incision where surgical procedure is conducted. It is a major cause of hospital morbidity, resulting in prolonged hospital stay, increased treatment costs, higher rates of re-operation, and even increased mortality rates. The etiological agents of superficial surgical site infections among post-operative patients attending a Teaching Hospital in Southeastern Nigeria were evaluated using standard microbiological methods. Seventy superficial surgical wound swab specimens were properly and aseptically obtained from post-operative patients after ethical clearance was duly obtained from the hospital's ethical committee. Pure microbial cultures were obtained after 24 - 48 hours incubation, aerobically at 25$^{o}$C, on blood, nutrient and Sabouraud' Dextrose agar plates. The isolates were identified based on their morphological, physiological, biochemical and molecular characteristics. Out of the 70 surgical swab specimens collected, 81% were bacterial isolates which included *Staphylococcus aureus* 41%, *Escherichia coli* 16%, *Pseudomonas aeruginosa* 14% and *Staphylococcus xylosus* 10%. The fungi isolates (19%) were *Candida tropicalis* 8% *and Trichosporon asahii* 11%. These isolates were probably endogenous and exogenous flora from the patients' and hospital' environments respectively; thereby necessitating the need for adequate patient care before, during and after surgical procedures.


*Keywords: Superficial; surgical wound; swab; infection; etiological agents.*

## 1. INTRODUCTION

"Surgical site infection (SSI) is an infection that occurs in the incision created by an invasive surgical procedure. According to Centers for Disease Control and Prevention (CDC), and the National Health-Care Safety Network (NHSN), these infections typically occur within 30 days of an operation at the site or part of the body where the surgery took place, or within a year if an implant is left in place and the infection is thought to be secondary to surgery" [1,2,3]. "SSI also known as post-operative wound infections are nosocomial infections accounting for 20% to 39% of all the infections acquired in hospitals" [4,5].

"According to the United States Centers for Disease Control (CDC), surgical site infections can be categorized into three: Superficial incision SSI, which is limited to the skin area where an incision was made; Deep incision SSI where the infection develops below the incised skin and spreads to affect surrounding muscles and other soft tissues; and Organ or organ space SSI that can disseminate to any part of the body to affect organs or organ spaces. The vast majority of SSI is superficial occurring within 30 days of the operation and involves the skin and subcutaneous tissue" [6,7].

"The majority of SSIs are caused by an endogenous infection, which is when the incision becomes contaminated with microorganisms derived from the patient's skin or from an opened internal organ. Exogenous infection occurs when external microorganisms contaminate the operative site during the procedure. Sources include surgical instruments, the theatre environment and the air. External microorganisms can also contaminate the wound at the time of incident, or gain access to the wound following surgery, before the wound has healed. Among the exogenous organisms commonly identified in surgical site infections are staphylococci and streptococci. However, the prevalence of highly virulent hospital-acquired microorganisms such as methicillin-resistant *S aureus* (MRSA) or extended-spectrum β-lactamase microbes isolated from surgical site infections is on the rise" [8]. "More rarely, SSIs can be caused by a distant source of infection within the body. In this instance, the microorganisms from the infection attach to prosthesis or other artificial implant within the operative site. This is often referred to as haematogenous seeding or spread" [9].

"The symptoms of surgical site infections typically appear 5 to 7 days post-procedure, however can develop up to 3 weeks after (especially if prosthesis is inserted). The common clinical features of surgical site infections include spreading erythema, localized pain, pus or discharge from the wound; and persistent pyrexia. In certain cases, superficial or even complete wound dehiscence can occur secondary to SSI developing" [10,11]. "Factors that increase the risk of surgical site infection include aging, poor glucose control, obesity, weak immune system, cancer, smoking and renal failure. Procedure-related risk factors include contamination of the surgical site, equipment, or personnel; improper hair removal; inadequate antibiotic prophylaxis; insufficient application of the skin prep; short duration of surgical pre-operative scrub; prolonged surgical time; poor operating room (OR) ventilation; history of prior infection or contaminated case; and prolonged peri-operative in-patient stay" [12,13,14].

"The standard approach to identifying the pathogens involved in a surgical site infection involves collecting a wound swab and culturing for different aerobic and anaerobic microorganisms. Apart from bacteria, wound swab specimens can also be used to identify fungal disease-causing agents. Ultrasonography is the single most useful radiological tool in the diagnosis and treatment of surgical site infections. An ultrasound scan can be used to determine the presence of collection or abscess within a surgical site, and guide its drainage. Antibiotic therapy remains the cornerstone principle in treating SSIs. Additionally, adequate wound site care, excision and drainage of abscess collection, and clinical surveillance are also critical aspects of the intervention. Empirical antibiotics covering a broad range of infective organisms should be started immediately after collecting swab specimens for lab testing to determine the specific organisms involved. Once the organism causing the SSI has been identified, antibiotic therapy should be modified according to the sensitivity patterns of the particular pathogen" [15,16].

"Surgical site infections contribute significantly to the overall cost of healthcare among surgical patients. Inhibiting its onset is therefore a top priority among healthcare providers. Prevention strategies can be implemented at all stages of surgical treatment (pre, intra, and post-op). Pre-op measures include use of prophylactic antibiotics; proper shaving the area to be incised as hair can serve as a nidus for infection; and encourage good lifestyle choices such as smoking cessation, reduced alcohol consumption and weight loss. Intra-op measures include adequate skin prep before incision; proper gowning and gloving; and observation of total asepsis. Post-op measures include regular wound inspection and dressing changes; paying special attention to surgical wounds in hard to reach areas like natural skin folds; assess tissue viability often and clean or debride as necessary; administer intravenous and oral antibiotics as indicated after assessing intra-op contamination status" [10,17,18].

The study aims at evaluating the etiological agents of superficial surgical site infections among post-operative patients attending a Teaching Hospital in Southeastern Nigeria.

## 2. MATERIALS AND METHODS

### 2.1 Study Area

The study was carried out at the Laboratory Unit of Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka, Anambra State, Southeastern Nigeria.

### 2.2 Sample Size

The sample size was estimated using Kish Leslie formula [19].

$$n = (Z_{0.95})^2 \left(\frac{P(1-P)}{D^2}\right)$$

Sample size was calculated based on prevalence of fungal and bacterial infection from previous studies [20].

Z = Standard normal deviation = 0.95 at 95%
P = Prevalence rate = 26%
D= Error margin = 5%

Sample size n, equals 69.9 approximately 70

### 2.3 Surgical Wound Swab Specimens' Collection

Seventy (70) surgical wound swab specimens were aseptically and carefully collected from patients with superficial surgical wounds after abdominal surgery at Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (COOUTH), Awka, Anambra State.

### 2.4 Isolation of Microorganisms

Plates of blood agar, Sabouraud dextrose agar supplemented with chloramphenicol (50 μg/mL) and nutrient agar supplemented with nystatin (100 U/mL) were inoculated with the swab specimens, using the spread plate method. The plates were aerobically incubated at 25°C for 24 - 48 hours and thereafter observed for microbial growth [21].

### 2.5 Pure Culture Isolation and Identification

The bacteria and fungi isolates were purified by sub culturing on fresh plates of nutrient agar and Sabouraud dextrose agar. They were identified based on their morphological, physiological, biochemical and molecular characteristics. The identification tests include macroscopic appearance of the colonies, Gram' stain reactions [22], Lactophenol cotton blue stain reaction, Growth on cornmeal tween-80 Agar [23], Germ tube test [24], Citrate utilization, Catalase, Coagulase, Indole and Oxidase tests [25], Motility [26], Voges-Proskauer (VP), sugar fermentation tests [27] and Nucleic acid sequence analysis [26].

## 3. RESULTS AND DISCUSSION

### 3.1 Microscopic Morphologies of Yeast Isolates and Germ Tube Test

This research investigated the microorganisms implicated in superficial surgical site infections among post-operative patients attending a Teaching Hospital in Southeastern Nigeria. Table 1 showed the morphological characteristics of the isolates after 24 to 48 hours growth on cornmeal tween-80 agar at 25ºC. *Candida tropicalis* produced chlamydospores, blastospores and pseudohyphae; while *Trichosporon asahii* produced arthroconidia. *C. tropicalis* was negative to germ tube test as shown below in Table 1.

**Table 1. Microscopic morphologies and germ tube test**

| Characteristic features | *C. tropicalis* | *T. asahii* |
|---|---|---|
| Germ tube | - | + |
| Chlamydospores | + | - |
| Pseudohyphae | + | - |
| Blastospores | + | - |
| Arthroconidia | - | + |

*+ = positive, - = negative*

### 3.2 Morphological and Biochemical Characteristics of Bacteria Isolates

Table 2 showed the morphological and biochemical characteristics of the bacteria isolates including Gram staining reaction, microscopic appearance, motility test, enzyme production tests such as catalase, oxidase, citrate and urease tests.

### 3.3 Sugar Fermentation Test

The ability of the isolates to ferment various carbohydrates with the production of acid and or gas as end products was shown in Table 3. Acid production was indicated by a yellowish colour change of the indicator (bromothymol blue), while gas production was indicated by gas accumulation in the submerged Durham tubes. The sugars include fructose, sucrose, lactose, glucose, maltose, D-xylose, galactose, cellobiose, dextrose, trehalose, raffinose and mannose. The isolates exhibited varied response to the various carbohydrates as shown below in Table 3.

### 3.4 Distribution of Microbial Isolates from Surgical Wound Swab Specimens

Out of the 70 surgical swab specimens collected, 81% (57) were bacterial isolates which included *Staphylococcus xylosus* 10% (7 isolates), *Pseudomonas aeruginosa* 14% (10 isolates), *Staphylococcus aureus* 41% (29) *and Escherichia coli* 16% (11 isolates). The total fungi isolated were 13, which is 19% of the total number of isolates. The fungi isolates were *Candida tropicalis* 8% (5 isolates) *and Trichosporon asahii* 11% (8 isolates) as shown below in Figs. 1 and 2. The findings are in line with Chidimma et al. [28], who isolated different species of bacteria from surgical wounds of about 160 surgery patients attending Enugu state University teaching Hospital.

Amongst these isolates, the most prevalent bacteria species were *Staphylococcus aureus* and *Pseudomonas aeruginosa.* A similar study was performed by Ezekiel et al. [20] affirming that *Staphylococcus aureus* is the most etiologic bacterial agent that is implicated in surgical wound infection. *Staphylococcus aureus* is mostly found in the environment. It is also a normal human flora, located on the skin and mucous membranes (most often the nasal area) of most healthy individuals. It does not normally cause infection on healthy skin. However, if allowed to enter the bloodstream or internal tissues, these bacteria may cause a variety of potentially serious infections [29]. Hence, the increase in prevalence of *Staphylococcus aureus* isolated from superficial surgical wound infections calls for more attention.

**Table 2. Morphological and biochemical characteristics of bacterial isolates**

| S/N | Shape | Gram | Catalase | Oxidase | Motility | Citrate | MR | $H_2S$ | Urease | Isolates |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Cocci | + | + | + | - | + | +- | - | + | *Staphylococcus xylosus* |
| 2 | Rod | - | + | + | + | + | - | + | + | *Pseudomonas aeruginosa* |
| 3 | Cocci | + | + | + | - | + | + | - | - | *Staphylococcus aureus* |
| 4 | Rod | - | + | - | + | - | + | - | - | *Escherichia coli* |

+ = *positive*, - = *negative*

**Table 3. Sugar fermentation test**

| Sugars | *S. xylosus* | *P. aeruginosa* | *S. aureus* | *E. coli* | *C. tropicalis* | *T. asahii* |
|---|---|---|---|---|---|---|
| Fructose | + | - | + | + | + | - |
| Sucrose | + | + | + | + | + | - |
| Lactose | + | - | + | + | - | - |
| Glucose | + | - | + | + | + | - |
| Maltose | + | - | + | + | + | - |
| D-xylose | + | - | - | + | + | + |
| Galatose | + | - | - | + | + | - |
| Cellebiose | - | - | + | - | + | - |
| Dextrose | + | + | - | - | + | - |
| Trehalose | + | + | + | + | + | - |
| Raffinose | - | - | - | - | - | - |
| Mannose | + | + | + | - | - | - |

+ = *positive*, - = *negative*

In the current study, *Escherichia coli* was the most common Gram-negative bacteria which is consistent with the findings of Ashoobi et al. [30] having *Escherichia coli* as the most common Gram-negative isolate identified from surgical wound infection. The results are also consistent with the reports by Christian et al. [31], who revealed the following; *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Streptococcus pyogenes* as bacteria commonly found in infected wounds.

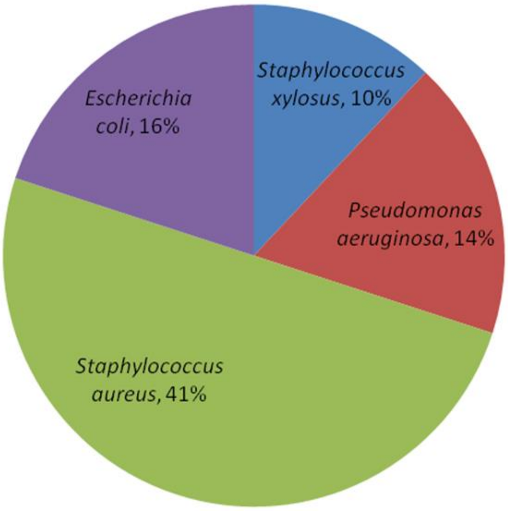


**Fig. 1. Frequency of bacterial isolates**

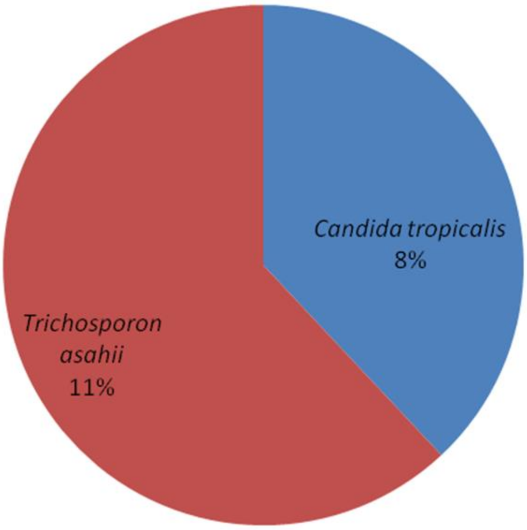


**Fig. 2. Frequency of fungal isolates**

Fungi are rarely etiological agents of surgical site infections; though *Candida tropicalis and Trichosporon asahii* were implicated as shown in Fig. 2. This corresponds with the report of Zoungrana et al. [32] which indicated that *Candida albicans, Nakaseomyces glabrata, Candida tropicalis* and *Pichia kudriavzevii* were implicated in surgical site infections. The work on *Trichosporon asahii* [33] revealed that the fungus causes a rare opportunistic infection in immune compromised individuals. Kruschewsky et al. [34] reported that *Trichosporon asahii* was isolated from patient after carpal tunnel surgery. The above findings call for the need for more efficient and effective means of managing surgical site infections.

## 4. CONCLUSION AND RECOMMENDATION

Superficial surgical site infection, a nosocomial infection, is a common post-operative problem accounting for high morbidity and mortality among patients. The etiological agents are mostly patients' endogenous normal flora and sometimes exogenous microbes from the environment. The need for proper and adequate patient care through application of pre, intra, and post-op preventive strategies is very vital in all surgical procedures. These measures would drastically reduce the high morbidity and mortality associated with surgical site infections.

## DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

## CONSENT AND ETHICAL APPROVAL

Ethical clearance was duly obtained from COOUTH Ethical Committee. These swab specimens were obtained with due consent from the patients. The specimens were collected between October, 2023 till April, 2024.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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