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Research Article

# Determination of Pathogenic Bacteria Isolated from Post-Operative Wound Infections and Their Antibiotic Resistance Pattern: A Hospital-Based Cross-Sectional Study

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## Abstract

Postoperative wound infections in surgical patients after surgeries, as well as the emergence and spread of drug-resistant strains, have been identified as a key challenge in the medical field and also can cause mortality, morbidity and economic burden. Therefore, this study investigated some common aerobic pathogenic isolates and their antibiotic sensitivity pattern in patients with post-surgical wound infections in a territory hospital in Dinajpur, Bangladesh. Bacteriological analysis was performed on wound swab samples collected from 15 patients with a medical assessment of post-surgical wound infections at Dinajpur Medical College. Wound swabs were collected from different surgical wound during dressing and inoculated on Nutrient agar, MacConkey agar, EMB agar, Blood agar, Cetrimide agar, and Mannitol Salt agar for isolation and identification of *Escherichia coli, Pseudomonas* spp. and *Staphylococcus* spp. The modified Kirby-Bauer disk diffusion technique was utilized for antibiotic sensitivity tests. Out of 15 samples, 10 (66.67%) samples were positive, of which 4 were male and 6 were female patients with age ranged from 15- ≥50 years. A high predominance of aerobic bacteria was observed and *Staphylococcus* spp. (42.11%), *E. coli* (31.58%) and *Pseudomonas* spp. (26.31%) were predominantly identified. The highest number of the positive case found in the age group ≥50 (40%) and the lowest positive cases were found in the age group 15-30 (10%) respectively. Among 19 bacterial isolates, higher growth of isolates (52.63%) were found in the age above ≥50, whereas the lowest growth of isolates was found in the age group 15-30 (5.27%) respectively. Sequencing the *16S rRNA* gene, *E. coli* was identified with a 1466 bp PCR band. Gram-positive bacteria were susceptible to Cloxacillin 7/8 (87.5%) and Gentamicin 6/8 (75%), whereas, Gram-negative isolates were most effective to Ofloxacin, Ciprofloxacin, Gentamicin and Amikacin 5/5 (100%). Hence, the most predominant bacteria in post-operative wound infection were *Staphylococcus* spp. and *E. coli*. The antibiotic sensitivity tests revealed that numerous and multi-drug resistant bacteria are involved in post-operative wound infection. Therefore, routine microbiological investigation of wound specimens and antibiotic susceptibility testing will be advised by the clinicians in treating wound infections.

**Keywords:** Aerobic Bacteria; Drug-Resistant; Post-Operative Wound Infections; Wound Swabs

## Introduction

Infection of a wound can be described as the invasion of microorganisms into tissues as a result of the collapse of systemic and local host defense mechanisms [1]. Probably, wound infection has been a significant consequence of surgery and trauma [2]. Invasion and multiplication of microorganisms in body tissue, which may be clinically inapparent or result in local cellular injury due to competitive metabolism, toxins, intracellular replication, or antigen-antibody response, is considered to be postoperative wound infection or surgical site infection (SSI) [3]. These infections can affect any organ or bodily region, although they most commonly occur after surgery [4]. Surgery site infections (SSIs) are the second most prevalent type of nosocomial infection in the United States, affecting 38%, Bangladesh affecting 22% of all surgical patients [5]. Infections of surgical incisions are a leading cause of patient harm, including higher mortality rates and illness, more extended hospital stays, and higher overall healthcare expenses [6]. Infections that develop in surgical incisions can cause considerable discomfort and, in some situations, require additional procedures to treat the problem [7]. These infections induce anxiety, patient discomfort, delayed healing, and mortality (NINSS: Surveillance of Surgical Site Infection in English Hospitals, 2002). The occurrence of SSI depends on the contaminating microorganisms and the patient's immune response (National Institute of Health and Clinical Excellence, 2008). Postoperative wound infection or SSI is caused by bacteria such as *Staphylococcus aureus*, *S. epidermidis*, *Streptococcus pyogenes*, *S. faecalis*, *S. pneumoniae*, *Pseudomonas aeruginosa*, *Escherichia coli* and other coliforms, *Proteus* spp., *Klebsiella* spp., *Moraxella* spp. [8]. Most of these bacteria come from the patient's endogenous flora, but sometimes they are acquired from an external source, such as the operating room air, surgical equipment, implants, gloves, or drugs used during the operative procedure [9]. MRSA is a primary nosocomial infection. Rapid MRSA diagnosis is critical for patients, health care providers, and epidemiology [8]. Surgical antimicrobial prophylaxis minimizes postoperative wound infections or SSIs. Surgical prophylaxis provides for 30–50% of all antibiotic prescriptions in hospitals, and 30–90% of these prescriptions are needless [10]. The selection of empiric antimicrobial therapy and infection control strategies in healthcare facilities relies on awareness of the normal infectious flora, effective antimicrobial drugs, and susceptibility patterns of the pathogenic bacteria [11]. Without proper identification of pathogenic bacteria from post-operative infection sites, non-prescribed antibiotics create complications. Therefore, this investigation aimed to determine predominant bacterial pathogens associated with post-operative wound infection and to characterize the antibiotic susceptibility profile of these bacteria in our territory.

## Materials and Methods

### Study area selection and settling

The study was conducted on 15 patients of different ages admitted in post-operative surgery with post-operative wound infections. After taking a relevant clinical history and sampling, these samples were processed in the department of microbiology, Hajee Mohammad Danesh Science and Technology University, Dinajpur. About 15 post-operative wound samples were collected from Dinajpur Medical College, Dinajpur. The samples were stored at appropriate conditions until analysis was performed.

### Isolation and identification of bacteria

Wound swabs were processed in the bacteriology laboratory within 1 hour of collection. All 15 samples were primarily cultured on a Nutrient agar plate and finally incubated at 37°C for 24 hours. Then selective media were used for bacterial cultures, such as MacConkey agar, EMB agar, Blood agar, Cetrimide agar, and Mannitol Salt agar (HI Media, India). All culture plates were sub-cultured and then incubated overnight at 37°C for 24 hours. Using previously published protocol, pure cultures were maintained in a bacteriological laboratory [12]. In this work, several morphological and biochemical examinations were applied, such as microscopic examination (Gram-staining), Catalase, MR-VP, TSI, Indole, MIU, and Citrate utilization. All tests were performed by conventional methods [13].

### Molecular detection

### Bacterial genomic DNA extraction

For molecular characterization, pure colonies of *E. coli* were isolated from post-operative wound samples. According to maker's guidance, DNA was extracted with a Robotic DNA extractor (Maxwell-16, Source: Promega-USA). The genomic DNA was tested for concentration and purity using Nano-Drop Spectrophotometer (ND-2000, Source: Thermo Scientific-USA). Finally, we stored DNA at -20°C for further use.

### Polymerase chain reaction (PCR) products analysis

Using universal primers, a PCR experiment was performed on genomic DNA. The forward and reverse primers have the following sequences: *E. coli* (27F), 5-'AGAGTTTGATCCTGGCTCAG- 3'and *E. coli* (1492R), 5'- TACCTTGTTACGACTT -3' DNA polymerase enzyme of high fidelity (ScientificTM, PhusionTM) was used to create the DNA fragment of the *16S rRNA* gene. The PCR procedure utilized 50 mL of DNA diluted to a concentration of 100 ng/mL, 25 mL of Phusion Master Combine, and 0.5 M of each primer. The ultimate volume of reactions was selected to be 50 L. The following temperature settings were used when running the thermal cycler: denaturation for 30 seconds at 95°C, tempering for 30 seconds at 56°C, extraction for 90 seconds at 72 °C, and final addition for 8 minutes at 72°C [14]. Finding the DNA band required 1% agarose gel. Ethidium bromide stain and electrophoresis were used to run the agar gel (Biometra standard power pack P 2T). Run for 60 minutes at 100 volts and 70 amps after that. The Thermo Scientific Gene Extraction Kit was then used to remove the bands from the gel. PCR products are seen using UV Tran's illuminator (UVITEC Cambridge, UK) [15].

### Sequence and phylogenetic analyses

PCR reaction run by Sanger sequencing method with an applied biosystem (Foster City, CA, USA) automatic DNA sequencer (ABI3130xl genetic analyzer) at National Institution of Biotechnology (NIB), Savar, Dhaka. The phylogenic tree was analyzed using molecular evolutionary genetic analysis software resulting from edited and analyzed sequences [16] using the neighbor-joining method [17]. The gene sequence was uploaded to the GeneBank database by BLASTN (http://www.ncni.nih.gov/BLAST) algorithm.

### Nucleotide sequence accession number

Using the accession number NR 026332, the *16S rRNA* gene sequences obtained in this research have been submitted to the GenBank database.

### Antibiotics susceptibility test

The Kirby-Bauer disk diffusion method [18] as recommended by the National Committee for Clinical Laboratory Standards, was used to determine the antibiotic susceptibility profile of the isolates [19]. Antibiotic sensitivity was tested using commercial antibiotic discs on Muller-Hinton agar (HI Media). Antibiotic disks were applied using sterile forceps. Then all plates were incubated at 37°C overnight. According to the manufacturer's instructions, the zone was measured using a millimeter scale following overnight incubation. For antibiotic sensitivity tests, 15 commercially available antibiotics were applied in this research. The antibiotics disc was Ciprofloxacin (5 µg), Vancomycin (30µg), Cefixime (5 µg), Kanamycin (30µg), Gentamicin (10 µg), Ofloxacin (30 µg), Cloxacillin (5 µg), Erythromycin (15 µg), Cotrimazole (5 µg), Amoxicillin (10 µg), Chloramphenicol (30 µg), Ceftriaxone (30 µg), Amikacin (30 µg), Ceftazidime (30 µg) Cefotaxime (5 µg). We purchased all antibiotic discs from Hi Media, India.

## Results

### Isolation and identification of pathogenic bacteria

Out of 15 post-operative wound pus samples, 10 (66.67%) samples showed growth, while 5(33.33%) samples showed no growth. A total of 10 positive samples, 6(60%) was obtained from female patients whereas, 4(40%) was from male patients (Figure 1).

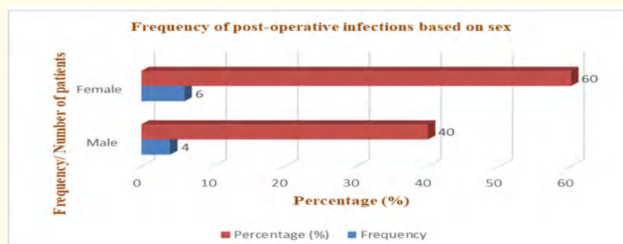


**Figure 1:** Frequency of post-operative infections based on sex.

In this research, *Staphylococcus* spp., *E. coli*, and *Pseudomonas* spp. were isolated and identified using different selective culture media and biochemical tests. *Staphylococcus* spp. produced yellow colony on Mannitol Salt agar, *Pseudomonas* spp. showed smooth, raised, irregular, and semi-translucent colonies with pigment (pyocyanin) production on Cetrimide agar, and *E. coli* produced green colonies with a metallic sheen on EMB agar (Figure 2).

Microscopically, *E. coli* is pink, rod-shaped, singular, pair, or short chain. Biochemical tests result showed in (Table 1).

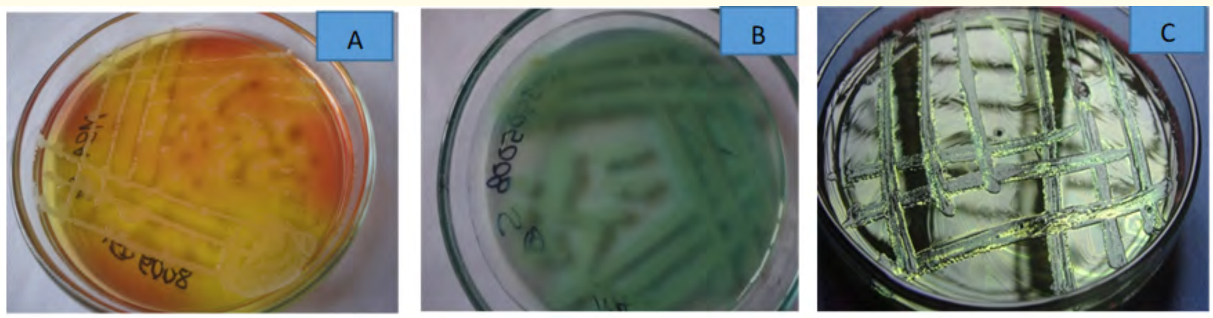


**Figure 2:** *Staphylococcus* spp. on MSA (A), Pseudomonas spp. on CA, and *E. coli* on EMB (C).

| **Sample** | **S-1** | **S-2** | **S-3** | **S-4** | **S-5** | | **S-6** | | **S-7** | **S-8** | **S-9** | **S-10** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | **$S_5C_1$** | **$S_5C_2$** | **$S_6C_1$** | **$S_6C_2$** | | | | |
| Oxidase Test | + | + | + | + | + | - | + | + | + | - | + | - |
| Catalase Test | + | + | + | + | + | + | + | + | + | + | + | + |
| MR Reaction | + | + | − | + | + | + | - | + | + | + | _ | + |
| VP Reaction | - | - | - | - | - | - | - | - | - | - | - | - |
| Citrate Test | - | - | + | - | - | - | + | - | - | - | + | - |
| MIU | - | - | - | - | - | + | − | − | − | + | − | + |
| Indole | - | - | - | - | - | + | - | - | - | + | - | + |
| TSI Test | Slant and Butt both acidic | Slant and Butt both acidic | Slant and Butt both alkaine | Slant and Butt both acidic | Slant and Butt both acidic | Slant and Butt both acidic | Slant and Butt both alkaine | Slant and Butt both acidic | Slant and Butt both acidic | Slant and Butt both acidic | Slant and Butt both alkaine | Slant and Butt both acidic |
| Results | *Staph* spp. | *Staph* spp. | *Pseudo-monas* spp. | *Staph* spp. | *Staph* spp. | *E. coli* | *Pseudo-monas* spp. | *Staph* spp. | *Staph* spp. | *E. coli* | *Pseudo-monas* spp. | *E. coli* |

**Table 1:** Biochemical characteristics of 10 culture-positive post-operative wound infections.

S: Sample; C: Colony; MN: Mannitol MR: Methyl red; VP: Voges-Proskauer; (+) = positive; (-) = negative.

A total of 19 bacterial isolates were obtained, of which 8 (42.11%) were Gram-positive, and 11 (57.89%) were Gram-negative bacteria. Out of 19 bacterial isolates, the highest prevalence was found in *Staphylococcus* spp. 8 (42.11%) followed by *E. coli* 6 (31.58%) and *Pseudomonas* spp. 5 (26.31%) respectively (Figure 3).

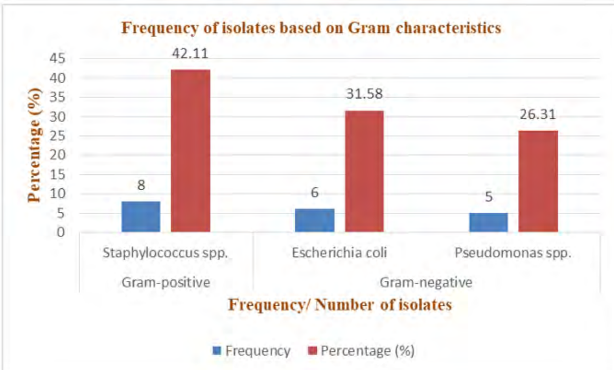


**Figure 3:** Frequency of post-operative infections based on Gram characteristics.

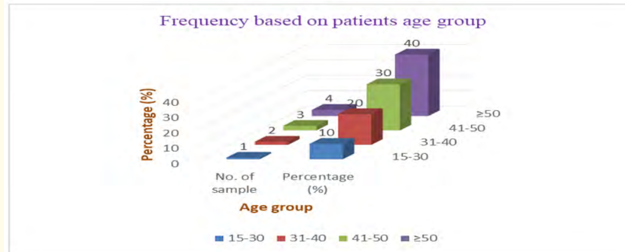


**Figure 4: (A):** Frequency of post-operative infections based on age group.

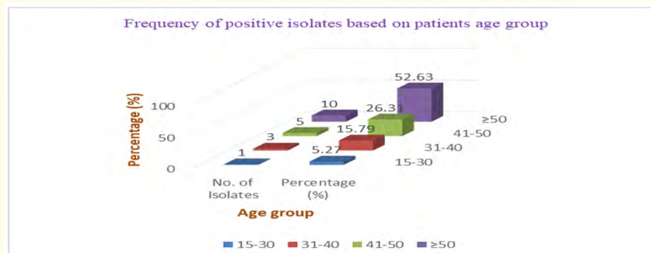


**Figure 4: (B):** Frequency of positive isolates based on age group.

Out of 10 positive post-operative infected patients, the highest number of the positive case found age group ≥50 (40%), followed by 41-50 (30%), and the lowest positive case was found in the age group 15-30 (10%), respectively. Among 19 bacterial isolates, the patients in the age group ≥50 had higher growth of isolates (52.63%), whereas the lowest isolates were found in the age group 15-30 (5.27%), respectively (Figure 4 A, B).

## Antibiotic sensitivity test of Gram-negative and Gram-positive bacteria

*Staphylococcus* spp., *E. coli*, and *Pseudomonas* spp. were treated with commercially available antibiotics, listed in Table 2. Among 8 gram-positive isolates, *Staphylococcus* spp. were highly sensitive to Cloxacillin 7/8 (87.5%), followed by Gentamicin 6/8 (75%), Erythromycin 6/8 (75%), Ciprofloxacin 5/8 (62.5%), Ofloxacin 5/8 (62.5%) whereas, highly resistant to Cefixime, Clotrimazole 6/8 (75%) followed by Vancomycin and Kanamycin 5/8 (62.5%) respectively (Table 2).

| Antimicrobial agents | Sensitive | | Intermediate | | Resistant | | Total |
|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % | |
| Ciprofloxacin (CIP) | 5 | 62.5 | 1 | 12.5 | 2 | 25 | 8 |
| Vancomycin (V) | 0 | 0 | 3 | 37.5 | 5 | 62.5 | 8 |
| Cefixime (CEF) | 0 | 0 | 2 | 25 | 6 | 75 | 8 |
| Kanamycin (K) | 0 | 0 | 3 | 37.5 | 5 | 62.5 | 8 |
| Gentamicin (GEN) | 6 | 75 | 1 | 12.5 | 1 | 12.5 | 8 |
| Ofloxacin (OF) | 5 | 62.5 | 0 | 0 | 3 | 37.5 | 8 |
| Cloxacillin (CX) | 7 | 87.5 | 0 | 0 | 1 | 12.5 | 8 |
| Erythromycin (E) | 6 | 75 | 1 | 12.5 | 1 | 12.5 | 8 |
| Clotrimazole (COT) | 1 | 12.5 | 1 | 12.5 | 6 | 75 | 8 |
| Amoxicillin (AMX) | 3 | 37.5 | 1 | 12.5 | 4 | 50 | 8 |

**Table 2:** Antibiotic sensitivity pattern of *Staphylococcus* spp.

Among 11 Gram-negative isolates, Ciprofloxacin and Gentamicin 6/6 (100%) was a drug of choice, followed by Chloramphenicol, Amikacin and Cefotaxime 5/6 (83.33%) for *E. coli*, whereas, Amoxicillin 1/6 (16.67%) was less effective drug with 5/6 (83.33%) resistant followed by Clotrimazole and Ceftriaxone 4/6 (66.67%), Ofloxacin and Kanamycin 2/6 (33.33%) respectively (Table 3).

| Antimicrobial agents | Sensitive | | Intermediate | | Resistant | | Total |
|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % | |
| Ciprofloxacin (CIP) | 6 | **100** | 0 | 0.00 | 0 | 0.00 | 6 |
| Chloramphenicol (C) | 5 | 83.33 | 1 | 16.67 | 0 | 0.00 | 6 |
| Ceftriaxone (CFZ) | 2 | 33.33 | 0 | 0.00 | 4 | 66.67 | 6 |
| Kanamycin (K) | 3 | 50.00 | 1 | 16.67 | 2 | 33.33 | 6 |
| Gentamicin (GEN) | 6 | **100** | 0 | 0.00 | 0 | 0.00 | 6 |
| Ofloxacin (OF) | 4 | 66.67 | 0 | 0.00 | 2 | 33.33 | 6 |
| Amikacin (A) | 5 | 83.33 | 1 | 16.67 | 0 | 0.00 | 6 |
| Cefotaxime (CEF) | 5 | 83.33 | 0 | 0.00 | 1 | 16.67 | 6 |
| Clotrimazole (COT) | 2 | 33.33 | 0 | 0.00 | 4 | 66.67 | 6 |
| Amoxicillin (AMX) | 1 | 16.67 | 0 | 0.00 | 5 | **83.33** | 6 |

**Table 3:** Antibiotic sensitivity pattern of *E. coli*.

For *Pseudomonas* spp. Ofloxacin and Amikacin 5/5 (100%) was the most effective antibiotic, followed by Ciprofloxacin and Gentamicin 4/5 (80%), Kanamycin and Cefotaxime 2/5 (20%), respectively. Chloramphenicol, Ceftriaxone, and Amoxicillin showed the highest resistance, 5/5 (100%), followed by Cefotaxime and Ceftazidime 3/5 (100%), Kanamycin 2/5 (40%), respectively (Table 4).

| Antimicrobial agents | Sensitive | | Intermediate | | Resistant | | Total |
|---|---|---|---|---|---|---|---|
| | No. | % | No. | % | No. | % | |
| Ciprofloxacin (CIP) | 4 | 80.00 | 1 | 20.00 | 0 | 0.00 | 5 |
| Chloramphenicol (C) | 0 | 0.00 | 0 | 0.00 | 5 | **100.00** | 5 |
| Ceftriaxone (CF) | 0 | 0.00 | 0 | 0.00 | 5 | **100.00** | 5 |
| Kanamycin (K) | 2 | 40.00 | 1 | 20.00 | 2 | 40.00 | 5 |
| Gentamicin (GEN) | 4 | 80.00 | 0 | 0.00 | 1 | 20.00 | 5 |
| Ofloxacin (OF) | 5 | **100.00** | 0 | 0.00 | 0 | 0.00 | 5 |
| Amikacin (A) | 5 | **100.00** | 0 | 0.00 | 0 | 0.00 | 5 |
| Cefotaxime (CEF) | 2 | 40.00 | 0 | 0.00 | 3 | 60.00 | 5 |
| Ceftazidime (CFZ) | 1 | 20.00 | 1 | 20.00 | 3 | 60.00 | 5 |
| Amoxicillin (AMX) | 0 | 0.00 | 0 | 0.00 | 5 | **100.00** | 5 |

**Table 4:** Antibiotic sensitivity pattern of *Pseudomonas* spp.

### Detection of *E. coli* with the sequencing of *16S rRNA* gene and phylogenic tree analysis

The universal forward primer 27F, which reads "(5' AGAGTTTGATCCTGGCTCAG 3')," and the reverse primer 1492R, which reads "(5' TACCTTGTTACG ACTT 3')," were used to amplify the *16S rRNA* gene region. The 1466 base pair PCR amplification is in (Figure 5).

The NCBI-GenBank database's BLAST results revealed the *E. coli* strain with accession no. NR_026332.1 0.00452. The phylogenic tree is presented in (Figure 6).

## Discussion

During the surgical wound dressing, postoperative wound swabs were collected from Dinajpur Medical College patients in this

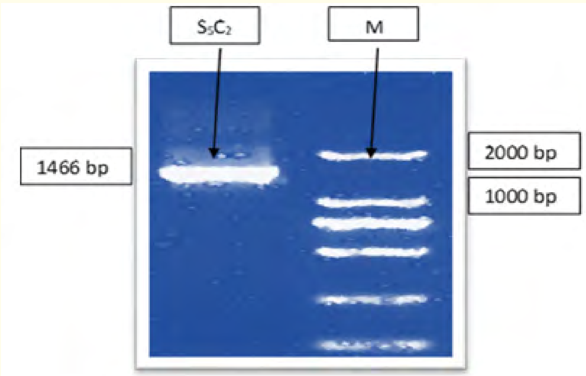


**Figure 5:** A 1466 bp fragment of the *16S rRNA* gene was amplified by PCR. (M: 2000 bp marker of DNA).

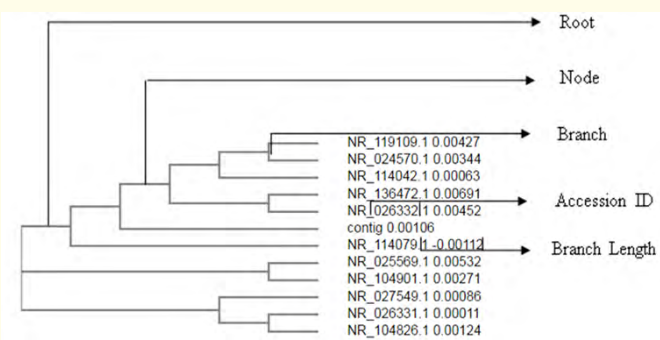


**Figure 6:** Phylogenetic tree based on *16S rRNA* gene of *E. coli* strain.

study. In our present study, 66.67% of wound samples were positive for bacterial growth. This isolation rate of bacterial pathogens is relatively similar to the 60.20% previously observed in Nepal [20] but higher (55.50%) than that previous study in Skopje [21]. According to our findings, 60% of male patients were identified, whereas female was 40%. According to Verma., *et al.* the high proportion of males in culture-positive cases is highly probable due to greater exposure to the environment [22] and greater chances of accidents while earning a living, as well as our social behavior in which males are favored over females and are rushed to hospitals if they become ill [23]. The highest number of the positive case found age group ≥50 (40%), followed by 41-50 (30%), and the lowest positive case found in the age group 15-30 (10%) respectively. This could be due to the patient's immune system weakening as they get older. In this study, A total of 19 bacterial isolates were identified, and the most predominant was found in *Staphylococcus* spp. (42.11%), related to [20]. In our research, *Staphylococcus* spp. (42.11%), *E. coli* 5(26.31%) and *Pseudomonas* spp. 6(31.58%) were identified as postoperative wound infections. This agrees with another study where *Staphylococcus* spp. (32.25%) and *E. coli* (31.63%) were predominant in Ethiopia, India, and Niger [24-26]. Normal flora of *Staphylococcus* spp. in the skin and anterior nares, which can infiltrate deep sites following surgery, may explain its high occurrence. In our research, among Gram-positive isolates (*Staphylococcus* spp.), the most effective antibiotic was Cloxacillin (87.5%), and Gentamicin (75%), and the least effective antibiotic was Clotrimazole. A similar result was observed in a study in Nigeria, where Cloxacillin was the most effective antibiotic, and Clotrimazole was the most resistant antibiotic [27]. Isolated *E. coli* were 100% sensitive to Ciprofloxacin and Gentamicin similar report was recorded in the previous study in Uganda, Ethiopia [28,29]. The isolated *Pseudomonas* spp. were 100% resistant to Chloramphenicol, Amoxicillin, and Ceftriaxone; similar resistance was reported elsewhere [29] but as reported from Nepal [30], all isolates were 100% susceptible for Ofloxacin, Amikacin, and Gentamicin. All the bacteria that were recovered had a high level of resistance to cefixime, amoxicillin, ceftriaxone, and clotrimazole. This resistance may be attributable to the medications' easy availability and non-prescription use. The antibiotics Amikacin, Ofloxacin, Gentamicin, and Erythromycin, showed promising results in our research against post-surgical wound infection in the study area, although they must be used with precaution. Further research is needed to identify extended-spectrum beta-lactamase (ESBL) producing *E. coli* strains that create a global health risk. The findings of this study will aid policymakers in assessing the efficacy of hospital infection control measures and in halting the spread of drug-resistant bacteria both within and outside of hospitals. It is tough to pinpoint the particular source of infections. Thus, studying how sterile equipment is cleaned and handled is another area to investigate.

## Limitations

Due to financial constraints, strict anaerobe bacteria and fungi were not separated in this investigation. Shortage of time period of research and financials restrictions, the sample size was not significant level and proportionate among surgical specialties. For this reason, no statistical tests were performed. Molecular characterization of most pathogenic isolates was not detected due to financial funds.

## Conclusion

The antibiotics Ciprofloxacin, Ofloxacin, Gentamicin, and Erythromycin should be the better choice for post-surgical wound infections treatment. We didn't study the impact of cleanliness on wound infection, although patient education on personal hygiene may improve wound healing and patient care. Although it's impossible to eliminate wound infections completely, their occurrence can be reduced to a minimum with preventative measures, clean surgical techniques, and appropriate wound care. To forestall the establishment and spread of antibiotic-resistant bacterial infections, it is crucial to conduct routine surveillance of bacteria and drug susceptibility.

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## Conflict of Interests

The author has no conflict of interest.

## Authors Contribution

Ishaq Hossion and Md. Shahin Mahmud carried out the experimental work. Nazmi Ara Rumi supervised the granted research. Md. Shahin Mahmud and Md. Aoulad Hosen helps with writing manuscripts. Md. Aoulad Hosen checked the plagiarism and grammatical errors. Md. Saiduzzaman and Fatema Tuj Zohora prepared the graph and other figures. Md. Shajedur Rahman prepared the tables. Md. Khaled Hossain evaluated the final manuscript. Md. Khaled Hossain and Nazmi Ara Rumi finally revised and prepared the manuscript. All authors read the entire manuscript thoroughly before final publication.

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