**Original Research Article**

**Cytokine patterns and Antibiogram of Bacteria isolates from Surgical site infection in Responses to Misuse of Antibiotic in Benin city, Edo state, Nigeria**

**ABSTRACT**

Antibiotic resistance has emerged as a critical global health challenge, exacerbated by the widespread misuse and overuse of antibiotics. The aim of this research was geared to determine the pattern of multidrug resistance isolates among patients with post operative surgical site infections and their immunological responses to misuse of antibiotics in Edo state, Nigeria using appropriate microbiological methods. Also, the levels of tumor necrosis factor alpha (pg/ml), interferon-gamma (pg/ml), and interleukin-10 (pg/ml) in post-surgical patients and control subjects were evaluated using enzyme link immunosorbent assay method. The prevalence of wound infection among study participants was (27%). *Escherichia coli* (30%) and *Pseudomonas aeruginosa* (50%) were most likely to be incriminated with wound infection closely followed by *proteus mirabilis* (15%) and the least was *staphylococcus aureus* (5%). The MDR bacterial isolates shows highest percentage of resistance to the penicillin and cephalosporin’s in the surgical wound infection patients. The levels of tumor necrosis factor alpha, interferon-gamma and interleukin-10 were also significantly higher in the post-surgical wound infection patient as compared with the control group (p=0.001) respectively. Multidrug resistance bacterial infections are more dangerous and pathogenic, and the observed elevation of inflammatory response may lead to difficulty in infection resolution, it could contribute to partially explaining why patients with MDR infections still have poor treatment outcomes even after receiving timely antibiotic treatment.

**Keywords**: Antibiotic, multidrug, resistance, interferon, interleukin-10, infection.

**INTRODUCTION**

The continuous emergence and rapid dissemination of antibiotic-resistant bacteria has become a significant public health concern worldwide, posing a critical challenge for the effective management of infectious diseases. According to the World Health Organization, one of the main risks to today's development, food security, and global health is antibiotic resistance. Every year, antibiotic-resistant illnesses claim the lives of at least 700,000 people worldwide; by 2050, that figure is predicted to reach 10 million.1 Multidrug-resistant (MDR) bacterial pathogens can pose significant health-care challenges by rendering most antimicrobials ineffective.2, 3 MDR pathogens can develop resistance to various antimicrobials through horizontal gene transfer and genetic mutations caused by drug overexposure.3 Antibiotic resistance causes more than 60,000 deaths in Nigeria each year.4

Antimicrobial resistance is defined as the ability of a microorganism to resist the action of the different antimicrobial agents. When this resistance occurs to multiple drugs, it is known as multidrug resistance (MDR).5 Different types of multidrug resistance mechanisms occur in microbes, like natural resistance in certain microbes against a particular antimicrobial, genetic mutation, or acquired resistance from other species.2

The patient's immune system and the organism's pathogenicity both have a significant impact on how an infection develops. On the other hand, any foul-smelling discharge from a closed surgical incision along with indications of tissue inflammation around it should be regarded as a wound infection (3–4) When compared to infections brought on by antibiotic-susceptible bacteria, infections produced by antibiotic-resistant pathogens in a clinical context are linked to higher rates of morbidity, death, and healthcare expenses.6 The overuse of antibiotics has put people's health in danger and put a financial strain on national healthcare systems.7 The unscrupulous selling of antibiotics without valid prescriptions or diagnostic testing is a significant contributing cause to antibiotic misuse.8

The development of post operative wound infections is due to three factors: the degree of bacterial contamination during the operation, the duration of the procedure, and the patient's underlying disease, such as immune deficiency, diabetes, and malnutrition. Multidrug-resistant bacteria isolates have presented significant challenges to the treatment of surgical wound infections worldwide.9 Exposing retinal and microglial cells to MDR bacterial strains results in increased levels of IL-6, IL-1α, IL-8, IL-10, tumor necrosis factor-alpha, and interferon- gamma.10 Thus, MDR-bacterial infections are more virulent, and an excessive inflammatory response may impede infection resolution.

Surgical site infections (SSIs) remain a significant cause of morbidity and mortality globally, contributing to prolonged hospital stays, increased healthcare costs, and poor patient outcomes. In Benin City, Edo State, Nigeria, the prevalence of SSIs is worsened by the misuse and overuse of antibiotics, which brings about the development of antibiotic-resistant bacteria. This issue is particularly pressing in resource-limited settings where infection control practices may be suboptimal, and access to second-line treatments is limited. Therefore, this study aimed to determine the pattern of multidrug resistance isolates among patients with post operative surgical site infections and their immunological responses to misuse of antibiotics in Edo state.

**MATERIALS AND METHODS**

**Study Area**

This study was conducted at Central Hospital, Benin City, Edo State, Nigeria. Edo State is situated at latitude 6.6342°N and longitude 5.9304°E, covering an area of 19,794 km². According to the National Population Census (2006), Edo State had a provisional population of 2,159,484, with Benin City estimated at 1,147,188 inhabitants.

**Study Design**

This was a descriptive cross-sectional study conducted at the post-operative ward of the Department of Surgery and Department of Gynaecology of Central Hospital Benin city (CHB), after obtaining an Ethical approval from the institutional review committee of the institute (Reference No: HA/737/24/C/0430285).

**Study Population**

The study involved 200 participants comprised of 100 patients who had post-operative surgical wounds and 100 subjects who served as control group. These patients were from the gynaecology department of the hospital. Clinical wound swab samples were collected from each patient.

**Sample Collection**

Random sampling of post-operative patients with surgical wounds was employed in this study. Samples were collected daily ensuring that patients with infections had an equal chance of being selected. Informed consent was obtained from all participants or their guardians before sample collection. The samples were collected by swabbing the wound site using sterile cotton swabs. Each swab was immediately placed into sterile screw-capped and a five (5ml) venous blood samples were collected from the medial cubital vein of the patients using a vacutainer and needle, that were then placed in plain containers. Serum were obtained after clot formation, retraction, centrifugation for 5minutes at 4000rpm and was used to determine the levels of tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), and interleukin-10 (IL-10) in post-surgical patients and control subjects using enzyme link immunosorbent assay method. The specimens were immediately transported within one hour of collection to the Medical Microbiology Laboratory at Benson Idahosa University (BIU) in Benin City, Edo State, for analysis. All collections were performed under strict aseptic conditions to avoid contamination.

**Microbiological analyses**

The wound swab specimens were inoculated on Blood agar, MacConkey agar, Nutrient agar, and Mannitol salt agar plates and were incubated aerobically and anaerobically at 370C for 24 hours. Duplicate blood agar plates were incubated anaerobically at 370C for 24 hours. All the media used (Blood agar, MacConkey agar, Nutrient agar, and Mannitol salt agar) were prepared according to the manufacturer’s directives.

**Isolation and characterization of isolates**

Macroscopic identification of bacterial isolates was initially performed based on their colonial morphology on MacConkey agar and nutrient agar plates. Key morphological characteristics observed included colony size, form, elevation, opacity, odor, and edge. For further identification, Gram staining was conducted on the colonies. The colonies on MacConkey agar were categorized into lactose-fermenting and non-lactose-fermenting colonies. Both lactose-fermenting and non-lactose-fermenting colonies were then subjected to a series of conventional biochemical tests. These tests included citrate utilization, urease production, indole production, oxidase test, motility test, and carbohydrate fermentation tests using sugars such as maltose, sucrose, and mannitol. These biochemical tests provided further confirmation of the bacterial species present.

**Antibiotics Susceptibility Testing**

Antimicrobial susceptibility testing was carried out on each isolate by the disc diffusion method using the Kirby- Bauer disc diffusion method in accordance with the National Committee for Clinical Laboratory Standards (NCCL, 2003) guideline to evaluate the sensitivity of the test organisms to the various antibiotics. Test isolates were grown on Nutrient agar and incubated at 37o C for 24 hours. Colonies were suspended into sterile normal saline and the inoculum density was adjusted to 0.5 McFarland turbidity standards. A sterile cotton wool swab was inserted into each test tube containing the standardized inoculum suspension, rotated with firm pressure on the inside wall of the test tube to remove excess fluid and then used to swab the surface of a freshly prepared dried Mueller- Hinton agar plate. The antimicrobial disc used included Ceftazidime (CAZ 30µg), Gentamycin (GN 30µg), Ofloxacin (OFL 5µg), Ciprofloxacin (CPR 5µg), Erythromycin (Ery 10ug), Imipenem (IMP 10ug), Oxacillin (OXA, 1ug), Cefuroxime (CRX 30ug), Cefixime (CXM 5ug), and Augmentin (AUG, 30ug) (Oxide). The discs were placed on the surface of the inoculated Muller Hinton agar plate and incubated at 370C for 24 hours. After incubation, diameters of zones of inhibition were measured to the nearest millimeter using a transparent meter rule. The clinical isolates diameter zones were compared with reference control organism held at Lahor Research Laboratories (*E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 6538) and interpreted as susceptible or resistant according to the CLSI (2017).

**Determination of Tumor necrosis factor-alpha (TNF-α), Interferon-gamma (IFN-γ), and Interleukin-10**

The levels of tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), and interleukin-10 (IL-10) in post-surgical patients and control subjects were evaluated using the ARG80930 Human M1/M2/MDSC Cytokine Multiplex ELISA Kit. Blood samples were collected, allowed to clot, and then centrifuged to obtain serum, which was stored at -20°C until analysis. ELISA kits specific for TNF-α, IFN-γ, and IL-10 were used following the manufacturer's instructions. Microtiter plates pre-coated with capture antibodies were blocked to prevent non-specific binding. Serum samples, standards, and controls were added to the plates and incubated. After washing, biotinylated detection antibodies were added, followed by streptavidin conjugated to horseradish peroxidase (HRP). The substrate solution was then added, and the reaction was stopped before measuring the optical density at 450 nm using a microplate reader. The concentrations of TNF-α, IFN-γ, and IL-10 were calculated based on standard curves, and statistical methods were used to compare the cytokine levels between post-surgical patients and control subjects.

**Statistical Analysis**

Data obtained from this research was analyzed using the Statistical Package for Social Sciences (SPSS) version 21.0 (IBM Inc., USA). The data was presented using mean +/- standard deviation and student t test was used for comparison between groups. P<0.05 was considered statistically significant.

**RESULTS**

A total of one hundred (100) post-operative wound swabs specimens from hospitalized patients at CHB and one hundred from apparently healthy individual were analyzed. The prevalence of wound infection among the study participants was (60%) (Table 1). The isolates from the samples were phenotypically characterized as shown in (Table 2). *Escherichia coli* 18(30%), *Pseudomonas aeruginosa* 30(50%) and were most likely to be associated with wound infection closely followed by *proteus mirabilis* 9(15%) and the least was *staph aureus* 3(5%) (Table 3). The MDR bacterial isolates showed highest resistivity to the cephalosporins and the penicillin such as Augmentin in the surgical wound infection patients

Among the penicillin class, Augmentin showed a high sensitivity rate with 50 isolates (83%) sensitive and 10 (17%) resistant, while Oxacillin had an equal distribution of resistance and sensitivity (50%). For macrolides, 30 isolates (50%) were sensitive to Erythromycin, and 30 (50%) were resistant. In the aminoglycoside class, Gentamycin had 50 isolates (83%) resistant and only 10 (17%) sensitive. The cephalosporin class demonstrated high sensitivity, with Ceftazidime showing 55 isolates (92%) sensitive and 5 (8%) resistant, Cefuroxime had 56 (93%) sensitive and 4 (7%) resistant, and Cefixime showed 54 (90%) sensitive and 6 (10%) resistant. Imipenem, a carbapenem, had the highest sensitivity with 58 isolates (97%) sensitive and only 2 (3%) resistant. Lastly, the quinolones, Ofloxacin and Ciprofloxacin, showed moderate resistance, with 20 isolates (33%) sensitive and 40 (67%) resistant to Ofloxacin, and 19 (32%) sensitive and 41 (68%) resistant to Ciprofloxacin (Table 4).

The antibiogram profile of bacteria isolated is summarized in Table 5. For *Pseudomonas aeruginosa* (n=30), all isolates (100%) were resistant to the tested antibiotics, with no sensitivity observed. *Escherichia coli* (n=18) showed a resistance rate of 67%, with 12 isolates being resistant and 6 (33%) sensitive. *Proteus mirabilis* (n=9) exhibited a lower resistance rate, with 3 isolates (33%) resistant and 6 (67%) sensitive. *Staphylococcus aureus* (n=3) demonstrated complete sensitivity, with 100% of the isolates sensitive and no resistance observed. Overall, out of 60 isolates, 45 (75%) were resistant, while 15 (25%) were sensitive to the antibiotics tested (Table 5).

Tumor necrosis factor alpha (TNF-alpha) levels in surgical subjects (512 ± 0.03 pg/ml) were significantly higher compared to the control group (14.69 ± 0.01 pg/ml) (p<0.05). Interferon-gamma levels were elevated in surgical subjects (234 ± 0.01 pg/ml) compared to controls (24.68 ± 2.67 pg/ml) (p<0.05). Interleukin-10 levels were significantly higher in surgical subjects (35.01 ± 0.01 pg/ml) than in controls (3.02 ± 0.02 pg/ml) (p<0.05) (Table 6).

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| **Table 1: Prevalence of surgical wound infection in CHB** | | | |
| **Variable** | **No. Examined** | **Prevalence** | **Percentage (%)** | |
| Positive | 100 | 60 | 60 | |
| Negative | 100 | 40 | 40 | |

**Table 2: Phenotypic Identification of bacteria isolates**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Identified bacteria** | **Gram stain** | **Oxidase** | **Catalase** | **Nitrate reductase** | **VP** | **MR** | **Indole** | **Citrate** | **Motility** | **Urease** |
| ***Escherichia coli*** | -ve bacilli | -ve | +ve | +ve | -ve | +ve | +ve | -ve | Motile | -ve |
| ***Proteus mirabilis*** | -ve bacilli | -ve | +ve | +ve | -ve | +ve | -ve | +ve | Motile | +ve |
| ***Pseudomonas*** | -ve bacilli | +ve | +ve | +ve | -ve | -ve | -ve | +ve | Motile | -ve |
| ***Staphylococcus aureus*** | +ve cocci in clusters | -ve | +ve | -ve | +ve | -ve | -ve | +ve | Non-motile | -ve |

**Keys: -Ve** = Negative, **+Ve =** Positive, **VP**: Voges-Proskauer test, **MR**: Methyl Red test, **Indole**: Indole test

**Table 3: Distribution of etiologic agents of wound infection**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Variable** | **No. infected** | ***E. coli***  **N (%)** | ***P. aeruginosa***  **N (%)** | ***Proteus mirabilis***  **N (%)** | ***S. aureus***  **N (%)** |
| No Isolated | 60 | 18(30.0) | 30 (50.0) | 9(15.0) | 3(5.0) |

**Keys: N** = Total number of bacteria infected (%)

|  |  |  |  |
| --- | --- | --- | --- |
| **Table 4: Susceptibility profile of isolates to tested antibiotics** | | | |
| **Class of Antibiotics** | **Type of antibiotics** | **CHB (%) No. tested =60** | |
|  |  | **R** | **S** |
| **Penicillin** | Augmentin (30µg) | 50(83) | 10(17) |
|  | Oxacillin (4µg) | 30(50) | 30(50) |
| **Macrolides** | Erythromycin (10µg) | 30(50.0) | 350(8.0) |
| **Aminoglycoside** | Gentamycin (30µg) | 10 (17) | 50(83) |
| **Cephalosporin** | Ceftazidime (30µg) | 55 (92) | 5(8.0) |
|  | Cefuroxime (30µg) | 56(93) | 4(7.0) |
|  | Cefixime (5µg) | 54(90) | 6(10) |
| **Carbapenem** | Imipenem (30µg) | 2(3.0) | 58(97) |
| **Quinolones** | Ofloxacin (5µg) | 20(33.0) | 40(67.0) |
|  | Ciprofloxacin(5µg) | 19 (32) | 41(68.0) |

**Keys: No**. = Number of bacteria tested, **R**= Number of bacteria resistant **(%) S=** Number of bacteria sensitive **(%)**

|  |  |  |  |
| --- | --- | --- | --- |
| **Table 5: Percentage Antibiogram of Bacteria Isolated** | | | |
| **Isolates** | **N** | **R (%)** | **S (%)** |
| *Pseudomonas aeruginosa* | 30 | 30 (100) | 0(0.0) |
| *Escherichia coli* | 18 | 12 (67.0) | 6(33.0) |
| *Proteus mirabilis* | 9 | 3 (33.0) | 6 (67.0) |
| *Staphylococcus aureus* | 3 | 0 (0) | 3 (100) |
| Total | 60 | 45(75%) | 15(25%) |

|  |  |  |  |
| --- | --- | --- | --- |
| **Table 6: Levels (mean ± SD) of Tumor necrosis factor alpha (pg/ml), Interferon-gamma (pg/ml) and Interleukin-10 (pg/ml) in post-surgical patients and control subjects** | | | |
| **Time intervals** | **TNF-alpha** | **Interferon-gamma** | **Interleukin-10** |
| Surgical subject | 512 ± 0.03 | 234 ± 0.01 | 35.01 ± 0.01 |
| Control | 14.69 ± 0.01 | 24.68 ± 2.67 | 3.02 ± 0.02 |
| P value | 0.001\* | 0.001\* | 0.001\* |

\* = significant

**DISCUSSION**

Antimicrobial resistance in Surgical site infection is becoming a worldwide alarming problem, resulting in longer hospital stay for patients as well as higher mortality and morbidity.11 A total of one hundred (100) post operative wound swabs specimens from hospitalized patients at CHB was analyzed. A total of 27 clinical bacterial isolates, was isolated which gave a prevalence rate of 27%. Although this is lower than an earlier report of 27.6% by Ehiaghe *et al.*.12 Also, when compared with the result of 70.1%.9 World Health Organization in 2011 gave a prevalence of 5 – 34% of SSI and this is in line with the result of this study. This gradual reduction in the prevalent rate of surgical site infection could be due to high hygienic or aseptic procedure been carried out by the Hospital personnel before surgical operation.

*Pseudomonas aeruginosa* (50%) was found to be the most common etiologic agent of surgical wound infections in CHB, followed by *Escherichia coli* (30%), *P. mirabilis* (15%), and *Staph aureus* (5%) (Table 4). However, it has been reported that *P. aeruginosa* is the most commonly associated with post-operative surgical wounds, possibly due to its high survival rates in the hospital setting.13, 14

All antibiotics used in this study displayed similar level of resistance to the isolates. This high resistance the isolates showed to the various antimicrobial agents used in this study may in part be due to various factors such as inappropriate usage of antibiotics and drug resistance mechanism possessed by the bacterial isolates. Cephalosporins and Penicillins have been found to be highly resisted by surgical wound pathogens. This was also in lined with the report result of Ehiaghe *et al*.12

The post-surgical wound infection patient had significantly higher levels of tumor necrosis factor alpha, interferon-gamma, and interleukin-10 than the control group. From the study, we detected that MDR-bacterial infection intensifies the inflammatory response of TNF, interferon gamma (a strong pro-inflammatory cytokine), and interleukin-10 (a strong anti-inflammatory cytokine) produced by activated macrophages, monocytes, and natural killer cells among the study populations for the first time in Benin City, Nigeria. Our results are in line with those observed in diseases where inflammatory cytokines are markedly increased, such as bacteremia, acute organ failure, post-surgical wound infections and multidrug-resistant tuberculosis.15,16 According to Ehiaghe, *et al.*,12 MDR-TB patients' mean levels of TNF-α and IL-10 were considerably greater than those of control subjects. The results of these investigations indicate that patients' ability to successfully control the microbial infection was diminished by MDR bacterial infections, as evidenced by the moderate rise in IL-10 response to MDR-bacterial isolates.

It had also shown that in patients with human immunodeficiency virus infection and other experimental infection have high levels of both pro and anti-inflammatory cytokines, which correlated with susceptibility to the pathogens and the severity of the associated disease.17 However, studies have shown that exposing retinal and microglial cells to MDR bacterial strains results in increased levels of IL- 6, IL-1α, IL-8, IL-10, tumor necrosis factor-alpha, and interferon-gamma.4, 10, 18 Thus, excessive inflammatory response observed in this present study may impede infection resolution, which may help to explain in part, the poor treatment outcome in patients with MDR infections in Benin city, Nigeria, even after prompt antibiotic treatment. Furthermore, understanding the drug resistance strategies possessed by the etiologic agents of surgical site infections will significantly improve chemotherapeutic approaches in the treatment of wound infections worldwide.

**CONCLUSION**

This study revealed a 60% prevalence of wound infection. The predominant bacterial isolates were *Pseudomonas aeruginosa* (50%) and *Escherichia coli* (30%), with lower occurrences of *Proteus mirabilis* (15%) and *Staphylococcus aureus* (5%). Antibiotic resistance was highest among cephalosporins and penicillins, with *Pseudomonas aeruginosa* showing 100% resistance. Sensitivity was observed mainly with Imipenem and cephalosporins. The antibiogram revealed that 75% of isolates were resistant to tested antibiotics, while 25% were sensitive. Additionally, inflammatory cytokine levels (TNF-alpha, Interferon-gamma, and Interleukin-10) were significantly elevated in surgical patients compared to controls, indicating a pronounced inflammatory response.

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