Original

Pattern of Multidrug Resistance Genes and Immunological Biomarkers in Post-Surgical wound infections in Nnewi, Nigeria

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Abstract

Background: Post-surgical wound infection (PSWI) can cause poor wound healing and longer period of hospitalization. The study evaluated the patterns of antibiotic multidrug-resistant genes and immunological biomarkers associated with PSWI in Nnewi, Nigeria.

Methods: Bacterial isolates that were difficult to identify using cultural and biochemical means were sent for sequencing. Enzyme linked immunosorbent assay method was used to evaluate Interleukin-10 (IL-10), Interleukin-4 (IL-4), tumor necrosis factor alpha (TNF-α,), interferon gamma (IFN-γ), and automated blood counting was used for haematological parameters. Polymerase chain reaction was used to detect the multidrug-resistant genes (MDR). Two hundred participants (100 patients with PSWI and 100 apparently healthy individuals (control group) aged between 17-70 years were recruited into the cross-sectional study using simple random sampling method. Data were analyzed using independent t-test and Pearson correlation with $p<0.05$ assumed to be statistically significant.

Results: The detection of Serratia marcescens, Ectopseudomonas mendocina, Providencia rettgeri, Providencia stuartii and Klebsiella grimontii using gene sequencing underscores a complex etiology in PSWI. We detected the presence of SHV, TEM, and gyrA MDR in patients with PSWI. The levels of neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), IL-10, IL-4, TNF-α and IFN-γ were significantly elevated in the test group when compared with the control group ($p<0.05$).

Conclusion: The study detected the presence of SHV, TEM, and gyrA MDR genes in the isolates. The significantly elevated levels of TNF-α, IFN-γ, IL-4, NLR, and PLR in cases of PSWI suggest that these markers could be useful in the early detection, monitoring, and management of such cases.

Keywords: *Serratia marcescens*, *Ectopseudomonas mendocina,* post-surgical wound infections, cytokines and multidrug resistant genes

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Introduction

Post-operative wound infections remain a significant global health burden, affecting millions of surgical patients annually and leading to substantial morbidity, mortality, and healthcare costs [1,2]. Such infections, collectively referred to as surgical site infections (SSIs), are among the most frequent healthcare-associated infections worldwide. Recent global estimates reveal that SSIs affect 2-5% of patients undergoing inpatient surgeries, with prevalence rates varying significantly by region and surgical procedure [3]. According to Mengistu *et al*., SSIs estimates varied among the WHO regions of the world and were high in the African region, accounting for 7.2%[4]. In low- and middle-income countries (LMICs) like Nigeria, SSIs are disproportionately higher due to factors such as limited healthcare resources, inadequate infection control measures, and poor patient health literacy [5].

SSI rates can exceed 20% in some surgical specialties, particularly in resource-limited hospitals where infection prevention practices are suboptimal [6]. The burden is exacerbated in rural areas, where access to adequately equipped healthcare facilities and trained personnel is limited, leading to poor postoperative outcomes [7].
Evidence highlights that SSIs contribute to prolonged hospitalizations and can triple the costs of care, placing financial strain on both patients and healthcare systems, particularly in LMICs. [8,9]

According to the study by Monahan *et al*., the additional cost of SSIs varied widely in both settings, with LMICs ranging from $174 to $29,610 and high-income European countries ranging from $21 to $34,000 which emphasizes the substantial cost burden imposed by SSIs in LMICs[10].

In Nigeria, where many patients face economic challenges, these infections further exacerbate healthcare inequities, as prolonged hospitalization and repeat interventions for SSIs are often financially burdensome [11].

Moreover, SSIs are a significant cause of mortality in surgical patients, particularly when associated with multidrug-resistant (MDR) pathogens [12]. Patients with SSIs are at heightened risk of developing complications such as sepsis, which can progress to organ failure and death if untreated [12]. Given the high morbidity and mortality associated with SSIs, particularly in resource-limited settings, addressing these infections is essential for improving patient outcomes and reducing preventable deaths related to surgical complications [13].
In Nigeria, the unregulated sale of antibiotics, coupled with limited public awareness about proper antibiotic use, has accelerated the emergence and spread of resistant bacteria in clinical settings [14]. SSIs involving antibiotic-resistant pathogens are notably challenging to treat, as standard prophylactic and therapeutic antibiotics are rendered ineffective, leading to prolonged infections and higher risks of adverse outcomes [15].
In LMICs, antibiotic resistance in SSIs is driven by factors including inadequate healthcare infrastructure, poor infection prevention practices, and limited access to diagnostic testing, which can lead to the indiscriminate use of broad-spectrum antibiotics [16]. This situation underscores the necessity for improved antibiotic stewardship in LMICs to control the spread of resistant pathogens and prevent SSIs from becoming untreatable [17].

Pseudomonas aeruginosa and *Escherichia coli* also play substantial roles in SSIs, particularly in cases involving high-risk surgical procedures [18]. Both pathogens are notorious for their ability to acquire resistance to multiple antibiotics, including carbapenems, due to genetic mutations and horizontal gene transfer [19]. The spread of MDR pathogens complicates treatment regimens and necessitates prolonged hospitalizations, which can increase healthcare-associated infection risks and elevate overall healthcare costs [20].

The resistance mechanisms in common SSI pathogens are complex, involving both chromosomal mutations and the acquisition of resistance genes via plasmids [21]. Genes such as aminoglycoside acetyltransferase (AAC), gyrase A, TEM, SHV, and BlaOxa contribute to multidrug resistance in bacteria like *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* [22]. AAC enzymes inactivate aminoglycosides while mutations in gyrase A confer fluoroquinolone resistance by altering target sites, reducing drug efficacy [23]. Similarly, TEM and SHV are β-lactamase genes that degrade Penicillin and cephalosporins, and BlaOxa confers resistance to oxacillin and carbapenems [24].

In addition to understanding resistance patterns, it is essential to assess the immunological responses associated with inappropriate antibiotic use, as this can

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influence infection outcomes. Studies suggest that antibiotic misuse may alter the neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR), which are biomarkers associated with inflammation and immune responses in infection [25,26]. These biomarkers are critical for predicting infection severity, as elevated NLR and PLR have been linked to poorer outcomes in bacterial infections. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) are additional haematological parameters that may be affected by inappropriate antibiotic exposure, influencing the host's ability to respond effectively to bacterial challenges [27].
The cytokine response to antibiotic misuse is also a pivotal area of concern, as cytokines play a crucial role in mediating inflammation and immune defense against infections. Studies have highlighted that cytokines such as interleukin-1β (IL-1β) and tumour necrosis factor-alpha (TNF-α) are elevated during severe infections, triggering an inflammatory cascade that helps to contain bacterial spread [28,29]. However, overuse of antibiotics may lead to dysregulation of these cytokines, which could either attenuate or exaggerate inflammatory responses, potentially delaying recovery. IL-4 and IL-10, which are anti-inflammatory cytokines, also play essential roles in moderating immune responses and preventing tissue damage; their balance with pro-inflammatory cytokines is critical for achieving effective infection resolution [30]. Unregulated antibiotic exposure has been associated with imbalanced cytokine levels, suggesting that antibiotic misuse could impair immune function and alter infection dynamics. [31]

Consequently, addressing antibiotic misuse is crucial for both controlling resistance and maintaining effective immune function in patients with post-operative infections. The study was aimed at evaluating the Pattern of Multidrug Resistance Genes (SHV, TEM, and gyrA) and Immunological Biomarkers (neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), Interluekin-10, IL-4, tumor necrosis factor alpha and interferon gamma) in post-surgical wound infections in Nnewi, Nigeria.

Methods

Study Design: This cross-sectional study participant included a total of 200 hospitalized Patients (100 males and 100 females) from the department of general surgery, obstetrics/gynecology and orthopedic wards of Nnamdi Azikiwe University Teaching Hospital, (NAUTH) Nnewi, Anambra state, Nigeria with ages ranging from 17-70 years. Participants were recruited using simple random sampling method. Participants were both age matched and sex matched to increase the power of the study and minimize gender bias. Venous blood and wound swab samples were collected from wound site under strict aseptic conditions. Specimens were transported immediately to the Immunology/Medical microbiology Department of Nnamdi Azikiwe University Teaching Hospital, (NAUTH) Nnewi for analysis within 3-4 hours of sample collection. Bacterial isolation and characterization were done using standard microbiological method while cytokine evaluation was done using enzyme linked immunosorbent assay method. The gene (SHV, TEM, and gyrA) expression analysis was performed using Polymerase chain reaction (PCR) with gene specific primers at Iykenson Molecular Diagnostic Centre, Awka. No DNA template controls which serve as quality control were used to normalize the polymerase chain reaction.

Study Site: The study was carried out at Nnamdi Azikiwe University Teaching Hospital Nnewi. Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, Anambra State, is one of Anambra State's two tertiary healthcare facilities, the other being Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (COOUTH), Amaku, Awka. As a federal institution, NAUTH has fulfilled its obligation for years by delivering healthcare services to Anambra people, Nigeria, and beyond, with the majority of patients being sent to the facility for quality care. NAUTH is a 2,000-bed capacity complex built on 100 hectares of land featuring state-of-the-art facilities, including a 240-bed Trauma and Emergency Complex, a 200-bed Pediatrics Emergency Complex, a 200-bed Medical Ward Complex, a 200-bed Surgical Ward Complex, a 120-bed Mother and Childcare Building, a Radiology Building, a General Outpatient Department Complex, and many more.

Study Population: The cross-sectional study randomly included a total of 200 hospitalized Patients from the department of general surgery, obstetrics/gynecology and orthopedic wards of Nnamdi Azikiwe University Teaching Hospital, (NAUTH) Nnewi, Anambra state,

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Nigeria with ages ranging from 17-70 years. Participants were recruited using basic random sampling, with no regard for departmental quotas. Data collection took place between September 2023 and October 2024.

Participants Recruitment: A total of 200 hospitalized patients (100 males and 100 females) with ages ranging from 17-70 years was randomly recruited for the study. Randomization of participants was achieved by balloting method in which participants have to pick numbers and those that picked even numbers were recruited into the study. Also, participants were age and sex matched. These processes were undertaken to minimize selection bias which could influence the outcome of the study, and this was purely as a result of randomization.

Sample size and Sample size calculation: The sample size was obtained using the formula of Naing *et al.* [32], which is $N = Z^2 \times P (1-P)/d^2$
Where: n=Minimum sample size; D =Desired level of significance (0.05); Z= Confidence interval (1.96); P = Prevalence rate of surgical wound infection. Prevalence rate of surgical wound infection according to WHO [33] is 11%. Thus, N = 150
However, a total of two hundred participants were recruited to increase the power of the study.

Inclusion Criteria: Male and female genders were included. Participants were age matched between the test group and control participants and those between the ages of 17 and 70 years were included in the present study.

Exclusion Criteria: The study excluded people who are under the age of 17 or who are above the age of 70, HIV positive patients, diabetics, hypertensive patients, tuberculosis patients and people with known immunological problems such as asthma, autoimmune diseases and patients who did not consent to the study. Due to challenges encountered in obtaining written informed consent from individuals below 17years, they were excluded from the study. Also, participants above 70 years were also excluded due to age associated diseases such as immunosuppressive conditions like arthritis etc

Ethical Considerations: In line with the Helsinki Declaration, approval for this study was obtained from the Human Research Ethics Committee of Nnamdi Azikiwe University Teaching Hospital, (NAUTH) Nnewi, Anambra state (NAUTH/CS/66/VOL.16/VER.3/322/2023/76).
The procedures involved in the study were explained to the subjects and written informed consent was obtained from each subject before enrolling in the study. They were assured of the confidentiality of the information obtained from them during and after the study. Also, participants had the right to withdraw at any point during the research without being penalized and there was no monetary remuneration attached.

Sample Collection
Simple random sampling of patients with post-operative surgical wounds were used in this cross-sectional study. An informed consent form was signed before sample collection. Samples were only collected from patients before surgical wound dressing. Two swabs sample (for duplicate testing) were collected using sterile swab sticks from each patient and placed inside an Amies transport medium which was stored at room. All collections were done under strict aseptic conditions. Specimens were transported immediately to the Laboratory for analysis within 3-4 hours of sample collection.

Patient data collection Structured questionnaires were used to extract data by verbal consent from the patients and case notes; the information included were; demographic data, existing chronic disease (such as diabetes mellitus), past medical history, current drug use, smoking, length of preoperative hospital stay, and antimicrobial prophylaxis. Seven experts from the Faculty of Medical Laboratory Science at Nnamdi Azikiwe University, Awka (Nnewi campus), validated the questionnaire's content by reviewing its items and approving its clarity. An excellent agreement coefficient was achieved from the seven independent experts who evaluated the content validity tool designed to evaluate the pattern of immunological biomarkers and multidrug-resistant genes in post-surgical wound infection in Nnewi, Nigeria. Consequently, its content can be regarded as validated.

Laboratory Methods

Full blood count (FBC) estimation and immune cell ratios determination

The absolute neutrophil count, absolute lymphocyte count and absolute monocyte count were estimated using a Biobase automated hematology analyzer. The analyzer works on the principle of laser beam

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multidimensional cell classification, flow cytometry for white cell count, and differentiation and haemoglobin concentration were measured by cyanide-free colorimetric method. Neutrophil-lymphocyte ratio (NLR) was determined as the absolute value of neutrophil divided by the absolute value of lymphocytes. Platelet-lymphocyte ratio (PLR) was determined as the absolute value of platelets divided by the absolute value of lymphocytes.

Estimation of Human Tumor Necrosis Factor Alpha (TNF-α) and interferon gamma

Human TNF-α and interferon gamma immunoassays were determined using an enzyme-linked immunosorbent assay (ELISA) method according to Chukwuagwu *et al.* [34]

Estimation of Human Interleukin 10 (IL-10) and IL-4

Human IL-10 and IL-4 immunoassays were determined using an enzyme-linked immunosorbent assay (ELISA) method according to Hillyer and Woodward [35]

Bacteriological analysis

The wound swab specimens were inoculated on Blood agar, MacConkey agar and Mannitol salt agar plates and were incubated aerobically at 37°C for 24 hours. Duplicate blood agar plates were incubated anaerobically at 37°C for 24 hours. Bacterial isolates were phenotypically identified morphologically and biochemically Cheesbrough, [36]. All the media used (Blood agar, MacConkey agar and Mannitol salt agar) were prepared according to the manufacturer's directives in connivance with the procedure described Cheesbrough [36]. Five of the clinical isolates that were difficult to identify using cultural and biochemical means were sent for sequencing.

Isolation and Identification

Macroscopy

Isolates were identified based on their colonial appearances on agar. *P. aeruginosa* produces pale-coloured colonies on MacConkey agar while *E. coli* produces pink colonies on MacConkey agar. On nutrient agar, *P. aeruginosa* produces greenish colonies while *E. coli* appears colourless and Staph aureus produce golden yellow colour on mannitol salt agar plate Cheesbrough [33]. Morphological characteristics such as size, form, elevation, opacity, odour and edge were performed for identification of the organisms However, prior to this, films were made from the swabs and colonies by Gram's-stained techniques, which acted as a guide to the possible identity of the organisms into Gram positive and Gram-negative organisms. After the primary identification of the organism based on the Gram staining techniques, the colonies on the MacConkey agar were classified into lactose and non-lactose fermenting colonies. The non-lactose fermenting colonies and Lactose fermenting colonies were further subjected to the necessary conventional biochemical tests such as citrate utilization, urea production, indole production, Oxidase test, motility test, sugar fermentation (maltose, sucrose and mannitol) test.

Bacterial DNA extraction

The DNA was extracted from the samples in a 1.5ml micro centrifuge tube using Quick DNA mini plus kit as recommended by the manufacture (Zymo Research, Germany). This procedure included lysis, binding, washing (DNA purification) and elution into DNA elution buffer (provided in the kit). The inoculated PBS was centrifuged and 200µl of the sample was dispensed in a microcentrifuge tube prior to adding 200µl of Biofluid and Cell Buffer and 20µl Proteinase K. Following incubation at 55°C for 10 minutes, 1 volume of Genomic binding buffer was added to the sample. Spin column technology was used via the use of Zymo-Spin IIC-XL Column which was inserted in a collection tube to wash the sample proceeding series of centrifugation at ≥ 12,000 x g speed for 1 minute with the use of Pre-wash and Wash Buffer. DNA extracts was eluted into 50µl of DNA Elution Buffer. DNA extracts were stored at -20°C until use.

Quick load One Taq One Step Polymerase Chain Reaction

Quick load One Taq one step PCR master (2X) with catalog number NEB MO486S was purchased from lnqaba Biotech Hartfield South Africa incorporated and used according to the manufacturer's instruction. The system components were thawed and mixed by inverting ten times. The PCR was performed in 50µl reaction mixture containing 25µl Quick load One Taq one- step PCR master mix (2x), 1µl of each gene-specific forward primer (10µM), 1µl of each specific reverse primer10µM), 13ml of nuclease free water and 10µl of DNA template was added last. The PCR was started immediately as follows: Initial denaturation at 94°C for 1minute, denaturation at 94°C for 30secs, annealing at

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Tm-5 for 30secs, extension at 72°C for 1minute, Go to the denaturation step for 39 cycles, final extension at 72°C for 15mins and final holding at 4°C forever.

Table 1. Primer used for PCR amplification

Gene Symbol	Primers 5I-3I (forward, reverse)
SHV F	TATCTACAGCAGCGCCAGTG
	CGCATCAAATGCCATAAGTG
gyrA F	ATGACTGATATCACGCTGCCA
	ATAACGCATCGCTGCCGGTGG
TEM	ATGAGTATTCAACATTTCCG
	CTGACAGTTACCAATGCT

Preparation Agarose Gel

One point five percent agarose gel was prepared by dissolving 1.5g in 100ml Tris EDTA Buffer. The mixture was then heated in a microwave for 5minutes to dissolve completely. It was then allowed to cool at 56°C and 6µl of ethidium bromide was added to it. The agarose gel was poured into the electrophoresis chambers with gel comb and be allowed to solidify.

Electrophoresis

A 5µl of the amplified PCR products was analyzed on 1.5% agarose gel containing ethidium bromide in Tris EDTA buffer. Electrophoresis will be performed at 90v for 6ominutes. After electrophoresis, the PCR products was visualized by Wealth Dolphin Doc UV transilluminator and photographed. Molecular weights were calculated using molecular weight standard of the maker.

Sequencing

The Ultra-pure DNA was sequenced with ABI3500XL analyzer with a 50 cm array, using POP7 at Inqaba Biotechnical Industries Ltd (Hatfield, South Africa).

Statistical analysis

Statistical package for social sciences (SPSS) version 26 was used for the statistical analysis. Results were expressed as mean± SD. Frequency and percentage were employed to obtain descriptive statistics of the participants. The data generated was analyzed using Student's t-test for two independent variables as the parametric statistical tool of choice because the data obtained was normally distributed and also, the study involved the two distinct study groups (test group and control group). The Pearson correlation was used to correlate different parameters. Values were considered statistically significant if p-value is <0.05. Sequences data generated were analyzed with Geneious software version 9.0.5 and phylogenetic tree were constructed using neighbor joining.

Results

The mean body mass index (BMI), waist circumference (WC) and waist-hip ratio (WHR) of the study participants did not differ significantly when compared between the test group (PSWI group) and control individuals (p>0.05) respectively (Table 2).

The distribution of bacterial isolates (n = 100) obtained from wound infections is depicted in Table 3. The most frequently identified pathogen was *Escherichia coli*, accounting for 40% of the isolates. *Pseudomonas aeruginosa* was slightly less prevalent, representing 38 % of the isolates, followed by *Staphylococcus aureus* at 14%. Table 4 illustrates the antibiotic susceptibility patterns of the clinical isolates. Among *P. aeruginosa* isolates, 79% showed resistance, while only 21% were susceptible. *E. coli* showed 75% resistance and 25% susceptibility. *Proteus mirabilis* and *S. aureus* isolates exhibited resistance rates of 75% and 71.4%, respectively. Overall, the findings demonstrate a high resistance rate (76%) across all isolates, indicating that wound infection pathogens are predominantly resistant to conventional antibiotics. This high resistance rate could lead to treatment complications and may necessitate the use of alternative or combination therapies to manage infections effectively. Additionally, the high resistance observed in both gram-positive and gram-negative organisms emphasizes the growing challenge of antibiotic resistance in wound infections. Figure 1 to 5 show underscores the complex aetiology of diverse bacterial species, such as *Serratia marcescens*, *Ectopseudomonas mendocina*, *Providencia rettgeri*, *Providencia stuartii* and *Klebsiella grimontii* underscores a complex etiology in wound infections.

The image demonstrates the presence of SHV genes in several *Pseudomonas aeruginosa* isolates. Specifically, lanes 1 through 12 exhibit distinct bands at the 100 bp mark, suggesting that these isolates contain SHV resistance genes. The consistency in band visibility across these lanes underscores a relatively high prevalence of SHV resistance genes among the tested isolates. Notably, the lanes marked "NC" (negative control) did not show any bands, confirming that the PCR assay did not suffer

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from contamination or non-specific amplification. The findings suggest that the SHV genes are widespread among the analyzed *Pseudomonas aeruginosa* samples, which aligns with the growing concern regarding the role of ESBL-producing strains in clinical settings. The observation that all sample lanes (1 to 12) produced bands at the 100 bp target indicates a potentially significant distribution of this resistance gene among the population. This high prevalence may point to horizontal gene transfer mechanisms, such as plasmid-mediated transmission, which facilitate the spread of resistance genes across bacterial populations and environments.

Plate 2 reveals that samples 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, and 13 displayed bands at the 1,000 bp position, indicative of the presence of TEM resistance genes. Samples 1 and 3, however, did not display bands at this position, suggesting these isolates lacked the TEM gene. The negative control (NC) also exhibited no amplification, affirming the absence of contamination in the PCR process. The prevalence of TEM resistance genes across most samples (11 out of 13) highlights the extensive dissemination of this resistance gene in *Pseudomonas aeruginosa* isolates. This observation is consistent with existing literature suggesting that TEM genes, often plasmid-mediated, can rapidly spread among bacterial populations due to horizontal gene transfer. Such a high rate of TEM-positive isolates may suggest a widespread resistance to β-lactam antibiotics in this clinical setting, underscoring the critical need for resistance monitoring.

The gel electrophoresis results in Plate 3 indicate that samples 1, 3, 4, 5, 7, 8, and 9 exhibit bands at 600 bp, confirming the presence of *gyrA* resistance genes. Samples 2 and 6, however, did not display any band at this location, suggesting an absence of *gyrA* genes in these isolates. The negative control (NC) was free of any amplification bands, verifying that no contamination influenced the results, thereby reinforcing the reliability of the positive results. The high occurrence of *gyrA* positive samples (7 out of 9) aligns with documented trends of increasing fluoroquinolone resistance in *E. coli*, which is a common pathogen responsible for various infections. Given the prevalence of *gyrA* genes in the samples, this finding highlights the extensive spread of fluoroquinolone resistance, likely due to overuse or misuse of these antibiotics in clinical settings. The isolates' ability to harbor *gyrA* resistance genes suggests a significant selection pressure favoring resistant strains, making infections caused by these strains challenging to treat.

The t-test comparisons between the test and control groups, as outlined in Table 4, identified significant differences in various hematological and inflammatory parameters. Age was notably higher in the test group (M = 43.79, SD = 15.21) compared to the control group (M = 30.24, SD = 12.84), with a highly significant p-value ($p < 0.001$), suggesting that the test group consisted of older individuals on average.

Hematological variables, including MCV and MCHC, also differed significantly between groups. MCV was lower in the test group (M = 80.17, SD = 7.52) compared to controls (M = 83.51, SD = 8.31), with a p-value of 0.035. Similarly, MCHC was significantly higher in the test group (M = 414.06, SD = 44.68) than in the control group (M = 390.00, SD = 29.09; $p = 0.002$). These differences imply altered red blood cell characteristics in the test group, potentially indicating an underlying clinical condition or physiological adaptation. Inflammatory markers displayed the most pronounced differences between groups. The test group showed significantly elevated levels of NLR (M = 2.75, SD = 2.04) compared to the control group (M = 0.89, SD = 0.69; $p < 0.001$). Similarly, PLR, IFG, IL-4, TNF, and IL-10 were all significantly higher in the test group, with p-values of less than 0.001 in each case. These heightened levels of inflammatory markers in the test group suggest an increased inflammatory response, which may reflect the impact of disease or a heightened immune activation.

The mean age, MCV, MCH and MCHC levels did not differ significantly compared between the male patients with PSWIs and male control group ($p>0.05$) respectively. However, there were significantly higher mean NLR (3.12±2.69 Vs 0.97±0.53; p=0.001), PLR (135.18±78.62 Vs 79.02±33.50; p=0.002), IFN-γ (139.72±8.19 Vs 30.60±7.19; p=0.001), IL-4 (17.72±4.28 Vs11.56±2.53; p=0.001), TNF-α (164.08±4.09 Vs 46.24±8.41; p=0.001) and IL-10 (32.36±9.36 Vs 16.80±5.87; p=0.001) levels in the male patients with PSWIs than in the male control group respectively (Table 5).

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The mean age, MCV, MCH and PLR levels did not differ significantly compared between the female patients with PSWIs and female control group (p>0.05) respectively. However, there were significantly higher mean MCHC (413.20±45.31 Vs 380.52±14.02; p=0.003), NLR (2.43±1.21 Vs 0.81±0.31; p=0.001), IFN-γ (127.72±12.05 Vs 28.12±4.73; p=0.001), IL-4 (17.20±5.02 Vs 12.76±3.09; p=0.001), TNF-α (143.44±7.34 Vs 37.32±8.91; p=0.001) and IL-10 (27.52±7.76 Vs 17.12±6.22; p=0.001) levels in the female patients with PSWIs than in the female control group respectively (Table 6).

The Pearson correlation analysis, summarized in Table 7, revealed several notable relationships among variables in the test group. Notably, MCV and MCH exhibited a strong positive correlation ($r = 0.65$, $p < 0.01$), suggesting that larger mean corpuscular volumes were consistently associated with higher mean corpuscular hemoglobin levels. Similarly, MCH and MCHC were positively correlated ($r = 0.76$, $p < 0.01$), indicating that higher hemoglobin content per cell was linked with increased hemoglobin concentration.

In contrast, inflammatory markers demonstrated weaker and more varied correlations with hematological parameters. For instance, PLR showed a moderate positive correlation with NLR ($r = 0.39$, $p < 0.01$), which could reflect a parallel relationship between these two markers of inflammation. TNF-α also showed a weak positive association with IFG ($r = 0.29$, $p < 0.05$), potentially indicating a connection between immune activity and metabolic growth factors. However, other markers such as IL-4 and IL-10 displayed minimal correlations with age, hematological variables, or inflammatory ratios, suggesting limited direct association in this context.

Table 2: Anthropometric indices of the study participants (Mean ± SD)

Variables	PSWI group	Control group	t-value	p-value
Body mass index (kg/m²)	23.40±1.08	22.93±1.49	1.024	0.137
Waist circumference (cm)	85.77±5.29	84.17±4.93	0.895	0.176
Waist-hip ratio	0.87±0.04	0.86±0.04	0.987	0.342

*Statistically significant at p<0.05

Table 3: Distribution of etiologic agents of wound infection

Isolates	N (%)
Escherichia coli	40(40)
Pseudomonas aeruginosa	38(38)
Proteus mirabilis	8(8)
Staphylococcus aureus	14(14)

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F**igure 1**: Phylogenetic analysis of a bacterium based on the nucleotide sequence of part of the 16S rRNA nucleotide sequence of the Isolate. The clinical isolate is *Serratia marcescens* with gene bank accession number PQ459411.1

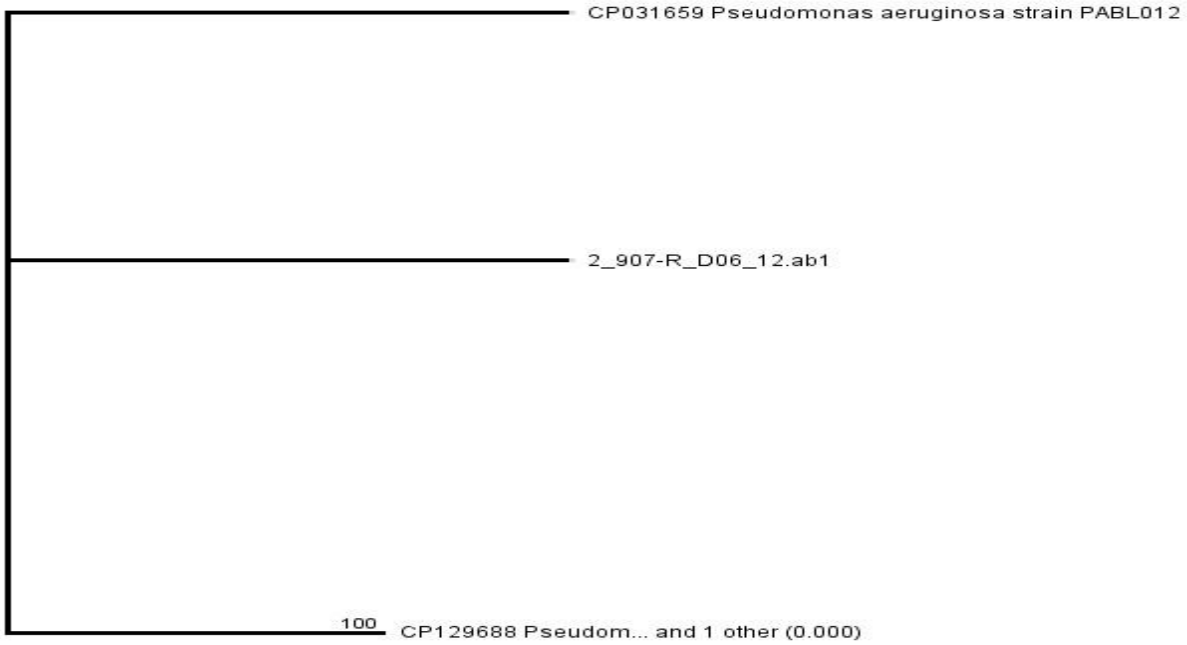

Figure 2: Phylogenetic analysis of a bacterium based on the nucleotide sequence of part of the 16S rRNA nucleotide sequence of the Isolate. The clinical isolate is *Ectopseudomonas mendocina* with gene bank accession number PQ4594412.1

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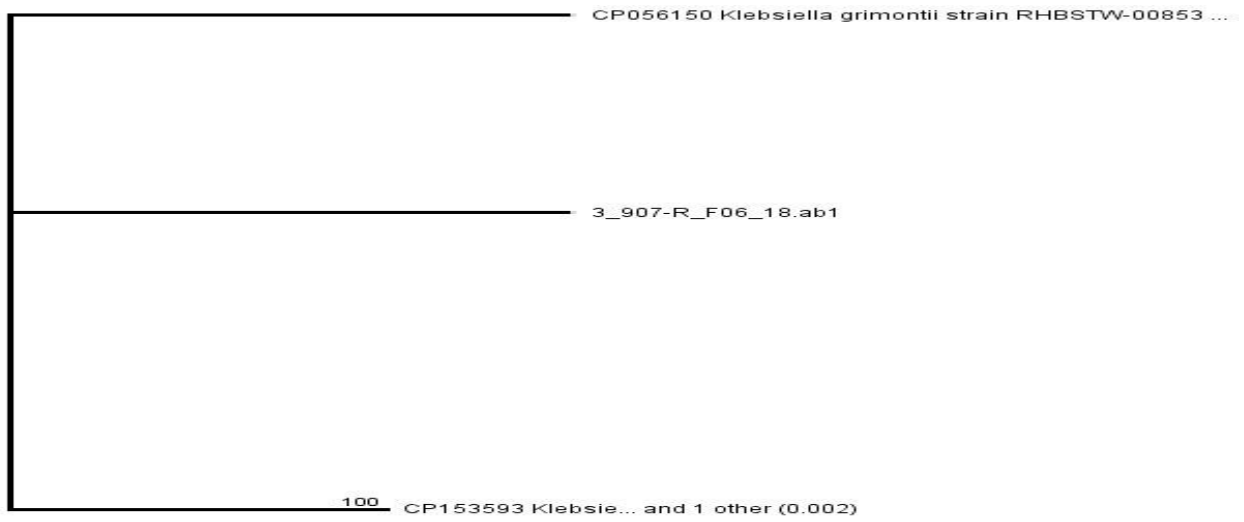

Figure 3: Phylogenetic analysis of a bacterium based on the nucleotide sequence of part of the 16S rRNA nucleotide sequence of the Isolate. The clinical isolate is *Klebsiella grimontii* with gene bank accession number PQ459413.1

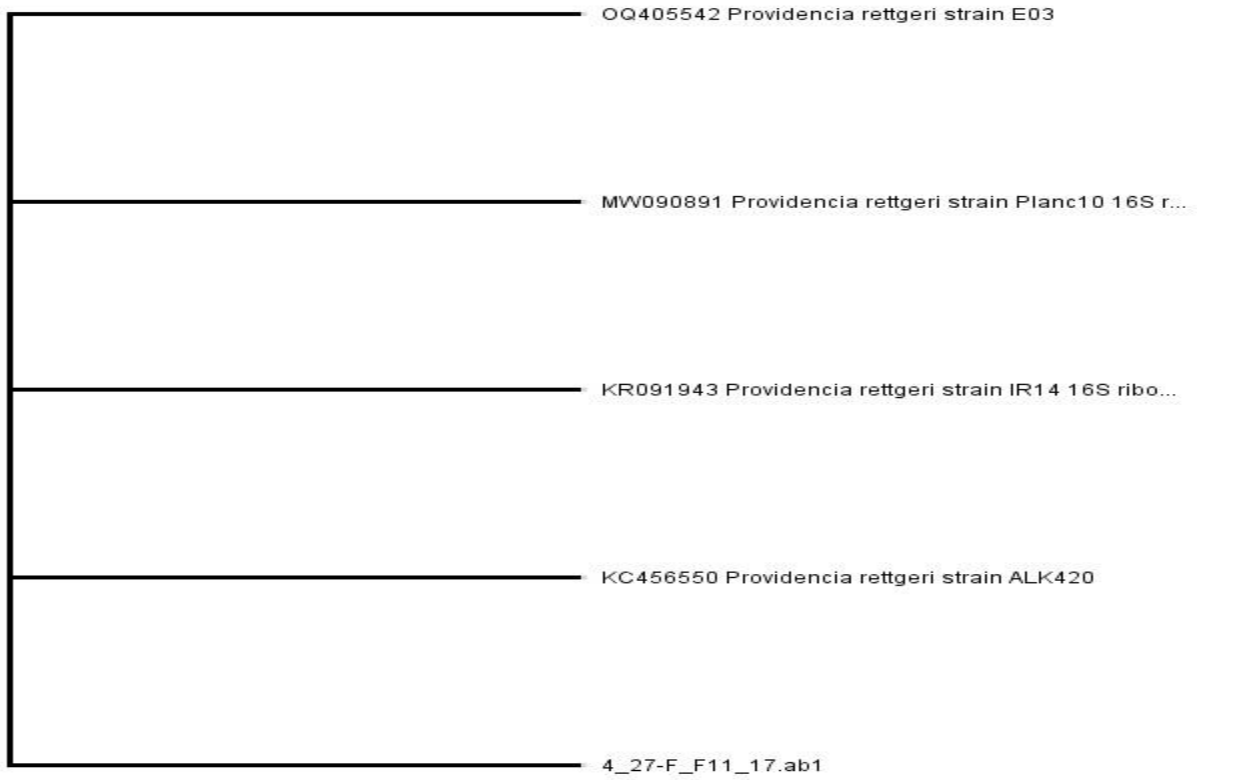

Figure 4: Phylogenetic analysis of a bacterium based on the nucleotide sequence of part of the 16S rRNA nucleotide sequence of the Isolate. The clinical isolate is *Providencia rettgeri* with gene bank accession number PQ459414.1

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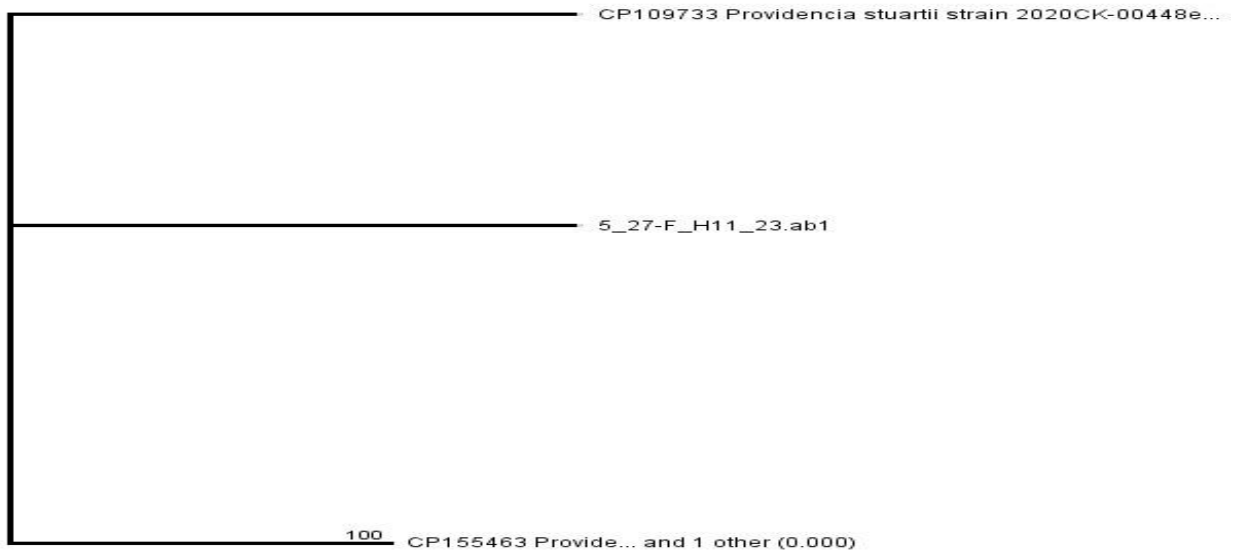

Figure 5: Phylogenetic analysis of a bacterium based on the nucleotide sequence of part of the 16S rRNA nucleotide sequence of the Isolate. The clinical isolate is *Providencia stuartii* with gene bank accession number PQ459415.1

Table 4: Antibiotic susceptibility pattern of clinical Isolates

Isolates	N	R (%)	S (%)
Pseudomonas aeruginosa	38	30 (79.00)	8 (21.00)
Escherichia coli	40	30 (75.00)	10 (25.00)
Proteus mirabilis	8	6 (75.00)	2 (25.00)
Staphylococcus aureus	14	10 (71.43)	4 (28.57)
Total	100	76(76.00)	24 (24.00)

Note: N = Number of isolates; R = Resistant; S = Sensitive

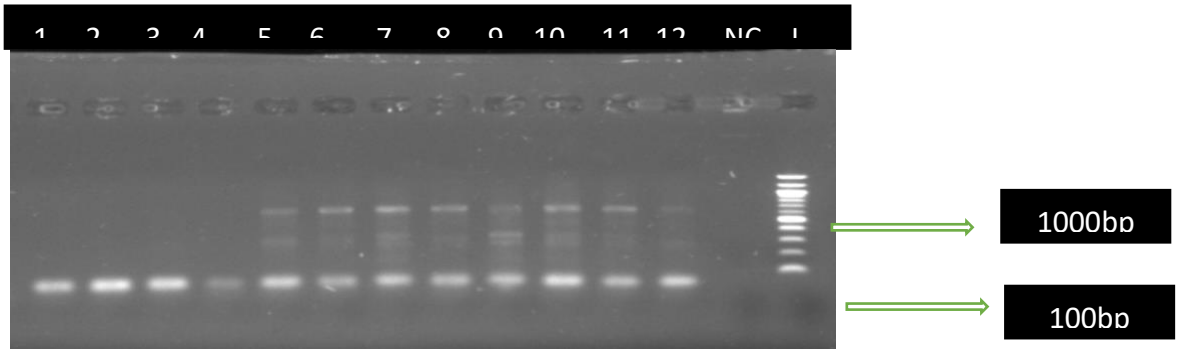

Plate 1: Polymerase chain reaction results of SHV resistant genes detected in *Pseudomonas aeruginosa* isolated analyzed on a 1.5% agarose gel electrophoresis stained with ethidium bromide. L is 100bp-1kb DNA ladder (molecular marker). Samples 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11and 12 are positive for SHV resistant genes with bands at 100 bp, NC is a no DNA template control.

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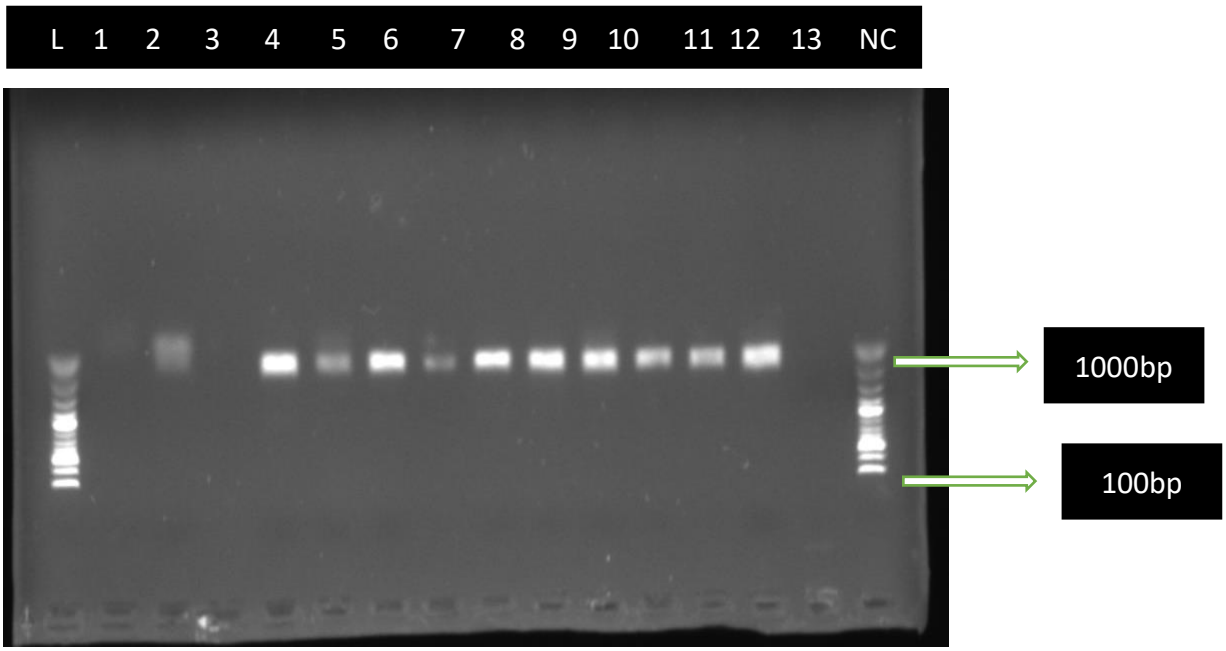

Plate 2: Polymerase chain reaction results of Tem resistant genes detected in *Pseudomonas aeruginosa* isolated from analyzed on a 1.5% agarose gel electrophoresis stained with ethidium bromide. L is 100bp-1kb DNA ladder (molecular marker). Samples 2, 4, 5, 6, 7, 8, 9, 10, 11, 12 and 13 are positive for Augmentin (TEM) resistant genes with bands at 1000bp. Samples 1 and 3 are negative for the resistant genes. NC is a no DNA template control.

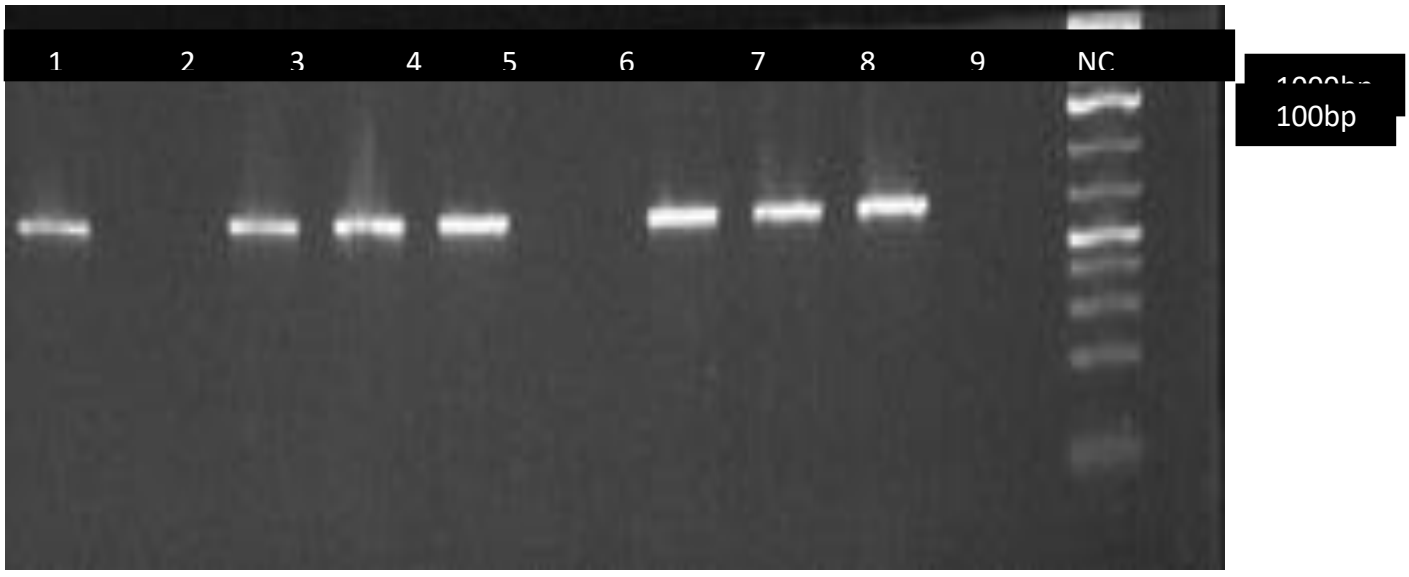

Plate 3: Polymerase chain reaction results of gyrA resistant genes detected in *Escherichia coli* isolated analyzed on a 1.5% agarose gel electrophoresis stained with ethidium bromide. L is 100bp-1kb DNA ladder (molecular marker). Samples 1, 3, 4, 5, 7, 8 and 9 are positive for ofloxacin (gyrA) resistant gene with bands at 600bp. Samples 2 and 6 are negative for genes. NC is a no DNA template control.

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Table 5: Comparison of Age, levels full blood count parameters and cytokine of post-surgical wound infection patients and individuals without post-surgical wound infection

Variables	PSWI (Mean ± SD)	Control Group (Mean ± SD)	p-value
Age (yrs)	33.79 ± 15.21	32.24 ± 12.84	0.345
MCV (fl)	80.17 ± 7.52	83.51 ± 8.31	0.035
MCH (pg)	33.24 ± 4.84	32.64 ± 4.52	0.517
MCHC(g/dl)	414.06 ± 44.68	390.00 ± 29.09	0.002
NLR	2.75 ± 2.04	0.89 ± 0.69	0.001
PLR	131.28 ± 74.86	88.40 ± 44.06	0.001
IFN-γ (pg/ml)	134.13 ± 11.68	29.36 ± 6.16	0.001
IL-4 (pg/ml)	17.42 ± 4.51	12.44 ± 2.79	0.001
TNF-α (pg/ml)	241.62 ± 38.26	42.64 ± 12.7	0.001
IL-10 (pg/ml)	26.94 ± 9.35	20.8 ± 5.13	0.001

*Statistically significant at $p<0.05$.

Table 6: Comparison of Age, levels full blood count parameters and cytokine of males with PSWIs and male control group

Variables	Males with PSWIs	Male control	t-value	P-value
Age (yrs)	35.44±13.83	34.56±11.98	1.194	0.412
MCV (fl)	81.58±7.50	83.97±7.51	1.124	0.266
MCH (pg)	33.92±4.70	33.62±4.89	0.218	0.828
MCHC(g/dl)	415.60±43.03	399.48±36.66	1.426	0.160
NLR	3.12±2.69	0.97±0.53	3.766	0.001
PLR	135.18±78.62	79.02±33.50	3.286	0.002
IFN-γ (pg/ml)	139.72±8.19	30.60±7.19	50.041	0.001
IL-4 (pg/ml)	17.72±4.28	11.56±2.53	6.185	0.001
TNF-α (pg/ml)	164.08±4.09	46.24±8.41	62.952	0.001
IL-10 (pg/ml)	32.36±9.36	16.80±5.87	7.037	0.001

*Statistically significant at $p<0.05$.

Table 7: Comparison of Age, levels full blood count parameters and cytokine of females with PSWIs and female control group

Variables	Females with PSWIs	female control	t-value	P-value
Age (yrs)	36.44±14.83	35.56±12.98	1.042	0.289
MCV (fl)	78.61±7.82	83.05±9.16	1.840	0.072
MCH (pg)	32.50±5.23	31.65±3.96	0.652	0.518
MCHC(g/dl)	413.20±45.31	380.52±14.02	3.187	0.003
NLR	2.43±1.21	0.81±0.31	6.465	0.001
PLR	131.05±48.76	75.84±21.55	1.815	0.076
IFN-γ (pg/ml)	127.72±12.05	28.12±4.73	38.461	0.001
IL-4 (pg/ml)	17.20±5.02	12.76±3.09	3.760	0.001
TNF-α (pg/ml)	143.44±7.34	37.32±8.91	45.925	0.001
IL-10 (pg/ml)	27.52±7.76	17.12±6.22	5.223	0.001

*Statistically significant at $p<0.05$.

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Table 8: Pearson Correlations between Age, MCV, MCH, MCHC, NLR, PLR, IFN-γ, IL-4, TNF-α and IL-10 for Test Subjects

Variables	r-value	P-value
MCV vs MCH	0.653**	0.000
MCV vs MCHC	0.028	0.841
MCV vs NLR	-0.004	0.978
MCV vs PLR	0.175	0.210
MCV vs IFN-γ	-0.049	0.729
MCV vs IL-4	0.042	0.764
MCV vs TNF-α	0.065	0.642
MCV vs IL-10	0.027	0.847
MCH vs MCHC	0.762**	0.000
MCH vs NLR	-0.081	0.564
MCH vs PLR	0.006	0.967
MCH vs IFN-γ	0.089	0.528
MCH vs IL-4	0.027	0.847
MCH vs TNF-α	-0.056	0.693
MCH vs IL-10	-0.102	0.469
MCHC vs NLR	-0.110	0.433
MCHC vs PLR	-0.139	0.322
MCHC vs IFN-γ	0.178	0.203
MCHC vs IL-4	0.009	0.947
MCHC vs TNF-α	-0.139	0.321
MCHC vs IL-10	-0.115	0.412
NLR vs PLR	0.385**	0.004
NLR vs TNF-α	-0.014	0.918
NLR vs IL-4	-0.083	0.554
NLR vs TNF-α	-0.080	0.571
NLR vs IL-10	-0.165	0.238
PLR vs IFN-γ	-0.244	0.079
PLR vs IL-4	-0.160	0.251
PLR vs TNF-α	-0.250	0.071
PLR vs IL-10	-0.231	0.096
IFN-γ vs IL-4	0.195	0.161
IFN-γ vs TNF-α	0.285*	0.039
TNF-α vs IL-10	-0.056	0.693
IL-4 vs TNF-α	0.041	0.773
IL-4 vs IL-10	0.010	0.943
TNF-α vs IL-10	0.185	0.170

Correlation is significant at $p < .05$ (*); $\mathbf{p} < .01$ (**).

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Discussion

The high prevalence of *E. coli* and *P. aeruginosa* among wound pathogens is notable, as these bacteria are often associated with severe infections that are resistant to standard antibiotic treatments. Such a distribution is consistent with epidemiological data indicating these pathogens as leading causes of healthcare-associated infections, including wound infections [37]. The dominance of Gram-negative bacteria in wound infections may complicate infection control, as these organisms are more resilient in the presence of antibiotics compared to Gram-positive species [38]. Their prevalence underscores the need for tailored infection management protocols that address the unique challenges posed by Gram-negative bacteria, including their capacity for horizontal gene transfer, which facilitates the spread of antibiotic resistance within clinical environments [39].

Furthermore, the presence of diverse bacterial species, such as *Serratia marcescens*, *Ectopseudomonas mendocina*, *Providencia rettgeri*, *Providencia stuartii* and *Klebsiella grimontii* underscores a complex etiology in wound infections, significantly influencing diagnostic and therapeutic strategies [40, 41]. This diversity suggests that wound infections are frequently polymicrobial, complicating diagnosis and treatment efforts [42]. The detection of less common pathogens often necessitates advanced molecular techniques, like PCR and next-generation sequencing, to accurately identify microbial populations within wounds [43]. A comprehensive understanding of wound microbiology enables clinicians to devise targeted therapies tailored to the specific bacterial composition of infections [44,45].

Table 3 presents antibiotic susceptibility patterns across isolates. Among *Pseudomonas aeruginosa* isolates, 79% were resistant, with only 21% showing susceptibility, while *E. coli* exhibited a similar resistance rate at 75%. Notably, *Proteus mirabilis* and *Staphylococcus aureus* showed resistance rates of 75% and 71.4%, respectively. These findings underscore a generalized resistance trend across both gram-negative and gram-positive pathogens, raising concerns about effective therapeutic options [46]. As such, the elevated resistance in wound infection isolates emphasizes the critical need for revised treatment strategies to manage AMR-associated infections [47]. Moreover, the study's antibiotic susceptibility results reflect a dire scenario where a substantial proportion of clinical isolates are resistant to conventional antibiotics. This trend, consistent across both gram-positive and gram-negative isolates, necessitates urgent investment in alternative therapies and infection control protocols. Antibiotic stewardship programs and targeted infection control measures could mitigate the spread of resistant strains [48].

Emerging antimicrobial resistance (AMR) among clinical pathogens such as *Pseudomonas aeruginosa* and *Escherichia coli* poses a significant global health threat [49, 50]. These pathogens frequently harbor extended-spectrum beta-lactamase (ESBL) genes, including SHV and TEM, and fluoroquinolone resistance genes like gyrA, which enable resistance to β-lactam and fluoroquinolone antibiotics [51]. This study details findings on the prevalence and distribution of SHV, TEM, and gyrA genes and contrasts these with observed antibiotic susceptibility patterns among isolates from clinical samples.

The PCR amplification in Plate 1 revealed the presence of SHV genes in all isolates (lanes 1–12) at the 100 bp mark, indicating a notable SHV gene prevalence among the isolates. This finding aligns with recent epidemiological studies, suggesting a pervasive distribution of SHV genes among *P. aeruginosa* populations, driven by horizontal gene transfer mechanisms [52]. As ESBLs confer resistance to β-lactam antibiotics, these results underscore a critical need for stringent surveillance in clinical settings [53].

In Plate 2, the PCR analysis highlighted TEM gene presence in most samples, with positive bands observed at the 1,000 bp mark across 11 out of 13 isolates. Negative amplification in samples 1 and 3 and the control (NC) confirmed assay specificity. The high prevalence of TEM-positive isolates aligns with previous studies indicating TEM gene's rapid dissemination through plasmid-mediated transfer [54]. This extensive spread of TEM genes across *P. aeruginosa* populations accentuates the urgency of antibiotic stewardship measures to curb the increasing resistance to β-lactam antibiotics [55].

Plate 3 illustrates that gyrA gene bands at the 600 bp position were present in 7 out of 9 samples, suggesting a widespread occurrence of fluoroquinolone resistance in *E. coli* isolates. Absence of gyrA in samples 2 and 6 suggests variability in resistance gene acquisition among isolates [56]. Fluoroquinolone misuse in clinical treatments

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has contributed to a significant selection pressure favoring gyrA-positive strains, supporting findings of prevalent gyrA-mediated resistance mechanisms across *E. coli* isolates [57].

The high prevalence of SHV, TEM, and gyrA resistance genes among clinical isolates signals an alarming trend towards multidrug resistance, especially among *P. aeruginosa* and *E. coli*. This distribution underscores the role of plasmid-mediated and chromosomal gene transfer in resistance proliferation. TEM genes, for instance, are extensively spread among pathogens due to their mobility, contributing significantly to the resistance burden [58]. These findings underscore the global patterns documented in regions with high antibiotic consumption, suggesting similar challenges in clinical management.

The independent t-test comparisons (Table 3) reveal statistically significant differences between the test and control groups across most of the examined parameters, indicating differential immune and inflammatory responses. Firstly, the average age in the test group (43.79 ± 15.21) was significantly higher than in the control group (30.24 ± 12.84; p = 0.000), potentially influencing variations in immune responses as aging correlates with immune function alterations. Notably, MCV values were lower in the test group (80.17 ± 7.52) compared to the control group (83.51 ± 8.31, p = 0.035), suggesting possible microcytic anemia tendencies, often linked to chronic inflammation or nutritional deficiencies. Similarly, MCHC levels were significantly elevated in the test group (414.06 ± 44.68) compared to controls (390.00 ± 29.09, p = 0.002), potentially reflecting compensatory mechanisms related to hypochromic microcytic anemia or underlying pathophysiological states [59]. However, MCH levels did not show significant differences (p = 0.517), indicating that the test group's hemoglobin mass per red blood cell was comparable to the controls, which may signify a lack of pronounced impact on erythropoiesis. Additionally, NLR and PLR values were notably elevated in the test group (2.75 ± 2.04 and 131.28 ± 74.86, respectively) compared to the control group (0.89 ± 0.69 and 88.40 ± 44.06, respectively), with p-values < 0.001. Elevated NLR and PLR are recognized as indicators of systemic inflammation and have prognostic value in various chronic inflammatory and autoimmune diseases [60, 61]. The significantly higher levels in the test group suggest heightened inflammatory status, possibly linked to chronic immune activation or infection.

In terms of cytokine profiles, IFN-γ levels in the test group (134.13 ± 11.68 pg/ml) were significantly elevated compared to controls (29.36 ± 6.16 pg/ml, p = 0.000), aligning with research linking higher IFN-γ with inflammatory conditions and metabolic disturbances [62]. Similarly, IL-4 and TNF-α levels were significantly elevated in the test group (17.42 ± 4.51 and 241.62 ± 38.26 pg/ml, respectively) compared to controls (12.44 ± 2.79 and 42.64 ± 12.7 pg/ml, p < 0.001). Elevated IL-4 may reflect skewed Th2 immune responses, often observed in chronic inflammatory diseases [63], while TNF-α elevation indicates a heightened pro-inflammatory response, implicated in chronic diseases and infections [64]. Finally, IL-10 levels were also significantly higher in the test group (26.94 ± 9.35 pg/ml) than in the control (20.8 ± 5.13 pg/ml, p = 0.000), suggesting an increased anti-inflammatory response possibly as a counter-regulatory mechanism to the heightened pro-inflammatory activity.

The findings present a distinct immunological profile between the test and control groups, with the test group exhibiting markers of heightened inflammation, immune dysregulation, and possibly chronic disease states. The elevated NLR, PLR, IFN-γ, and TNF-α levels collectively support the presence of an inflammatory milieu, aligning with studies showing that chronic diseases often upregulate these markers as part of the body's response to ongoing immune activation [65]. The upregulated IL-4 in the test group suggests that Th2-driven immune responses.

Despite the observed trends, the elevated IL-10 in the test group indicates an attempt by the body to modulate excessive inflammatory responses, a phenomenon often observed in chronic inflammation, where anti-inflammatory cytokines act to prevent tissue damage. However, while IL-10 elevation offers some anti-inflammatory buffering, its effectiveness may be limited by the overpowering pro-inflammatory cytokines such as TNF-α, creating a potentially unsustainable immunological balance.

These findings underscore the importance of monitoring hematological and cytokine profiles in identifying inflammatory and immune dysregulation

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early, especially in populations at risk for chronic diseases. Further research with larger sample sizes and longitudinal follow-ups is essential to elucidate the causal pathways linking these parameters to disease progression.

The analysis as depicted in table 4 revealed statistically significant correlations among various parameters, suggesting intricate relationships within hematologic and immune responses. MCH correlated positively with both MCV ($r = 0.65$, $p < .01$) and MCHC ($r = 0.76$, $p < .01$), indicating that subjects with higher mean corpuscular hemoglobin also exhibited increased mean corpuscular volume and mean corpuscular hemoglobin concentration. These findings align with research linking red blood cell indices to efficient erythropoiesis, as MCV, MCH, and MCHC have demonstrated interdependence in identifying anemias and other hematologic anomalies [60].

Furthermore, PLR correlated positively with NLR ($r = 0.39$, $p < .01$), reflecting a common inflammatory pattern where elevated platelet and neutrophil counts coexist, often linked to immune responses against infections or inflammatory conditions. This positive relationship corroborates evidence that elevated PLR and NLR markers serve as indicators of chronic inflammation and may aid in assessing systemic inflammatory burden in clinical settings. Additionally, TNF-α demonstrated a weak positive correlation with IFN-γ ($r = 0.29$, $p < .05$), suggesting a relationship between pro-inflammatory cytokines and chronic inflammatory responses associated with surgical wound infections. Contrary to expectations, age did not significantly correlate with most immune markers, except for a minor positive association with TNF-α ($r = 0.21$), although not reaching significance. This may suggest that the study's age range did not encompass wide enough age differences to capture typical age-related immune changes.

The positive correlation between PLR and NLR is noteworthy given that both parameters are associated with systemic inflammation and immune activation. Elevated levels of NLR and PLR are common indicators in chronic inflammatory conditions, including post-surgical wound infections, emphasizing the clinical relevance of these ratios in evaluating patient inflammatory status. These findings support the integration of NLR and PLR as cost-effective biomarkers in clinical risk stratification. In addition, the weak positive correlation between TNF and IFN-γ underscores the role of pro-inflammatory cytokines in metabolic inflammation. TNF is recognized for its role in exacerbating insulin resistance, and its association with IFN-γ aligns with findings indicating an interdependent relationship between metabolic and inflammatory processes

Summary of key findings:

i. High prevalence of SHV, TEM, and gyrA genes among clinical isolates of *Pseudomonas aeruginosa* and *Escherichia coli*, indicative of multidrug resistance (MDR).
ii. Detection of SHV genes in all isolates, indicating a significant prevalence linked to β-lactam resistance.
iii. TEM genes were present in most samples, with a noted spread due to plasmid-mediated gene transfer.
iv. Detection of gyrA in 7 out of 9 samples, suggesting common fluoroquinolone resistance among *E. coli* isolates.
v. Significant resistance across both Gram-negative and Gram-positive pathogens, particularly in *Pseudomonas aeruginosa*, *E. coli*, *Proteus mirabilis*, and *Staphylococcus aureus*.
vi. Elevated inflammatory markers in test group samples, particularly neutrophil-to-lymphocyte (NLR) and platelet-to-lymphocyte ratios (PLR).
vii. Elevated cytokine levels in test groups, especially IFN-γ, IL-4, TNF-α, and IL-10, signifying a heightened pro- and anti-inflammatory response to the MDR genes (SHV, TEM, and gyrA genes).
viii. Significant correlation between MCH, MCV, and MCHC, indicating interdependence among red cell indices in inflammatory responses.
ix. Positive correlation between PLR and NLR, reflecting a systemic inflammatory pattern.

Strengths and Limitation of the Study

The design of this study, which used a random sample technique to reduce the problem of selection bias, is its strongest point. Additionally, the study's subjects were matched for both sex and age, which lessens the impact of these factors on the study's findings. Furthermore, we used novel technological (PCR) methods to look into and identify the genes that cause multidrug resistance in people with PSWIs.

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The present study investigated the pattern of multidrug resistance genes and immunological biomarkers in post-surgical wound infections in Nnewi, Nigeria and the results may not be definitely generalised to the adolescents due to differences in metabolic status. Also, our study has some other notable limitations: the small number of participants, short duration of study, and the use of one-point sample collection. It seems that increasing the sample size and ensuring participants follow-up in a longitudinal study may give better results. The non-investigation of other parameters such as oxidative stress markers and adiponectin which could have further added more insight to the current study can be pointed out.

Conclusion

The findings of this study highlight a widespread presence of SHV, TEM, and gyrA multi drug resistant genes in clinical isolates of *P. aeruginosa* and *E. coli*, affirming the high resistance rates observed in antibiotic susceptibility testing. Also, the detection of other diverse bacterial species such as *Serratia marcescens*, *Ectopseudomonas mendocina*, *Providencia rettgeri*, *Providencia stuartii* and *Klebsiella grimontii* using gene sequencing underscores a complex etiology in wound infections. Elevated cytokine levels in test groups, especially IFN-γ, IL-4, TNF-α, and IL-10, signifying a heightened pro- and anti-inflammatory response. The association between resistance genes and inflammatory responses provides insight into the added immune burden posed by AMR. These findings underscore the need for stringent antibiotic stewardship and alternative therapeutic strategies to manage infections effectively.

Recommendation/suggestions for further studies

In view of the current findings, a follow up study (longitudinal study) or a national study is recommended for better insight and understanding of the MDR interplay on the immune response.

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