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ORIGINAL RESEARCH ARTICLE

Prevalence and Phenotypic Characterization of Bacteria Isolated from Patients with Breast Cancer at Rasheed Shekoni Federal University Teaching Hospital, Dutse, Jigawa State, Nigeria

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ABSTRACT
Breast cancer is the leading cause of cancer-related mortality in Nigeria, with patients frequently experiencing immunosuppression due to the disease and its treatments. This condition often leads to bacterial infections and dysbiosis, particularly in ulcerated or necrotic lesions, thereby complicating healing and clinical outcomes. This study investigated the prevalence and antibiotic susceptibility of aerobic bacteria in breast cancer patients at Rasheed Shekoni Federal University Teaching Hospital, Dutse, Jigawa State, Nigeria. A total of 272 breast swab samples were collected from patients with ulcerated or discharging lesions. Microbiological analysis revealed that 43.4% (118) of the samples had positive bacterial growth, while 56.6% showed no growth. Among the isolates, Gram-negative bacteria were more prevalent (59.3%) than Gram-positive species (40.6%). *Staphylococcus aureus* was the most frequently identified species (30.5%), followed by *Escherichia coli* (26.3%), *Proteus* spp. (19.5%), and *Klebsiella* spp. (13.5%). Antibiotic susceptibility testing using the Kirby-Bauer disc diffusion method indicated significant multidrug resistance (MDR). Gram-positive isolates were resistant to rifampicin andStreptomycin, while Gram-negative isolates showed extensive resistance to older agents and several cephalosporins. However, both groups remained sensitive to Ciprofloxacin and gentamicin. The findings highlight a predominant colonization of opportunistic pathogens in breast cancer lesions, which may impede wound healing. The study underscores the need for regular microbiological assessments, evidence-based antibiotic administration, and improved wound care protocols to manage infections and combat the growing challenge of antibiotic resistance in oncology settings.

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INTRODUCTION

Breast cancer is a disease that is characterized by the abnormal growth of cells in the breast (CDC, 2020). Breast cancer is currently the most common type of cancer worldwide, with 2.6 million cases recorded (WHO, 2021). The prevalence of breast cancer, the most common cancer in women globally, has been steadily increasing in Nigeria in 2023, accounting for almost 23% of all new cancer cases in the nation. It is the most frequently diagnosed cancer in women and the leading cause of cancer-related deaths among women worldwide. Globally, it is estimated that there were 1.68 million new diagnoses (23% of all new cancer diagnoses in women) and 0.52 million deaths (15% of all cancer deaths in women) from invasive breast cancer, corresponding to age-standardized incidence and mortality rates of 43.3 and 12.9 per 100 000, respectively. Breast cancer has been reported as the most common cause of cancer-related deaths in Nigeria, accounting for 18.1% of all cancer deaths in the country (Sung *et al.*, 2021).

According to the GLOBOCAN 2020 report (Sung *et al.*, 2021), the International Agency for Research on Cancer (IARC) reported 28,380 new cases of breast cancer in 2020, accounting for 22.7% of all new cancer cases (Agodirin *et al.*, 2023). The immunosuppressive effects of chemotherapy or radiation therapy can weaken the body's defense mechanisms, making patients more susceptible to infections by opportunistic pathogens (Delgado and Guddati, 2021; Samonis *et al.*, 2014). This indicates that the threat of breast cancer is currently growing at an alarming rate. Because cancer and its treatment weaken the immune system, bacterial infections are among the most common and potentially fatal complications of breast cancer. These infections can arise when pathogenic organisms are introduced into the site of infection or when the normal microbiota of the cancerous region is altered (Sajmina *et al.*, 2021). When this occurs, the malignant cells can quickly spread to nearby tissues, worsening the patient's condition. Pathogenic organisms can readily

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release toxins or other dangerous proteins that promote their proliferation and have detrimental effects on cancer patients.

It is well known that tumor-related and iatrogenic immunosuppression, characterized by the traditional clinical triad of severe neutropenia, fever, and headache, makes cancer patients more vulnerable to bacterial infections and their systemic spread (Kubeček *et al*., 2021; Bassam *et al*., 2023).

Cancer patients frequently experience infections, which can cause treatment regimen disruptions, prolonged hospital stays, higher medical expenses, and decreased survival (Sevitha *et al*., 2021). In patients with solid malignancies, including breast and cervical cancer, acute bacterial infections have a detrimental effect on survival and raise mortality (Kafayat *et al*., 2023; Oliveira *et al*., 2016). It is essential to monitor the prevalence of bacterial infections within this population, identifying the specific groups of organisms involved, associated morbidity rates, risk factors, and the attack rates of these infections. (Chiamaka *et al*., 2025). Bacterial infections remain a global therapeutic challenge despite the widespread availability of antibiotics (Okeke *et al*., 2022). A thorough understanding of the constantly evolving spectrum of infections is essential for effective infection prevention, diagnosis, and treatment (Sevitha *et al*., 2021).

Infection in fungating breast cancer wounds may arise from both endogenous and exogenous sources. Endogenous sources include the patient's own flora, with *S. aureus* being a common pathogen due to nasal carriage, which can spread through hand contact and subsequently infect other body sites (Eiji *et al*., 2022). Exogenous sources of infection can arise from external contamination, such as exposure to contaminated water or soil during an injury, or from hospital-associated pathogens during medical procedures, wound dressing, or handling of dressing materials (Fromantin *et al*., 2013). Additionally, wound infections in healthcare settings are often polymicrobial, with *S. aureus* frequently coexisting with other microorganisms. This underscores the complexity of managing wound infections, as polymicrobial infections may require multiple antibiotics to effectively target all pathogens (Amitabha *et al.,* 2023). Antimicrobial resistance is a serious risk to patient care because it causes high rates of morbidity and death, lengthens hospital stays, and places a heavy financial burden on both the patient and the healthcare system (Nazneen *et al*., 2016). Breast cancer patients have a high prevalence of antimicrobial resistance due to frequent and irrational antibiotic use and extended hospital stays. Therefore, it is imperative to optimize the use of antibiotics in breast cancer patients in order to prevent further increases in antibiotic resistance (Bray *et al*., 2018). Due to the increased and improper usage of antibiotics, bacterial isolates' patterns of antimicrobial sensitivity have evolved in recent years. A growing degree of resistance to the majority of the antibiotics used for empirical therapy makes treating bacterial infections in women with breast cancer clinically difficult. Multidrug-resistant isolates of *K. pneumoniae*, *P. aeruginosa*, *A. baumannii*, *E. coli*, *P. mirabilis*, and *S. aureus* are becoming more common in clinical settings (Montazeri *et al*., 2020). There has never been a reported study done in this field in Nigeria (Jigawa). Therefore, it was crucial to identify bacterial pathogens and determine antibiotic susceptibility patterns to provide clinicians with the appropriate information for choosing treatment regimens and to implement proper infection control guidelines for patients with breast cancer at Rasheed Shekoni Federal University Teaching Hospital, Dutse, Jigawa State, Nigeria.

MATERIALS AND METHOD

Materials

Sterile Swab sticks, Petri plates, ice box, disinfectant, cotton wool, hand gloves (latex), normal saline, inoculating loop, distilled water, glass slide, cover slip, Gram's stain, biochemical test reagents, and antibiotic sensitivity discs were purchased from Ado Jones Ibrahim Taiwo Road, Kano. Media (Blood agar, MacConkey agar, Mannitol salt agar, Eosin methylene blue agar, and nutrient agar and Muller Hinton agar) (Himedia Laboratory, Pvt. Ltd., India), other reagents (Sigma-Aldrich Laboratories Pvt. Ltd., USA). An analytical balance, Incubator, Laminar flow cabinet, and Autoclave were assessed at the Microbiology Laboratory, Federal University Dutse, Jigawa State, Nigeria.

Ethical consideration

Ethical approval was obtained from the Research and Ethical Committee of the Rasheed Shekoni Federal University Teaching Hospital, Dutse, Nigeria, before the commencement of the research, with Ethical approval number RSFUDTH/GEN/226/V.II. Before sampling, participants completed and signed informed consent forms.

Research design and period

This was a hospital–based cross-sectional study of inpatients and outpatients. The patients or their guardians completed the demographic questionnaires at Rasheed Shekoni Federal University Teaching Hospital, Dutse, from February, 2024 to October, 2025. The study involved both clinical specimen collection from breast cancer patients and laboratory-based microbiological analysis for the isolation and characterization of bacterial species.

Study area

The study was carried out at the Microbiology Laboratory of the Department of Microbiology and Biotechnology, Federal University Dutse, Jigawa State, Nigeria, after obtaining ethical clearance from the chairman Medical Advisory Committee. Patient data was gathered using structured questionnaires and an informed consent form.

Sample size and Sampling technique

Two hundred and seventy-two (272) hospitalized, consenting patients with breast cancer were enrolled using a simple random sampling technique. They were then promptly transported in an ice packed thermos flasks to the Microbiology Laboratory of the Department of Microbiology and Biotechnology, Federal University Dutse, Jigawa State, Nigeria, for Microbiological analysis.

Sociodemographic data and Swab specimen collection

To investigate the study's goals, a pretested questionnaire based on hypothesized risk factors was developed and modified to address the objectives. Then, sociodemographic traits and other relevant data were collected. Breast swab samples were aseptically collected with a sterile swab stick from consenting breast cancer patients who presented with ulcerated or discharging lesions during their clinical visits (Chiamaka *et al*., 2025). Each sample represented a distinct patient to ensure diversity and avoid duplication. The hospital laboratory technician collected these samples in accordance with standard operating procedures (Mba and Okeke, 2022).

Culture and Isolation of bacteria from breast swabs

The collected swabs were first inoculated aerobically onto blood agar and then incubated at 37 °C for 24 hours. The cultures from the Blood agar were then cultured on Mannitol Salt agar, MacConkey agar, Eosin methylene blue, and Nutrient agar from one end of the plates to the other, and then used to streak it across the line to spread it sideways using a flamed wire loop, respectively, and then incubated at 37^0 C for 24 hours (Ochei and Kolhatkar, 2007). The colonies were subcultured to produce single colonies after the plates were examined for potential growth and morphological characteristics during a 24-hour period (Chiamaka *et al*., 2025). The sub-cultured isolates were characterized using established microbiological methods, including colonial morphology, Gram stain characteristics (Nasiru *et al*., 2024), and biochemical tests, including indole tests, catalase production test, citrate utilization test, and coagulase test (Davis and Pezzlo, 2023). Incubation was extended by 24 hours for cultures that showed no or negligible growth before a negative culture result was reported. The confirmed pathogen cells were preserved in nutrient broth and refrigerated until use (Muhammad *et al*., 2020; Sevitha *et al*., 2021).

Standardization of Bacterial Inoculum

The confirmed isolates were subcultured onto sterile nutrient agar plates and incubated at 37 °C for 24 hours. The subcultured isolates were inoculated into sterile test tubes containing 5 mL of normal saline and compared with a 0.5 McFarland turbidity standard at a matching scale of 1.5×10^8 CFU/mL (Cheesbrough, 2006; Haris *et al*., 2019).

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was performed using the Kirby-Bauer disc diffusion method as described by the Clinical and Laboratory Standards Institute (CLSI, 2020) to determine their susceptibility to the most commonly prescribed antibiotics, using commercially prepared antibiotic discs for both Gram-negative and Gram-positive multidiscs. This method was selected because it is widely used, cost-effective, reliable, and suitable for routine clinical microbiology laboratories in resource–limited settings. Sterile cotton wool swabs were dipped into the 0.5 McFarland standards (1.5×10^8 CFU/mL) of the test bacteria; the excess fluid was removed by pressing and rotating the swabs against the walls of the tubes. The contents were then streaked onto Mueller-Hinton agar (MHA) plates (Nasiru *et al*., 2024). The inoculated plates were allowed to stand for 5 minutes. Sterile forceps were used to ensure adequate spacing between disks to prevent overlapping zones of inhibition. Gram-positive multidisc containing: Ciprofloxacin (10 μg), rifampicin (20 μg), gentamycin (10 μg), amoxicillin (20 μg),Streptomycin (30 μg), Ceftazidime (30 μg), erythromycin (30 μg), azithromycin (10μg), Cefuroxime (30μg),, and Levofloxacin (20 μg), were placed onto inoculated plates. Gram-negative antibiotic multidisc containing Ofloxacin (10 μg), pefloxacin (10 μg), Ciprofloxacin (10 μg), Augmentin (30 μg), gentamycin (10 μg), streptomycin (30 μg), ceporex (10 μg), Cefuroxime (30 μg), ceftriaxone (30 μg), and Ceftazidime (30 μg), were also placed onto the surface of the inoculated agar plates (Chiamaka *et al*., 2025). The plates seeded with test bacteria but without an antibiotic disc were used as the a control. The plates were allowed to stand for 30 min (pre-diffusion) and then incubated at 37°C for 18-24 hours. After incubation, the diameters of the zones of growth inhibition around each disc were measured in millimeters using a standard transparent ruler, and the results were interpreted as susceptible, intermediate, or resistant according to CLSI (2025) guidelines. The experiments were conducted in triplicate.

Statistical analysis

Chi-square test was used to determine whether there was a statistically significant difference in the distribution of the isolated bacterial species. Results with $P< 0.05$ were considered significant. Descriptive statistics were used to examine data from laboratory testing. Frequency and percentage distributions of bacterial isolates were displayed in tabular form. The relative abundance of different bacterial species was determined to assess their prevalence across the samples.

RESULTS

Demographic Characteristics of Study Participants from whom the samples were collected

Patient demographics were collected from consenting patients receiving treatment and dressings at Rasheed Shekoni Federal University Teaching Hospital, Dutse. The prevalence of potential behavioral and predisposing factors was displayed for a total of 272 participants (N = 272) (Table 1). According to the study's sociodemographic results, the majority of participants (70%) were between the ages of 35 and 55. Farmers and traders accounted for the largest share of participants (65%), followed by housewives (21%) and civil servants (14%). However, the largest group (38%) had no formal education, and only 12% had completed tertiary education. In terms of predisposing factors, a high percentage (67%) reported having a family history of the condition, and a large majority (89%) reported starting their menstruation at an older age, and 68% reported starting their menopause at an older age. Additionally, 77% of the study participants reported having dense breast tissue, while 61% and 60% of the subjects reported self-medication and inadequate drug dosing practices, respectively.

Table 1: Sociodemographic Characteristics of Study Participants

Parameter	Category	Freq.	%	Chi-square	P- value	Significance
Age Range	0–34	54	20	170.566	9.163e-38	***
	35–55	191	70	170.566	9.163e-38	***
	56–68	27	10	170.566	9.163e-38	***
Occupational status	Civil servant	38	14	125.301	6.182e-28	***
	Farmers/Traders	177	65	125.301	6.182e-28	***
	House wives	57	21	125.301	6.182e-28	***
Settlement	Urban	87	32	35.309	2.814e-09	***
	Rural	185	68	35.309	2.814e-09	***
Education level	Non formal	104	38	42.353	3.377e-09	***
	Primary	80	29	42.353	3.377e-09	***
	Secondary	56	21	42.353	3.377e-09	***
	Tertiary	32	12	42.353	3.377e-09	***
Family history	Yes	182	67	31.118	2.429e-08	***
	No	90	33	31.118	2.429e-08	***
Beginning period at a young age	Yes	243	89	168.368	1.682e-38	***
	No	29	11	168.368	1.682e-38	***
Beginning menopause at an old age	Yes	181	68	29.779	4.841e-08	***
	No	91	32	29.779	4.841e-08	***
Dense breast tissue	Yes	209	77	78.368	8.554e-19	***
	No	63	23	78.368	8.554e-19	***
Taking hormones	Yes	188	69	39.765	2.865e-10	***
	No	84	31	39.765	2.865e-10	***
10 Self-medication practice	Yes	165	61	12.368	4.368e-04	***
	No	107	39	12.368	4.368e-04	***
Drug dosage practice	Complete	108	40	11.529	6.850e-04	***
	Incomplete	164	60	11.529	6.850e-04	***

Keys: X^2 = Chi Square, *** = statistically Significant at $p < 0.001$, %= percentage

Table 2: Cultural and Microscopic Characteristics of Some Bacteria from Breast Cancer

Isolate	BA	MCA	EMB	MSA	GR	Shape	Presumptive Organism
BS 003	Golden yellow	Pale pink	_	Large yellow colonies	+	Cocci	*Staphylococcus aureus*
BS026	Golden yellow	Pale pink	_	Pink-red colonies	+	Cocci	Coagulase-negative *Staphylococcus*
BS 123	Greyish white	Pink-red colonies	Green metallic sheen	_	_	Rods	*E. coli*
BS 128	Creamy white	_	Colorless colonies	_	+	Rods	*Bacillus* spp
BS 193	Swarming growth	Colorless colonies	Pale colonies	_	_	Rods	*Proteus* spp
BS 271	Grey, shiny, mucoid	Pink – red colonies	Pink, mucoid colonies	_	_	Rods	*Klebsiella* spp

Keys: "+" = Gram-positive; "–" = Gram-negative; spp. = species, BA = Blood agar, MCA= MacConkey agar, EMB= Eosin methylene blue agar, MSA= Mannitol salt agar, GR= Gram reactions, - = no visible growth.

The morphological appearance of the bacterial isolates from breast cancer wound swab samples.

A two hundred and seventy two 272 swab samples were analyzed, and only 118 (43.4%) of the 272 breast swab samples examined in this study were found to be culture positive, while 154 (56.6%) were determined to be culture negative. Of the 118 positive samples, 70 (59.3%) had Gram negative bacteria, and 48 (40.6%) had Gram positive pathogens. The identification of these pathogens was performed on the basis of colony morphology with different media include Blood agar (BA), Mannitol salt agar (MSA), MacConkey agar (MCA), Eosin Methylene blue agar (EMB), Nutrient agar (NA), and Gram's staining reaction (GR). The isolates showed different colony morphologies. These features aided in presumptive identification, as shown in Table 2 and Figure 1.

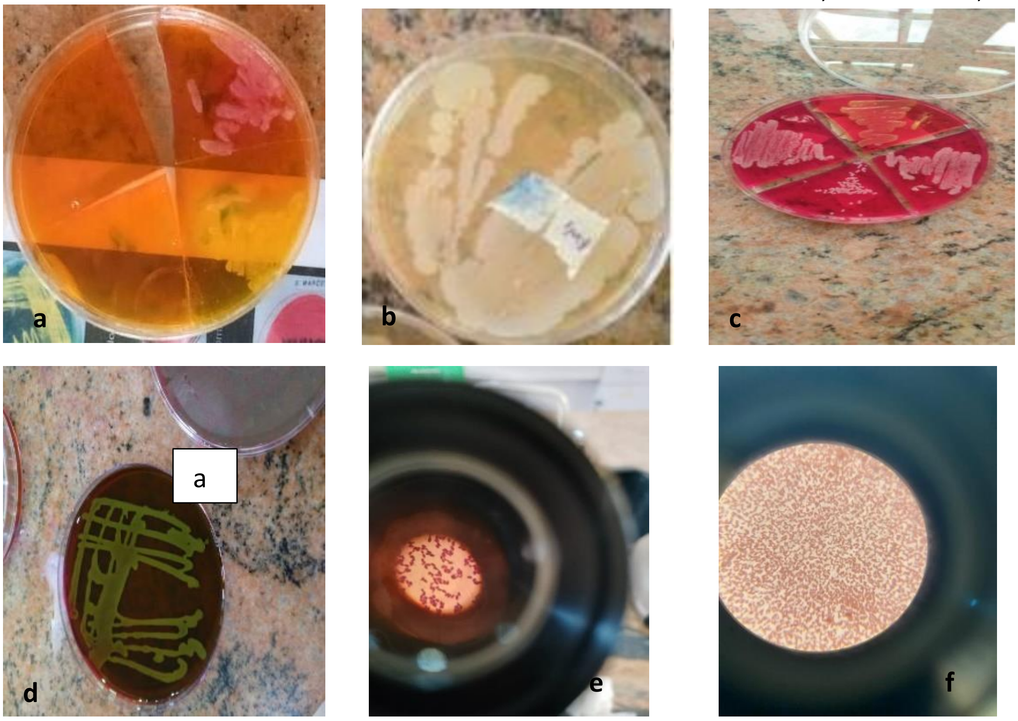

Figure 1: Cultural characteristics of *Staphlocoscuss aureus* on MSA plate (A), *Bacillus* spp grown on NA plate (B), *Klebsiella* spp growth on MCA (C), *E. coli* sub-cultured on EMB plate (D), Microscopic appearance of Gram-positive and negative isolates from breast cancer (E and F), respectively.

Table 3: Biochemical Characteristics of Some Bacterial Isolates from Breast Cancer

Isolate	Cat	Coa	Ox	Ind	Cit	Ur	MR	Identified Organism
BS003	+	+	-	-	+	-	-	*S. aureus*
BS026	+	-	-	-	+	-	-	CoNS
BS0123	+	-	-	+	-	-	+	*E. coli*
BS0128	+	-	-	-	+	+		*Bacillus* spp
BS193	+	-	-	+	+	+	+	*Proteus* spp.
BS271	+	-	-	-	+	+	+	*Klebsiella* spp

Keys: Cat = Catalase; Coa = Coagulase; Ox = Oxidase; Ind = Indole; Cit = Citrate; Ur = Urease; "+" = Positive reaction; "–" = Negative reaction.

Table 4: Frequency Distribution of Some Bacterial Isolates from Breast Cancer

Isolates	Organisms	Frequency (n)	Percentage (%)
BS003	*S. aureus*	36	30.5
BS026	CoNS	7	5.9
BS 123	*E. coli*	31	26.3
BS 128	*Bacillus* spp	5	4.2
BS 193	*Proteus* spp.	23	19.5
BS 271	*Klebsiella* spp	16	13.5
	Total	118	100%

Keys: n = Number of isolates; % = Percentage occurrence; spp. = species.

Biochemical features of the bacterial isolates from breast cancer wound swab samples.

A total of 118 isolates representing six distinct bacterial species were recovered based on their biochemical characteristics (Table 3). The isolates were further identified using a combination of biochemical tests, including Catalase, Coagulase, Oxidase, Indole, Citrate, and Urease tests to confirm the identity of the test bacteria. The most prevalent Gram positive and Gram negative bacteria were *Staphylococcus aureus* and *E. coli*, respectively.

The prevalence of organisms isolated from breast cancer wound swab samples.

The frequency and percentage prevalence of the bacterial species recovered in this research are shown in Table 4. *Staphylococcus aureus* and *Escherichia coli* accounted for 30.5% and 26.3% of the isolates, respectively; *Proteus species* accounted for 19.5%; *Klebsiella species*, 13.5%; and coagulase-negative *Staphylococcus* and *Bacillus species*, 5.9% and 4.2%, respectively. The predominance of *E. coli* and *S. aureus* indicates that these organisms are important in the bacterial colonization of breast cancer lesions in the research area. Their great frequency further confirms that breast wound infections are polymicrobial in nature.

Table 5: The Zone of Inhibition of Antibiotics on Multidrug-Resistant Gr-Positive Bacteria from Breast Cancer

Antibiotic (µg)	BS 69	BS 128	Mean ± SD
RD (20)	11	25	18.00 ± 9.90
CTZ (30)	23	14	18.50 ± 6.36
S (30)	10	9	9.50 ± 0.71
AZM (10)	19	11	15.00 ± 5.66
AMX (20)	10	12	11.00 ± 1.41
CPX (10)	26	18	22.00 ± 5.66
E (30)	10	10	10.00 ± 0.00
LEV (20)	11	9	10.00 ± 1.41
CN (10)	20	17	18.50 ± 2.12
CEF (30)	18	12	15.00 ± 4.24

Key: RD-Rifampicin, CTZ-Cetazidime, S-Streptomycin, AZM- Azithromycin, Amx- Amoxil, CPX- Ciprofloxacin, E-Erythromycin, LEV-Levofloxacin, CN- Gentamycin, CEF-Cefuroxime. BS 69 *Bacillus spp* (A), BS 128 *Bacillus spp* (B).

Table 6: The Zone of Inhibition of Antibiotic on Gram Negative Bacteria from Breast Cancer

Antibiotic (µg)	BS 53	BS100	BS123	BS193	BS263	BS271	Mean ± SD
OFX (10)	24	11	10	11	30	10	16.00 ± 8.74
AU (30)	12	21	22	12	15	20	17.00 ± 4.56
PEF (10)	20	12	10	25	9	15	15.17 ± 6.24
CTZ (30)	8	21	19	22	10	17	16.17 ± 5.85
CN (10)	19	18	19	20	20	22	19.67 ± 1.37
CPX (10)	20	28	26	26	26	27	25.50 ± 2.81
CEP (10)	26	10	24	19	12	26	19.50 ± 7.09
TRX (30)	25	11	10	20	20	25	18.50 ± 6.60
S (30)	10	10	10	9	8	10	9.50 ± 0.84
CEF (30)	18	20	12	12	20	11	15.50 ± 4.28

Key: OFX-Ofloxacin, CTZ-Cetazidime, S-Streptomycin, AU- Augmentin, PEF- Pefloxacin, CPX- Ciprofloxacin, TRX-Ceftriaxone, CEP-Ceporex, CN- Gentamycin, CEF-Cefuroxime. BS 53: *E. coli*, BS 100: *Proteus spp*, BS 123: *E. coli*, BS 193: *Proteus spp*, BS 263: *E.coli* and BS 271: *Klebsiella spp*.

Table 7: Antibiotics Susceptibility Profile Interpretation of the Gram Positive Bacteria from Breast Cancer

Antibiotic (µg)	*Bacillus* spp (A)	Bacillus spp (B)
RD (30)	R	S
CTZ (30)	S	R
S (30)	R	R
AZM (10)	S	R
AMX (30)	R	R
CPX (10)	S	R
E (30)	R	S
LEV (20)	R	S
CN (10)	S	S
CEF (30)	S	R

Key: RD-Rifampicin, CTZ-Cetazidime, S-Streptomycin, AZM- Azithromycin, Amx- Amoxil, CPX- Ciprofloxacin, E-Erythromycin, LEV-Levofloxacin, CN- Gentamycin, CEF-Cefuroxime. S = Susceptible, R = Resistant.

Table 8: Antibiotics Susceptibility Profile Interpretation of the Gram-Negative Bacteria from Breast Cancer

Antibiotic (µg)	BS 53	BS 100	BS 123	BS 193	BS 263	BS 271
OFX (10)	S	R	R	R	S	R
AU (30)	R	S	S	R	R	R
PEF(10)	S	R	R	S	R	R
CTZ (30)	R	S	R	S	R	R
CN (10)	S	S	S	S	S	S
CPX (10)	R	S	S	S	S	S
CEP (10)	S	R	S	S	R	S
TRX (30)	S	R	R	S	S	S
S (30)	R	R	R	R	R	R
CEF(30)	S	S	R	R	S	R

Key: OFX-Ofloxacin, CTZ-Cetazidime, S-Streptomycin, AU- Augmentin, PEF- Pefloxacin, CPX- Ciprofloxacin, TRX-Ceftriaxone, CEP-Ceporex, CN- Gentamycin, CEF-Cefuroxime. BS 53: *E. coli*, BS 100: *Proteus* spp, BS 123: *E. coli*, BS 193: *Proteus* spp, BS 263: *E.coli* and BS 271: *Klebsiella* spp. S = Susceptible, R = Resistant.

Antibiotic Susceptibility of Multidrug-Resistant Gram-Positive Isolates

All bacterial isolates (118) obtained in this study were subjected to antibiotic susceptibility testing using the disc diffusion method. Out of the total bacteria tested, eight (8) multidrug-resistant pathogens were recorded, consisting of two Gram positive and six Gram negative bacteria. The result of the susceptibility pattern of the Gram positive bacteria isolated from breast cancer (Table 5) showed the diameter (in mm) of the zone of inhibition (ZOI) results for ten (10) antibiotics against two multidrug-resistant bacterial species according to CLSI. (2024) guidelines. The results revealed that BS (69) *Bacillus* spp (A) was resistant to rifampicin (20 µg), Streptomycin (30 µg), Azithromycin (10 µg), Amoxil (20 µg), Erythromycin (30 µg), and Levofloxacin (20 µg) while the isolates demonstrate sensitive to Ciprofloxacin (10 µg), gentamycin (10 µg), Ceftazidime (30 µg), and Cefuroxime (30 µg). While BS 128 *Bacillus* spp (B) showed multidrug resistance to four antimicrobial agents, such as Amoxil (30µg), Erythromycin (30µg), Levofloxacin (20µg), and Streptomycin (30µg), while sensitive to Rifampicin (30µg), Ceftazidime (30µg), Azithromycin (10µg), Ciprofloxacin (10µg), Gentamycin (10µg), and Cefuroxime (30µg). Ciprofloxacin appears to be the most effective broad-spectrum agent, with an inhibition zone of 26 mm, followed by Cefazidime at 23 mm against the tested gram-positive organisms. Streptomycin and Levofloxacin showed poor inhibition, suggesting potential resistance against the isolates. Based on the findings, the test bacteria were resistant to most of the antibiotics tested.

Antibiotic Susceptibility of Multidrug-Resistant Gram-Negative Isolates

However, the results from the present study (Table 6) of gram-negative bacterial isolates revealed that BS 53 *E. coli* was found to be resistant to Augmentin (30µg), Ciprofloxacin (10µg),Streptomycin (30 µg), and Ceftazidime (30 µg), while it showed sensitivity to Ofloxacin (10 µg), Pefloxacin (10 µg), Gentamycin (10 µg), Ceporex (10 µg), Cefriaxome (30 µg), and Cefuroxime (30 µg). BS 100 *P*roteus spp was shown to be resistant to Peflacine (10 µg), Streptomycin (30 µg), Ofloxacin (10 µg), and Cefuroxime (30 µg), while revealed to be sensitive to Ceftazidime (30 µg), Gentamycin (10 µg), Ceporex (10 µg), Ceftriaxone (30 µg), and Cefuroxime (30 µg). BS 123 *E. coli* was most resistant to Ofloxacin (10 µg), Pefloxacin (10 µg), Ceftazidime (30 µg), Streptomycin (30 µg), and Cefuroxime (30 µg) while sensitive to Gentamycin (10 µg), Augmentin (30 µg), Ciprofloxacin (10 µg), Ceporex (10 µg), and Cefriaxone (30 µg). Whereas BS 193 *Proteus spp* was resistant to Ofloxacin (10 µg), Augmentin (30 µg), Streptomycin (30 µg), and Cefuroxime (30 µg) while sensitive to gentamycin (10 µg), Peflacine (10 µg), Ceftazidime (30 µg), Ciprofloxacin (10 µg), Ceporex (10 µg), and Ceftriaxone (30 µg). On the other hand, BS 263 *E. coli* was found to be resistant to Augmentin (30 µg), Peflacine (10 µg), Ceftazidime (30 µg), Ceftriaxone (30 µg) and Streptomycin (30 µg) while revealed to be sensitive to Ofloxacin (10 µg), Gentamycin (10 µg), Ciprofloxacin (10 µg), Ceftazidime(30 µg) and Ceporex (10 µg) Finally, BS 271 *Klebsiella spp* was shown to be resistant Streptomycin (30 µg), Ceftazidime (30µg), Peflacine (10µg), Cefuroxime (30µg) and Ofloxacin (10µg) and was Sensitive to Cefriaxone (30µg), Ciprofloxacin (10µg), Ceporex (10µg), Augmentin (30µg) and Gentamycin (10µg). Based on the results, Ciprofloxacin remained consistently effective across all tested gram-negative species, with the highest zone diameter ranging from 20 mm to 28 mm. Streptomycin, on the other hand, showed very low efficacy, with diameter zones ranging from 8 mm to 10 mm, indicating high resistance.

Antibiotic Resistance Profiles of Gram-Positive Bacteria

The results of the antibiotics susceptibility pattern of Gram positive bacteria from breast cancer at RSFUDTH are shown among the bacteria tested (Table 7).

BS 69 *Bacillus spp* (A) showed high resistance to five antimicrobial agents from different groups, such as Rifampicin (30 µg), Streptomycin (30µg), Amoxil (30 µg), Erythromycin (15 µg), and Levofloxacin (5µg). BS 128 *Bacillus spp* (B) was the most resistant bacterium among all the Gram positive bacteria tested against Ceptazidimes (30 µg), Streptomycin (30 µg), Azithromycin (10µg), Amoxil (30µg), Ciprofloxacin (10µg), and Cefuroxime (30µg).

Antibiotic Resistance Profiles of Gram-Negative Bacteria

However, the results from the present study (Table 8) of gram-negative bacterial isolates revealed that *Providencia spp* was found to be resistant to Augmentin (30µg), Ciprofloxacin (10µg),Streptomycin (30 µg), and Ceftazidime (30 µg), while it showed sensitivity to Ofloxacin (10 µg), Pefloxacin (10 µg), gentamycin (10 µg), Ceporex (10 µg), Cefriaxome (30 µg), and Cefuroxime (30 µg). *P*roteus spp was shown to be resistant to Peflacine (10 µg), Streptomycin (30 µg), Ofloxacin (10 µg), and Cefuroxime (30 µg), while revealed to be sensitive to Ceftazidime (30 µg), Gentamycin (10 µg), Ceporex (10 µg), Ceftriaxone (30 µg), and Cefuroxime (30 µg). *E. coli* was most resistant to Ofloxacin (10 µg), Pefloxacin (10 µg), Ceftazidime (30 µg), Streptomycin (30 µg), and Cefuroxime (30 µg) while sensitive to Gentamycin (10 µg), Augmentin (30 µg), Ciprofloxacin (10 µg), Ceporex (10 µg), and Cefriaxone (30 µg). Whereas *Proteus spp* was resistant to Ofloxacin (10 µg), Augmentin (30 µg), Streptomycin (30 µg), and Cefuroxime (30 µg) while sensitive to gentamycin (10 µg), Peflacine (10 µg), Ceftazidime (30 µg), Ciprofloxacin (10 µg), Ceporex (10 µg), and Ceftriaxone (30 µg). On the other hand, *E. coli* (NGH 263) was found to be resistant to Augmentin (30 µg), Peflacine (10 µg), Ceftazidime (30 µg), Ceftriaxone (30 µg) and Streptomycin (30 µg) while revealed to be sensitive to Ofloxacin (10 µg), Gentamycin (10 µg), Ciprofloxacin (10 µg), Ceftazidime(30 µg) and Ceporex (10 µg) Finally, *Klebsiella spp* was shown to be resistant Streptomycin (30 µg), Ceftazidime (30µg), Peflacine (10µg), Cefuroxime (30µg) and Ofloxacin (10µg) and was Sensitive to Cefriaxone (30µg), Ciprofloxacin (10µg), Ceporex (10µg), Augmentin (30µg) and Gentamycin (10µg). Based on the results, Ciprofloxacin remained consistently effective across all tested gram-negative species, with the highest zone diameter ranging from 20 mm to 28 mm. Streptomycin, on the other hand, showed very low efficacy, with diameter zones ranging from 8 mm to 10 mm, indicating high resistance.

DISCUSSION

Patients with breast cancer are particularly susceptible because of immunosuppression brought on by the disease itself, as well as from invasive procedures like radiation, chemotherapy, and surgery (Amitabha *et al.*, 2023). These circumstances foster an atmosphere that is favorable to opportunistic infections, which can have a major impact on patient outcomes, lengthen hospital stays, and raise morbidity and mortality. A physiologically and socially crucial stage of a woman's life, when cumulative hormonal exposure, reproductive variables, and genetic predisposition interact to raise the risk of cancer, is reflected in the study's finding that the majority of breast cancer patients are between the ages of 35 and 55. This result is consistent with the study on breast cancer and related bacterial pathogens by Chiamaka *et al.* (2025): a study of breast cancer ulcers in patients from the National Hospital Cancer Center, Abuja.

According to the findings of Bray *et al.* (2020) and Sung *et al.* (2021), large-scale epidemiological studies consistently show that breast cancer incidence rises sharply after the third decade of life and peaks in middle age, particularly in low- and middle-income countries like Nigeria, where screening programs are limited.

In line with the findings of Narod and Salmena (2021), Chiamaka *et al.* (2025) previously reported a significant association between breast cancer and family history, further highlighting the role of inherited genetic mutations, particularly in tumor suppressor genes such as BRCA1 and BRCA2. In resource-limited settings, the lack of genetic screening exacerbates this risk because high-risk individuals remain undetected until disease manifestation (Okeke *et al.*, 2022).

In addition to biological factors, the sociodemographic profile showed that people with low levels of education and those who live in rural areas predominate. These elements have significant effects on the course of cancer and the results of infections. Living in a rural area is often associated with reliance on traditional medicine, delayed diagnosis, and restricted access to healthcare services. Understanding disease symptoms, following treatment plans, and using preventive healthcare services are all further hampered by low literacy levels. These findings are consistent with those of Doaa (2020) and Chiamaka *et al.* (2025). Women from rural and low-education backgrounds are more likely to present with advanced-stage breast cancer, which increases vulnerability to secondary infections due to tissue necrosis and immunosuppression, according to studies conducted throughout sub-Saharan Africa (Tadesse *et al.*, 2022; WHO, 2023). According to Salam *et al.* (2023), the research population's high rates of self-medication, insufficient antibiotic doses, and uncontrolled access to antimicrobials are particularly alarming. Antimicrobial-resistant bacteria are more likely to arise and persist in the selective environment created by these methods. Nevertheless, the results are different from those of Doaa (2020).

Antibiotics exert selection pressure in evolution, eradicating susceptible bacterial populations while promoting the growth of resistant strains. Communities become reservoirs of organisms resistant to drugs over time as a result of this process. Poor antimicrobial stewardship in rural African settings accelerates the development of resistance, especially among enteric and opportunistic infections, according to recent surveillance data. These results are in line with earlier studies by Tacconelli *et al.* (2022) and Tadesse *et al.* (2022). A synergistic risk environment is created when immunosuppression, breast cancer, and antibiotic abuse come together. Prophylactic antibiotics, invasive procedures, and frequent hospital stays are common for cancer patients, all of which exacerbate resistance selection. Therefore, sociodemographic factors actively shape the microbial ecology of cancer care, rather than merely influencing cancer incidence. These results emphasize the necessity of integrated public health approaches that concurrently target antimicrobial stewardship, education, and cancer prevention. Patient outcomes in vulnerable populations will continue to be compromised by the combined burden of cancer and antibiotic resistance in the absence of such measures (WHO, 2023; Salam *et al.*, 2023).

The present study revealed that bacteria are commonly associated with breast cancer lesions among patients attending Rasheed Shekoni Teaching Hospital, Dutse. The predominant isolates were *Staphylococcus aureus* and *Escherichia coli*, accounting for 30.5% and 26.3 % of the total isolates, respectively. This is consistent with previous research by Savitha *et al.* (2021) and Onyeaghala *et al.* (2023), which found that *S. aureus* and *E. coli* were similarly prevalent in ulcerated or necrotic breast tissues. The identification of *Staphylococcus aureus*, *Proteus, Klebsiella, Escherichia coli*, and *Bacillus species* in breast cancer patients highlights the polymicrobial and opportunistic nature of infections associated with breast cancer. These organisms are not primary pathogens in healthy individuals but readily exploit compromised host defenses. This was consistent with discovery of Doaa (2020), the most often isolated bacteria in malignant tumors were *Proteus* and *Bacillus* spp. Given that *S. aureus* was found in almost one-third of the samples, likely that this bacterium contributes significantly to secondary infections in women with breast cancer. Its presence may be linked to contamination during wound dressing, weakened skin barriers, or inadequate hygiene. *S. aureus* is a known opportunistic pathogen capable of producing toxins and enzymes such as coagulase, hemolysin, and leukocidin, which contribute to tissue damage and delayed wound healing. Similarly, *E. coli* was isolated in a higher proportion. Although primarily a gut commensal, *E. coli* can act as an opportunistic pathogen when introduced into wounds. Its presence in cancerous lesions has been linked to immune suppression and hospital-acquired infections (Liu *et al.*, 2023). Some strains of *E. coli* carry genotoxic pks islands that encode colibactin, a toxin known to induce DNA damage and potentially influence tumor progression (Cao *et al.*, 2024). The detection of coagulase-negative *Staphylococcus* (CONS) in 5.9% of samples may represent skin commensals or opportunistic colonizers, consistent with the findings of Arega *et al.* (2017). The isolation of *Klebsiella* spp. (13.5%) also suggests contamination from enteric sources or nosocomial transmission. This bacterium is known for its capsule-mediated resistance and biofilm formation, which complicate antibiotic therapy, findings that are also in line with those of Arega *et al.* (2023). Another significant finding is the identification of *Bacillus* species (4.2%). The isolates, previously thought to be soil-associated or environmental organisms, are now more widely recognized as emerging opportunistic infections, particularly in immunocompromised hosts. They have a survival advantage in harsh environments, such as inflammatory or necrotic cancer tissues, because of their capacity to produce spores, withstand desiccation, and tolerate harsh conditions. According to recent genetic research, these organisms may have virulence-associated proteins and stress-response genes that help them colonize and remain in human hosts. The results of this investigation are consistent with those of Kwon *et al.* (2023). These findings emphasize the importance of comprehensive diagnostic approaches, including molecular confirmation, to accurately identify pathogens and guide effective treatment. Inappropriate treatment and subpar clinical outcomes may result from a failure to identify emerging opportunistic pathogens (WHO, 2023; Kwon *et al.*, 2023).

The antibiotic susceptibility patterns observed among Gram-positive isolates, particularly *Bacillus spp.* (BS 69 and 128), revealed a concerning level of multidrug resistance. Resistance to commonly prescribed antibiotics, such as macrolides, beta-lactams, and some fluoroquinolones, suggests the presence of adaptive survival mechanisms, including target-site modification, efflux pump overexpression, and enzymatic drug inactivation. These mechanisms are increasingly reported among Gram-positive bacteria in both community and hospital settings. These findings are in agreement with the report by WHO. (2023) and Bassetti *et al.* (2021). The relatively higher susceptibility observed with Ciprofloxacin and Ceftazidime indicates that these agents may still retain efficacy against certain Gram-positive opportunistic pathogens (WHO, 2023). Ciprofloxacin targets DNA gyrase and topoisomerase IV, enzymes essential for DNA replication, while Ceftazidime interferes with cell wall synthesis (Kubeček *et al.*, 2021). The preserved activity of these antibiotics suggests that resistance-conferring mutations in these pathways may be less prevalent or associated with fitness costs that limit their spread. Similar susceptibility trends have been reported in oncology wards, where selective pressure differs from general hospital environments (Iskandar *et al.*, 2025). From an evolutionary perspective, the resistance patterns observed resemble an "arms race" between bacterial populations and antimicrobial agents. In cancer patients, repeated and prolonged antibiotic exposure creates a chronic selective environment that accelerates bacterial adaptation. Each antibiotic course acts as a genetic bottleneck, favoring resistant clones that subsequently dominate the microbial population (Salam *et al.*, 2023). This phenomenon is particularly pronounced in immunocompromised hosts, where bacterial clearance is impaired, allowing resistant strains to persist and disseminate. This study aligns with the findings of Tacconelli *et al.* (2022). Clinically, the presence of multidrug-resistant Gram-positive organisms complicates infection management in breast cancer patients (Okeke *et al.*, 2022). Empirical therapy becomes increasingly unreliable, leading to treatment delays, prolonged hospitalization, and increased mortality. These findings reinforce the necessity of routine susceptibility testing and individualized therapy in cancer settings.

The Gram-negative isolates showed widespread resistance to several antibiotics, especially older antibiotics such as Streptomycin and cephalosporins (Singh *et al.*, 2024). Due to their intrinsic and acquired resistance mechanisms, Gram-negative bacteria are increasingly linked to infections that are challenging to treat. This resistance profile aligns with global trends. The outer membrane of Gram-negative bacteria acts as a molecular "shield," preventing intracellular drug accumulation and limiting antibiotic penetration (Pitout & Laupland, 2022). Ciprofloxacin's consistent effectiveness against multiple isolates indicates that fluoroquinolones remain a valuable treatment option (Salam *et al.*, 2023). However, the presence of extended-spectrum beta-lactamases (ESBLs) and other resistance determinants is a worry due to the emergence of resistance in *Proteus* species and *E. coli.* Many beta-lactam antibiotics can be hydrolyzed by ESBL-producing organisms, making routine treatments ineffective and requiring the use of last-resort medications (Satlin *et al.*, 2020). Gram-negative bacteria have several defense mechanisms, including plasmid-mediated resistance genes, efflux pumps, and alterations in porins (Kubeček *et al.*, 2021). These characteristics provide exceptional flexibility, especially in settings with high antibiotic exposure. Long hospital stays and frequent antibiotic use increase the likelihood of horizontal gene transfer in cancer patients, hastening the spread of resistance (Tacconelli *et al.*, 2022). The results of this investigation support international concerns about the increasing prevalence of drug-resistant Gram-negative infections in oncology settings (Eiji *et al.*, 2022). Higher rates of morbidity, mortality, and medical expenses are linked to certain infections.

The antibiotic resistance profile of Gram-positive isolates, especially Bacillus (BS 69 and 128), validates their designation as multidrug-resistant (MDR) organisms. This result aligns with the earlier research conducted by Amitabha *et al.* (2023). The cumulative effect of extended antibiotic exposure in hospital and community settings is reflected in resistance to several antibiotic classes, including β-lactams, macrolides, fluoroquinolones, and aminopenicillins (WHO, 2024). The ecological conditions for the selection and maintenance of such resistant organisms are ideal in cancer care settings, which are characterized by immunosuppression, frequent antibiotic use, and extended hospital stays (Bassetti *et al*., 2021).

The preserved susceptibility to gentamicin observed in this study suggests that aminoglycosides may still retain therapeutic relevance; however, their nephrotoxic and ototoxic potential limits widespread use, especially in oncology patients receiving nephrotoxic chemotherapeutic agents (Iskandar *et al*., 2025). Furthermore, aminoglycosides exhibit poor tissue penetration in biofilm-associated infections, further constraining their efficacy. These findings reinforce the necessity of susceptibility-guided therapy and antimicrobial stewardship to slow resistance evolution and preserve remaining therapeutic options (Iskandar *et al*., 2025). There is a global crisis of Gram-negative antimicrobial resistance, as evidenced by patterns of resistance in *Escherichia coli*, *Proteus* species, and *Klebsiella pneumoniae* (WHO, 2023). In line with the widespread prevalence of extended-spectrum β-lactamases (ESBLs) and aminoglycoside-modifying enzymes, these organisms showed substantial resistance to streptomycin, a number of cephalosporins, and β-lactam/β-lactamase inhibitor combinations. Gram-negative bacteria have an inherent structural advantage, according to Gambhir *et al*. (2022). Their outer membrane functions as a molecular "armor," limiting the entry of antibiotics and promoting rapid resistance acquisition (Pitout and Laupland, 2022). Repeated exposure to antibiotics increases selective pressure in cancer patients, hastening the emergence of resistance (Okeke *et al*., 2022). This study's finding of streptomycin's almost universal inefficiency highlights the negative effects of past abuse and uncontrolled access. Due to decades of unchecked use, streptomycin, once a mainstay antibiotic, has become functionally obsolete in many low- and middle-income nations (O'Neill, 2020). Ciprofloxacin, on the other hand, maintained very high efficacy among Gram-negative isolates, indicating either a significantly lower rate of abuse or a more recent introduction into regional therapeutic practices. The results of this investigation supported those of Salam *et al*. (2023). Concerns about the duration of this therapeutic window are raised by global patterns of increasing fluoroquinolone resistance (Tacconelli *et al*., 2022).

These findings are concerning in terms of public health. According to Satlin *et al*. (2020), gram-negative MDR infections are currently the primary cause of infection-related death in cancer patients globally. Surgical wounds, necrotic tumor tissue, and indwelling devices provide as entry points for these microorganisms in patients with breast cancer (Chiamaka *et al*., 2025). When MDR pathogens and immunosuppression combine, common diseases become potentially fatal.

These results highlight the significance of regular microbial culture and sensitivity testing in the treatment of infection among patients with breast cancer. Clinicians can better treat wounds, avoid subsequent infections, and administer tailored medications by identifying the bacterial flora. Overall, the bacterial spectrum obtained in this study reflects a mixed infection pattern, dominated by *Staphylococcus* and *Enterobacteriaceae*, which are frequently associated with cancer-related wounds. The predominance of bacteria supports earlier suggestions that tumor necrosis and tissue breakdown provide favorable conditions for microbial colonization.

CONCLUSION

Bacterial infections are a prevalent issue and one of the causes of breast cancer patients' mortality. These infectionss are caused by a variety of bacteria (Gram–positive and Gram-negative). The study concludes that breast cancer lesions are significantly colonized by bacteria, with *Staphylococcus aureus* and *Escherichia coli* being the most prevalent. The identified isolates are known for their ability to cause serious opportunistic infections, particularly in immunocompromised patients. These findings highlight the importance of routine microbiological assessment of cancer-related wounds. Early identification of bacterial species may support better clinical decisions, guide antimicrobial therapy, and improve patient outcomes. Lastly, molecular characterization of the resistant isolates is needed to provide a more accurate definition of bacterial burden in breast cancer patients.

CONFLICT OF INTEREST

The authors declare that no conflicting interests exist.

AUTHORS' CONTRIBUTION

This study was carried out in collaboration among the authors. Haris, N. G., and Danjuma L. designed and supervised the study. Bashir, S. F, Musa. H. M. and Haris, N. G. conceptualized the study, collected the samples, and conducted the laboratory analysis. Haris, N.G., Danjuma. L., Prepared and edited the manuscript. All the authors edited the manuscript and approved the publication.

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