Antibiogram Analysis and Biofilm Formation of Bacteria from Patients with Breast Cancer at Rasheed Shekoni Federal University Teaching Hospital Dutse, Jigawa State, Nigeria

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ABSTRACT

Breast cancer remains the most common malignancy among women worldwide and is the leading cause of cancer-related deaths in Nigeria. Patients often face heightened risks of bacterial infections due to immunosuppression from the disease itself and treatments such as chemotherapy, surgery, and radiation. Microorganisms growing in a biofilm are associated with chronic and recurrent human infections and are highly resistant to antimicrobial agents. This study investigated the antibiotic susceptibility and biofilm-forming capabilities of bacteria isolated from breast cancer patients at Rasheed Shekoni Federal University Teaching Hospital, Dutse. 118 bacterial isolates from six different species were examined in this cross-sectional investigation. Biochemical assays and colony morphology were used for identification. The Kirby-Bauer disc diffusion method was used to assess antibiotic susceptibility in accordance with CLSI standards. The gold-standard Tissue Culture Plate Method (TCPM) was used to quantify biofilm formation. S. aureus, CoNS, E. coli, Bacillus species, Proteus species, and Klebsiella species were among the pathogens found. Gram-positive isolates showed multidrug resistance (MDR) to rifampicin and streptomycin but remained sensitive to ciprofloxacin and gentamicin. Gram-negative isolates exhibited extensive resistance to older agents like streptomycin and several cephalosporins, whereas ciprofloxacin and gentamicin remained the most reliable treatments. Critically, 100% of tested MDR isolates were "strong" biofilm producers (OD > 0.68), with Klebsiella species recording the highest optical density (2.74). The universal presence of strong biofilms among MDR isolates poses a serious clinical challenge because biofilms shield bacteria from host immunity and increase antibiotic tolerance up to 1,500-fold. These findings highlight the need for routine susceptibility testing and enhanced antimicrobial stewardship in oncology.

Keywords: *Cancer, Malignancy, Antimicrobial, Biofilm, Chemotherapy*



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INTRODUCTION

Cancer is one of the most complex and devastating diseases known to humanity, characterized by the uncontrolled proliferation of abnormal cells that invade surrounding tissues and can metastasize to distant parts of the body (WHO, 2024). It is not a single illness, but rather a group of conditions that are characterized by a breakdown of regular cellular growth regulation. Cancer affects both people and animals and can develop in almost any organ of the body. It has been characterized as a complex illness impacted by lifestyle, environmental, and hereditary factors (Bastiaannet & Pilleron, 2025). According to Liu *et al*. (2023), breast cancer arises when aberrant cells in the breast start to divide uncontrolled, creating a tumor that may infiltrate surrounding tissues or migrate to other regions of the body.

The disease can occur in both men and women, though it is about 100 times more common in women. Risk factors include genetic mutations (such as BRCA1 and BRCA2), hormonal imbalance, obesity, infections, radiation exposure, alcohol intake, and aging (German *et al*., 2023). Breast cancer is currently the most common type of cancer worldwide, with 2.6 million cases recorded (WHO, 2021). In 2023, the incidence of breast cancer, the leading cause of cancer in women worldwide, has continued to rise in Nigeria and now accounts for about 23 % of all new cases of cancer in the country. It is the most commonly diagnosed cancer in women and the most common cause of cancer death in women worldwide. Globally, invasive breast cancer is estimated to have caused 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), corresponding to age-standardized incidence and mortality rates of 43.3 and 12.9 per 100,000, respectively.

According to the GLOBOCAN 2020 (Sung *et al*., 2023), the latest 2020 report from the International Agency for Research on Cancer (IARC) recorded 28,380 new BC cases which represent 22.7% of new cancers (Agodirin *et al*., 2023). The majority of breast cancers are sporadic, resulting from interactions between genetic vulnerability and environmental variables, however a tiny percentage of instances are caused by hereditary factors. The significance of microbes in the onset and spread of cancer has drawn more attention in recent years. However, new research indicates that bacteria may also affect immune regulation, the tumor microenvironment, and the response to treatment in malignancies that were not previously thought to be infection-related (D'Afonseca *et al*., 2024).Due to numerous factors that make immunosuppressed cancer patients more susceptible to infection, including neutropenia during chemotherapy, altered gut flora from frequent antibiotic administration, and skin disruption and tissue epithelial surface damage from cytotoxic chemotherapeutic agents, infection is a persistent and serious issue for patients with breast cancer. Multidrug-resistant bacterial infections have emerged as a significant global health concern (WHO, 2023). Multidrug-resistant organisms have emerged in some infections in the current environment. 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). Multidrug resistance has been increased throughout the world that is considered a public health threat (Makharita *et al*., 2020) sale of antimicrobials leads to the emergence and rapid dissemination of resistant bacterial strains. Rapid and reliable detection of pathogens and their antibiotic susceptibility patterns is crucial in the management of breast cancer patients (Algammal *et al*., 2021). Breast cancer patients exhibit increased susceptibility to infections caused by both gram-positive cocci, such as methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant Enterococci (VRE), as well as gram-positive bacilli, including extended-spectrum beta-lactamase (ESBL) producing Enterobacteriaceae and carbapenems-resistant Pseudomonas aeruginosa (Makharita *et al*., 2020). *Staphylococcus aureus* remains one of the most common pathogens isolated from breast cancer patients, with MRSA accounting for approximately 50-60% of *S*. *aureus* isolates in many healthcare institutions.

Gram-negative bacteria, especially those belonging to the Enterobacteriaceae family, such as Escherichia coli and Klebsiella pneumoniae, have become major causes of bloodstream infections in neutropenic patients. These organisms often carry resistance genes encoding ESBL enzymes, carbapenemases, and other resistance mechanisms that limit therapeutic options (Algammal *et al*., 2021). Pseudomonas aeruginosa, an opportunistic pathogen that can develop additional resistance during therapy. The clinical manifestation of bacterial infections in patients with breast cancer is generally different from that of immune-competent persons. These infections often appear with mild signs and symptoms that may be obscured by side effects of treatment or cancer-related symptoms. Early diagnosis is difficult in neutropenic individuals because they may acquire severe infections

without usual inflammatory responses such fever, leukocytosis, or purulent discharge (Klastersky *et al*., 2016). High clinical suspicion and strong diagnostic techniques are required since the lack of neutrophils impairs the production of pus and may conceal the severity of illness. With fatality rates ranging from 15 to 50% depending on the causative organism, patient characteristics, and promptness of adequate medication, bloodstream infections are the most dangerous infectious complication in cancer patients. Compared to gram-positive infections, gram-negative bacterial sepsis usually manifests as more severe hemodynamic instability and frequently progresses quickly to septic shock and multi-organ failure (Agrebi *et al*., 2020). As a tactic to maximize antibiotic use, reduce the emergence of resistance, and enhance patient outcomes in cancer settings, the idea of antimicrobial stewardship has grown in popularity. Evidence-based prescription guidelines, laboratory-supported decision-making, frequent monitoring of resistance patterns, and training campaigns aimed at healthcare professionals are all components of successful stewardship programs (Dellit *et al*., 2017).

Multidrug resistant organism (MDRO) infections have become a major global public health concern. These bacteria are resistant to several antibiotic classes, which makes treatment options more difficult and raises rates of morbidity and death (CDC, 2023). Antibiotic abuse and misuse, inadequate infection control procedures, and a halt in the discovery of novel antibiotics are some of the causes contributing to the rise in MDROs (Smith, 2020). Biofilm is an aggregation of bacteria that resembles a community. Bacterial attachment to a specific surface, irreversible binding, and the creation of a hydrated matrix of proteins and polysaccharides are all part of the dynamic process of bacterial biofilm development (Noor *et al*., 2013). Inert surfaces like medical equipment and dead tissues like pieces of dead bone are examples of surfaces that encourage the formation of biofilms (Abdallah *et al*., 2011). The bacteria in the biofilm emit antigens that cause the production of antibodies. Even in individuals with strong immune responses, these antibodies are insufficient to eradicate the bacteria entrenched in the biofilm (Sultan and Nabiel, 2019). Through a variety of pathogenic mechanisms, biofilm formation promotes bacterial virulence by facilitating adhesion to solid surfaces, phagocytosis avoidance, and gene exchange among the biofilm's constituents, resulting in more virulent strains. Additionally, biofilms can shield bacteria from antimicrobial drugs, leading to infections that are resistant and have significant therapeutic implications (Ruchi *et al*., 2015).The mechanisms by which biofilms escape the effects of antimicrobial agents include: inability of the agent to reach the bacteria present at the deep part of the biofilm, the slowly growing bacteria in the biofilm shows decrease susceptibility to the agents, and some of the bacteria exist in a programmed protected phenotype that is generated in response to surface attachment (Sultan and Nabiel, 2019). Therefore, this study seeks to determine the antibiotic susceptibility patterns and biofilm production of bacteria isolated from breast cancer patients at RSFUTH, Dutse to provide a baseline information for evidence-based antibiotic administration in the treatment of bacterial infections associated with breast cancer.

MATERIALS AND METHOD

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 cross-sectional observational study of in- and out patients with breast cancer attending clinics at RSFUTHD from February, 2024 to October, 2025.

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 of the hospital.

Sample Collection

A total of one hundred and eighteen (118) isolates representing six distinct bacterial species were obtained from the principle investigator of the ongoing research on breast cancer at Microbiology Laboratory, Federal University Dutse. Each sample represented a distinct patient to ensure diversity and avoid duplication.

Identification of the Bacterial Isolates

The collected isolates were first inoculated aerobically on to blood agar, Mannitol Salt agar, MacConkey agar, Eosin methylene blue and Nutrient agar and then incubated at 37^0 C for 24 hours (Okeke *et al*., 2022). The sub-cultured isolates were characterized using established microbiological methods, including colonial morphology characteristics such as the size, surface, color, elevation, texture and transparency, Gram stain characteristics (Nasiru *et al*., 2024), and biochemical tests, including indole tests, coagulase test, catalase production test, citrate utilization test, oxidase test and methyl red test (Davis and Pezzlo, 2023). The confirmed cells of pathogens were preserved in nutrient broth and kept in refrigerator until use (Muhammad *et al*., 2020;

Sevitha *et al*., 2021).

Standardization of Bacterial Inoculum

The confirmed isolates were sub-cultured onto sterile nutrient agar plates incubated at 37^0 C for 24 hours, the sub-cultured isolates were inoculated into a sterile test-tubes containing 5ml of normal saline and compared with 0.5 McFarland turbidity standard on the turbidity scale that marched with 1.5 $\times 10^8$ CFU/mL (Cheesbrough, 2006; Haris *et al*., 2019).

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was performed using Kirby-Bauer disc diffusion method as described by Clinical and Laboratory Standard Institute (CLSI, 2020) to determined their susceptibility to the most commonly prescribed antibiotics using commercially prepared antibiotic discs for both Gram negative and Gram positive multidisc. This method was selected because it is widely used, cost effective, reliable, and suitable for routine clinical microbiology laboratories in resource limited settings. A sterile cotton wool swabs were dipped into the 0.5 McFarland standards (1.5 $\times 10^8$ CFU/mL) of the test bacteria, the excess fluid was removed by pressing and rotating the swabs against the wall of the tubes. The contents was then streaked on the surface of Mueller Hinton agar (MHA) plates (Nasiru *et al*., 2024). The inoculated plates were allowed to stand for 5 minutes. Using sterile forceps, ensuring 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), peflacine (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. The plates seeded with test bacteria, but without antibiotic disc were used as control. The plates were allowed to stand for 30 min (pre-diffusion), and then incubated at 37°C for 18- 24 hours. After the incubation, the diameter of zones of growth inhibition around each disc were measured in millimeters using a standard transparent ruler, and the results were interpreted as either susceptible, intermediate, or resistant as in according with guidelines by CLSI, (2025). The experiments were conducted in triplicate.

Determination of Biofilm Production using Tissue Culture Plate Method

This study used the tissue culture plate quantitative approach, which Sultan and Nabiei. (2019) describe as the gold standard test for biofilm formation and recovery. A loopful of freshly cultured isolates was inoculated in 10 ml of trypticase soy broth with 1% glucose. The inoculated broth was then incubated at 37^0 C for 24 hours. Bacterial suspensions were further diluted 1:100 with fresh medium. Individual wells of sterile polystyrene tissue culture plate, composed of 96 flat bottom wells, were filled with 200 uL of the diluted cultures (Afreenish *et al*., 2011). The control organisms were also incubated, diluted and added to tissue culture plate. Negative control wells contained inoculated sterile broth. The plates were incubated at 37^0 C for 24 hours. After incubation, the plate was gently tapped to remove the content of the wells followed by washing with 200 µl of phosphate buffer saline (pH 7.2). The washing step was repeated four times to remove any free bacteria present in the wells. Sodium acetate (2%) were added to the wells and kept for 30 minutes in order to fix the biofilms formed by bacteria attached to the wells and staining of the fixed biofilms was conducted using crystal violet (0.1%). After 30 minutes, the wells were thorough washed by deionized water to remove any extra stain. Optical density (OD) of stained adherent biofilm was quantify at wavelength of 550 nm using 30 % acetic acid in water as the blank (O' Toole, 2011). Biofilms formation was interpreted as none/weak, moderate or strong as described in previous studies as follows (Panda *et al*., 2019).

Data Analysis

Statistical analysis was carried out on data obtained in the study. Analysis of Variance (ANOVA) and Duncan Multiple Range Test (DMRT) was used to test for significance and means separation between the organisms respectively. This was done using a computer-based Programme-SPSS version 25. Results were represented in tables and charts.

RESULTS

Biochemical features of the bacterial isolates from breast cancer swab samples

A total of one hundred and eighteen (118) isolates representing six distinct bacterial species were recovered, the identification of these pathogens were 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 were further identified using a combination of biochemical tests including Catalase, Coagulase, Oxidase, Indole, Citrate, Methyl red and Urease tests to confirm the identity of the test bacteria (Table 1).

Table 1: Biochemical Characteristics of Some Bacterial Isolates from BC

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 MR= Methyl red; "+" = Positive reaction; "–" = Negative reaction.

Antibiotic Susceptibility of Gram-Positive Isolates

The result of the susceptibility pattern of the gram positive bacteria isolated from breast cancer (Table 2), showed the diameter (in mm) of the zone of inhibition (ZOI) results for ten (10) antibiotics against two bacterial species according to CLSI. (2024) guidelines.

Table 2: The Zone of Inhibition of Antibiotic on Gram 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-Ceptazidimes, S-Streptomycin, AZM- Azithromycin, Amx- Amoxil, CPX-Ciprofloxin, E- Earythromycin, LEV-Levofloxacin, CN-Gentamycin, CEF-Cefuroxime. BS 69 *Bacillus spp* (A), BS 128 *Bacillus spp* (B).

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) Ceptazidimes (30 µg) and Cefuroxime (30 µg).While BS 128 *Bacillus* spp (B) showed multidrug resistance to four antimicrobial agent such as Amoxil (30µg), Earythromycin (30µg), Levofloxacin (20µg) and Streptomycin (30µg) while sensitive to Rifampicin (30µg), Ceptazidimes (30µg), Azithromycin (10µg), Ciprofloxacin (10µg), Gentamycin (10µg) and Cefuroxime (30µg). Ciprofloxin appear to be the most effective broad spectrum agents with inhibition zone of 26 mm, followed by Ceptazidimes 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 Gram-Negative Isolates

However, the results from the present study (Table 3) 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 showed sensitive to Ofloxacin (10 µg), Peflacine (10 µg), Gentamycin (10 µg), Ceporex (10 µg), Cefriaxome (30 µg) and Cefuroxime (30 µg). BS 100 *P*roteus spp was shown 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), Peflacine (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 the tested gram negative species with the highest zone diameter ranging from 20 mm – 28 mm. Streptomycin on the other hand, showed very low efficacy, with diameter zones ranging from 8 mm – 10 mm, indicating high resistance.

Antibiotic Resistance Profiles of Gram-Positive Bacteria

All bacteria isolates (118 isolates) obtained were subjected to antibiotics susceptibility testing using the disc diffusion method. The results of the antibiotics susceptibility pattern of gram positive bacteria from breast cancer at RSFUDTH shown among the bacteria tested (Table 4). BS 69 *Bacillus spp* (A) showed high resistance to five antimicrobial agent from different groups such as Rifampicin (30 µg), Streptomycin (30µg), Amoxil (30 µg), Earythromycin (15 µg) and Levofloxacin (5µg). BS 128 *Bacillus spp* (B) was the most resistant bacteria among all the gram positive bacteria tested against Ceptazidimes (30 µg), Streptomycin (30 µg), Azithromycin (10µg), Amoxil (30µg), Ciprofloxacin (10µg) and Cefuroxime (30µg).

Table 3: 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-Ceptazidimes, S-Streptomycin, AU- Augmentin, PEF- Peflacine, 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 4: Antibiotics Susceptibility Profile Interpretation of the Gram Positive Bacteria from Breast Cancer.

Antibiotic (µg)	*Bacillus* spp (A)	*B*acillus 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-Ceptazidimes, S-Streptomycin, AZM- Azithromycin, Amx- Amoxil, CPX- Ciprofloxin, E-Earythromycin, LEV-Levofloxacin, CN- Gentamycin, CEF-Cefuroxime. S= Susceptible, R= Resistant.

Table 5: 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-Ceptazidimes, S-Streptomycin, AU- Augmentin, PEF- Peflacine, 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 Resistance Profiles of Gram-Negative Bacteria

The Gram-negative bacterial isolates from breast cancer samples demonstrate a multidrug –resistant profile, with resistance observed against most β-lactam antibiotics (Augmentin, Ceftazidime, Cefuroxime, Ceftriaxone, and Streptomycin). However, Gentamicin (CN) and Ciprofloxacin (CPX) showed uniform sensitivity across all isolates, indicating they remain the most reliable treatment options in this study. Ceporex (CEP) and Cefuroxime (CEF) displayed variable sensitivity, suggesting strain-specific effectiveness (Table 5).

Biofilm Formation Characteristics

Antimicrobial resistant bacteria continue to be a major challenge for treating infections among breast cancer. The ability of the test organisms to produce biofilm is considered one of the main causes of antimicrobial resistance. Bacteria embedded in the biofilm can survive a higher concentration of antimicrobials up to 1500 folds

Table 6**:** Biofilm Formation Based on Optical Density of Tissue Culture Plate Method.

Average OD Value	Biofilm Formation
< 0.17	None
0.17 – 0.34	Weak
0.35- 0.63	Moderate
> 0.68	Strong

Key: ODc=Average OD of negative control + 3 × SD of negative control.
OD: Optical density, ODc: OD cut off value, SD: Standard deviation (Kala *et al*., 2020).

Table 7: Biofilm Formation Characteristics of Bacteria Isolated from Breast Cancer (Average OD value > 0.68).

Code	OD	Biofilm Interpretation				Organism
		None	Weak	Moderate	Strong	
BS 53	1.27	-	-	-	+	*E. coli*
BS 69	1.34	-	-	-	+	*Bacillus* spp
BS 100	1.63	-	-	-	+	*Proteus* spp
BS 123	0.96	-	-	-	+	*E. coli*
BS 128	1.46	-	-	-	+	*Bacillus* spp
BS 193	0.96	-	-	-	+	*Proteus* spp
BS 263	1.09	-	-	-	+	*E. coli*
BS 271	2.74	-	-	-	+	*Klebsiella* spp

Key: - = (Absent), += (Present
ODc=Average OD of negative control + 3 × SD of negative control. BS = Breast swab
OD: Optical density, ODc: OD cut off value, SD: Standard deviation (Kala *et al*., 2020).

than those needed to eliminate free bacteria (Tenke *et al*., 2024). In the present study eight (8) multidrug resistance bacteria isolated from patients with breast cancer were subjected to tissue culture plate method (TCPM) for phenotypic detection of biofilm production as shown in (Tables 6 and 7). The TCPM, the gold standard method, detected biofilm formation in 8 bacterial isolates (100%). The highest biofilm production was found among *Klebsiella spp* with optical density value of 2.74 and lowest value was recorded among *E*. *coli* and *Proteus spp* with both optical density value of 0.96 which are still above the 0.68 threshold value for strong biofilm formation. It was revealed that none of the test organisms were categorized as “None”, “Weak”, or “Moderate” biofilm producer.

DISCUSSION

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 (Kubecek *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 is in line 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 ones like streptomycin and several 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 is in line with global trends.

Gram-negative bacteria's outer membrane acts as a molecular "shield," preventing intracellular drug buildup and limiting antibiotic penetration (Pitout & Laupland, 2022). Ciprofloxacin's consistent effectiveness against a number of isolates indicates that fluoroquinolones are still 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, such as plasmid-mediated resistance genes, efflux pumps, and porin alterations (Kubecek *et al*., 2021). These characteristics provide exceptional flexibility, especially in settings where antibiotic exposure is high. 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 worries about the increasing prevalence of Gram-negative infections that are resistant to drugs in oncology settings (Eiji *et al*., 2022). Higher rates of morbidity, mortality, and medical expenses are linked to certain infections.

The Gram-positive isolates' profile of antibiotic resistance, 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 indicated by resistance to several antibiotic classes, such as β-lactams, macrolides, fluoroquinolones, and aminopenicillins (WHO, 2024). Through mutation and horizontal gene transfer, these bacteria accumulate resistance determinants throughout time, acting as "genetic archivists" in terms of evolution. The ecological circumstances for the selection and maintenance of such resistant organisms are perfect in cancer care settings, which are marked by immunosuppression, frequent antibiotic administration, 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 in Gram-negative antimicrobial resistance, as evidenced by the patterns of antibiotic resistance seen in *Escherichia coli*, Proteus species, and Klebsiella pneumoniae (WHO, 2023). In line with the widespread synthesis of extended-spectrum β-lactamase (ESBL) and aminoglycoside-modifying enzymes, these organisms showed substantial resistance to streptomycin, a number of cephalosporins, and β-lactam/β-lactamase inhibitor combos. Gram-negative bacteria have an inherent structural advantage, according to Gambhir et al. (2022). Their outer membrane functions as a molecular "armor," limiting antibiotic entrance and promoting quick 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 over the duration of this therapeutic window are raised by global patterns that show 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. In the absence of vigorous antimicrobial stewardship, surveillance, and infection control measures, the clinical environment runs the risk of reverting to a time before antibiotics, when simple infections were incurable. One of the study's most therapeutically significant findings is the universal strong biofilm development seen in all MDR isolates (Ciofu *et al*., 2022).

Polysaccharides, proteins, lipids, and extracellular DNA make up the extracellular polymeric substance (EPS) matrix that biofilms, which are intricate, multicellular bacterial communities, are embedded in. According to Harika *et al*. (2020), this matrix serves as a microbial "citadel," significantly lowering antibiotic penetration and protecting germs from host defense responses. Antibiotic sensitivity is further decreased by the various metabolic states that bacterial cells adopt within biofilms, such as dormancy (Ciofu *et al*., 2022).

The remarkably high optical density seen in Klebsiella species is consistent with its well-established status as a chronic hospital pathogen and a prolific biofilm producer.

The results of the study of Harika *et al*. (2020) are in agreement. The development of biofilms in cancer patients has significant clinical consequences. According to Sultan and Nabiel (2019), biofilms have the ability to colonize necrotic tumor tissue, surgical sites, and medical equipment, forming persistent infection reservoirs that are resistant to conventional antibiotic therapy. This explains why in vitro susceptibility results and poor clinical outcomes are frequently at odds. According to Tenke *et al*. (2024), biofilm-associated bacteria may withstand antibiotic concentrations up to 1,000–1,500 times higher than those of planktonic cells, making traditional dosage schedules useless. Furthermore, biofilms promote horizontal gene transfer, which speeds up the dissemination of resistance genes within microbial populations (Fromantin *et al*., 2013). Biofilms are a cooperative survival strategy that enables bacteria to act as multicellular entities rather than solitary cells from an ecological perspective. Biofilm persistence is significantly increased in individuals with immunocompromised breast cancer due to reduced immune surveillance (Caigoy *et al*., 2022).

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