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Breast Cancer and Associated Bacterial Pathogens: Study of Breast Cancer Ulcers in Patients from National Hospital Cancer Center, Abuja, and their Antibiotic Sensitivity Profile

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Abstract

Background: Bacterial infections are ubiquitous and promote dysbiosis, resulting in disease conditions that may be resolved through antimicrobial therapy in some hosts but may persist for long durations in others, thereby significantly increasing the carrier's risk of developing cancer over time. The consequences range from immune suppression to the development of acute resistance to anticancer and/or antibacterial therapeutics, and subsequently, a higher risk of progression to active cancers. This study identified the bacterial spectrum implicated in breast cancer wounds or ulcers, their antimicrobial susceptibility profiles, and the resistance genes embedded in their genomes.

Methods: A total of two hundred and forty breast cancer wound/ulcer swab samples (150 breast wound swabs and 90 endo-cervical swabs) from symptomatic patients attending the National Hospital Cancer Clinic, Abuja, were screened for bacterial infection, using standard microbiological cultural methods, and biochemical tests for the identification of isolates. Randomly selected samples (36 breast wound swabs and 19 endocervical swabs) were also analyzed using next-generation gene sequencing for the identification and detection of resistant genes within the sequenced genome.

Results: Prevalence of one hundred and twenty-three bacterial species were observed: *Escherichia coli*, 25(20.3%) > *Staphylococcus aureus*, 21(17.1%) > *Klebsiella pneumoniae*, 19(15.4%), *Pseudomonas aeruginosa*, 15(12.2%) > *Enterococcus faecalis* 7(5.7%), and *Staphylococcus epidermidis* 5(4.1%). Among the array of antibiotics tested, Ciprofloxacin was the most effective against both Gram positive with sensitivity percentage of *S. epidermidis* (83.3 %); *S. aureus* (81%); *E. faecalis* (80%) and Gram-negative organisms *P. aeruginosa* (86.7%); *K. pneumoniae* (78.9%); *E. coli* (60%) while Ceftriaxone had the least activity with resistance percentage of *K. pneumoniae* (78.9%); *E. coli* (72%); *P. aeruginosa* (66.7%) Gram-negative isolates and for the Gram-positive isolates *S. epidermidis* (83.3 %); *E. faecalis* (80.0%); *S. aureus* (71.4%). For the sequenced samples, the prevalent pathogens were *Acinetobacter baumannii* (53%) > *Alcaligenes faecalis* (30%) > *Pseudomonas aeruginosa* (7%) > *Enterococcus faecalis*, *Corynebacterium striatum* (4% respectively) > *Proteus mirabilis* (2%) for breast cancer lesions while for cervical cancer lesions, *Staphylococcus epidermidis* (16%) > *Pseudomonas mendocina* (14%) > *Enterococcus faecalis*(13%) > *Bacillus cereus*(11%) > *Escherichia coli*, *Staphylococcus aureus* and *Ligilactobacillus agilis* (10% respectively) > *Gardnerella vaginalis* and *Pseudomonas aeruginosa* (8% respectively) were prevalent.

Conclusion: This study reveals that bacterial pathogens involved with breast cancer infection and resistance to commonly used antibiotics could cause prolonged cancer therapy treatment and a huge threat to successful eradication of cancer, and the need for regular checks for bacterial infection as a means of effective infection and cancer management.

Keywords: breast cancer, wound pathogens, Gram-positive & negative bacteria, Antimicrobial susceptibility, Resistance pattern, Resistant genes.

INTRODUCTION

Cancer is a broad term that describes a disease resulting from the uncontrolled growth and division of cells. According to the World Health Organization (WHO), it is the leading cause of death worldwide, accounting for an estimated 9.6 million deaths in 2018, especially in developing countries like Nigeria, with a huge financial cost estimated at 1.16 trillion dollars per year as of 2010 (WHO, 2018). Over time, it has been discovered that certain groups of bacteria are associated with cancer, and these are called cancer-causing bacteria- oncogenic bacteria (Chang and Parsonnet, 2010; Eyvazi *et al.*, 2020). Cancer-causing bacteria are infectious organisms that are known or suspected to cause cancer or promote the progression of cancer. Although cancer-associated bacteria have long been considered to be opportunistic (i.e., infecting tissues after cancer has already established itself), there is some evidence that some bacteria may be directly carcinogenic. Anything that increases defective mitosis rates is going to increase mutations that can lead to cancer (Hosea *et al.*, 2024). Genomic alterations are considered the main cause of cancer establishment, which are inducible through various pathogenic infections, of which about 20% of global cancer is attributed to infections (Eyvazi *et al.*, 2020).

Bacterial infection is one of the most common life-threatening complications of cancer and its treatment because cancer and its treatment weaken the immune system, and these bacterial infections can occur when the normal microbiota of the cancerous region is altered or pathogenic organisms are introduced into the site of infection (Sajmina *et al.*, 2021). When this happens, the cancerous cells can easily spread to adjacent tissues and cause more harm to the patient. The organisms that are pathogenic can easily release toxins or other harmful proteins that enhance their growth and cause harmful effects on cancer patients.

Bacteria represent the most common pathogen in cancer-associated infections, and bacterial sepsis continues to be a leading cause of morbidity and toxic death in children and the elderly receiving intensive therapy for cancer (Kafayat *et al.* 2023). It is well acknowledged that oncology patients are more susceptible to bacterial infections and their systemic spread due to tumor-related and iatrogenic immunosuppression (a state of ill health or adverse effect caused by medical treatment), which is comprised of the classic clinical

combination of severe neutropenia, fever, hypotension, and headache (Kubeček *et al.*, 2021; Bassam *et al.*, 2023). Acute bacterial infections negatively impact survival and increase mortality in patients with solid cancers such as breast and cervical cancer malignancy (Kafayat *et al.* 2023; Oliveira *et al.*, 2016).

Examining the microbial makeup of cancer patients is crucial for enhancing our understanding of disease pathogenesis and informing therapeutic interventions. Genomic alterations, widely recognized as primary drivers of cancer development, can be induced by various pathogenic infections. Consequently, elucidating the role of bacteria in carcinogenesis and the detrimental impact of bacterial infections in cancer patients has shifted from being a scientific luxury to an urgent necessity, especially in a developing country like Nigeria. Infection remains a primary associated cause of death among breast cancer patients, with bacteria most commonly accounting for infection-associated death, hence the need for this study. 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.

Therefore, this study seeks to determine the prevalence of bacterial pathogens among breast cancer patients attending the National Hospital Cancer Center and to characterize their antibiotic sensitivity profiles.

MATERIALS AND METHODS

Ethics approval and Consent to participate

Ethical approval was obtained from the Research and Ethical Committee of the National Hospital, Abuja, Nigeria, before the commencement of the research, with Ethical number NHA/EC/091/2021. Before sampling, participants completed and signed informed consent forms.

Research Design

A cross-sectional observational study design.

Study Area

The study was carried out at the National Hospital, Abuja, after obtaining ethical clearance from the hospital's ethics board (ethical number NHA/EC/091/2021). An informed consent form and well-structured questionnaires were used to collect patient information.

SAMPLING

A simple random sampling technique was employed to collect one hundred and fifty (150) breast cancer wound swab samples using sterile swab sticks from consenting patients attending National Hospital Abuja Cancer Center and immediately transferred in an ice bag to the microbiology and parasitology laboratory of the hospital for culture and isolation.

SAMPLE SIZE DETERMINATION.

The minimum sample size for the study will be calculated using the formula for a cross-sectional comparative study (Charan & Biswas, 2013):

$$n = [Z_{\alpha}^2 P (1-P)] / d^2$$

Where: n = Minimum sample size;

Z = Confidence level (95% ie 1.96);

P = Prevalence rate of 19.4% (Fentie *et al.*, 2018);

d = Precision significance level (5% ie 0.05)

$$n = 1.96^2 \times 19.4(1-19.4)/0.05^2$$

$$n = 3.8416 \times 0.194 \times 0.806 / 0.0025$$

$$= 240.275$$

$$\sim 240 \text{ Samples}$$

Inclusion criteria:

- ❖ Female patients diagnosed with breast cancer who are willing to participate in the study and have given their consent.
- ❖ Female patients diagnosed with breast cancer who have signs of infection.
- ❖ Breast cancer patients ages 18 years and above.
- ❖ Breast cancer patients with fungating wounds were coming for dressing during the duration of the study.

Exclusion Criteria:

- ❖ Breast cancer patients who didn't consent to participating in the study.
- ❖ Breast cancer patients without signs of infection.
- ❖ Patients on antibiotic treatment were not included in the study (to avoid artificially inflating the number of resistant isolates).

Culture and Isolation of bacteria from wound swabs.

On arrival at the laboratory, the swab sticks were used to make a smear on already prepared chocolate, blood and MacConkey agar plates, using a flamed wire loop the smear was spread out, after which the, blood and MacConkey agar

plates were incubated at 37°C for 24 hours, and the chocolate agar plates in an enclosed metal lid chamber to achieve an anorexic environment. After 24 hours, the plates were observed for possible growth and morphological features, and the colonies were subcultured to obtain single colonies. The sub-cultured isolates were characterized using established microbiological methods, including colonial morphology, Gram stain characteristics (Cheesbrough, 2017), and biochemical tests, including indole tests, catalase production test, citrate utilization test, and coagulase test (Davis and Pezzlo, 2023). The Vitek 2 compact principle was also used to identify isolates that were not well defined after subculturing.

Antimicrobial susceptibility testing.

Antimicrobial screening according to CLSI (2020) was performed to determine the susceptibility of the isolates to selected antibiotics. The susceptibility of isolates to antibiotics were demonstrated using specific antibiotics such as penicillin (30µg - Amoxicillin clavulanate, 20µg - Ampiclox), cephalosporins (30µg- Cefotaxime, 30µg - Cefuroxime, 30µg - Ceftriaxone sulbactam), carbapenems (10µg - Imipenem, 10µg - Meropenem), fluoroquinolones (30µg - Nalidixic acid, 5µg - Ciprofloxacin, 5µg - Levofloxacin, 5µg - Ofloxacin), aminoglycosides (10µg - Gentamycin), macrolides (30µg - Azithromycin, 15µg - Erythromycin). Inhibition zone diameters were measured in millimeters, and susceptibility was scored as resistant, intermediate, or sensitive according to CLSI guidelines. The Kirby-Bauer Disc diffusion method was used, in which antibiotics diffuse from a confined source through Mueller-Hinton agar and create a concentration gradient. A loopful of the isolated bacteria was inoculated into nutrient broth and incubated for 24 hours to check for turbidity, which indicates microbial growth. Afterward, the culture was diluted to 0.5 McFarland standard. A sterile swab stick was dipped into the standardized bacterial solution in a test tube and then strained a bit to remove excess solution. The swab stick was then streaked onto the already prepared Mueller-Hinton plates, and the selected antibiotic discs were placed on the inoculated agar plates using flamed forceps. The plates were allowed to stand for 5 minutes to ensure proper contact and diffusion of the antibiotic discs, after which they were incubated for 24 hours, and the zone of inhibition was measured using a transparent meter rule and recorded in millimeters.

RESULTS

Demographical details of patients from whom the samples were collected were shown.

A total of one hundred and fifty breast cancer wound swab samples were collected from

consenting patients attending the National Hospital Cancer Center for dressing and treatment, as shown in Table 1 below. All were females aged 18 - 65 years.

Table 1: Socio-demographic characteristics of study participants.

Demographic Characteristics	Frequency	Percentage (%)
Age in years		
0 - 34	31	21
35 - 55	104	69
56 - 68	15	10
Educational level		
No formal education	16	11
Primary	18	12
Secondary	84	56
Tertiary	32	21
Occupational status		
Civil servants	30	20
Farmers/Traders	98	65
House wives	22	15
Locality		
Rural	102	68
Urban	48	32
Behavioural practices for breast cancer patients (Use of contraceptives)		
Yes	115	77
No	35	23
Mastectomy		
Yes	142	94.7
No	8	5.3

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

A hundred and twenty-three bacterial isolates were recovered, representing nineteen different species, with the most prevalent being

Escherichia coli. Most patients had single microbial infections, and a few had dimicrobial infections, with the most common association being *Staphylococcus aureus* and *Escherichia coli*. This is represented in Figure 1.

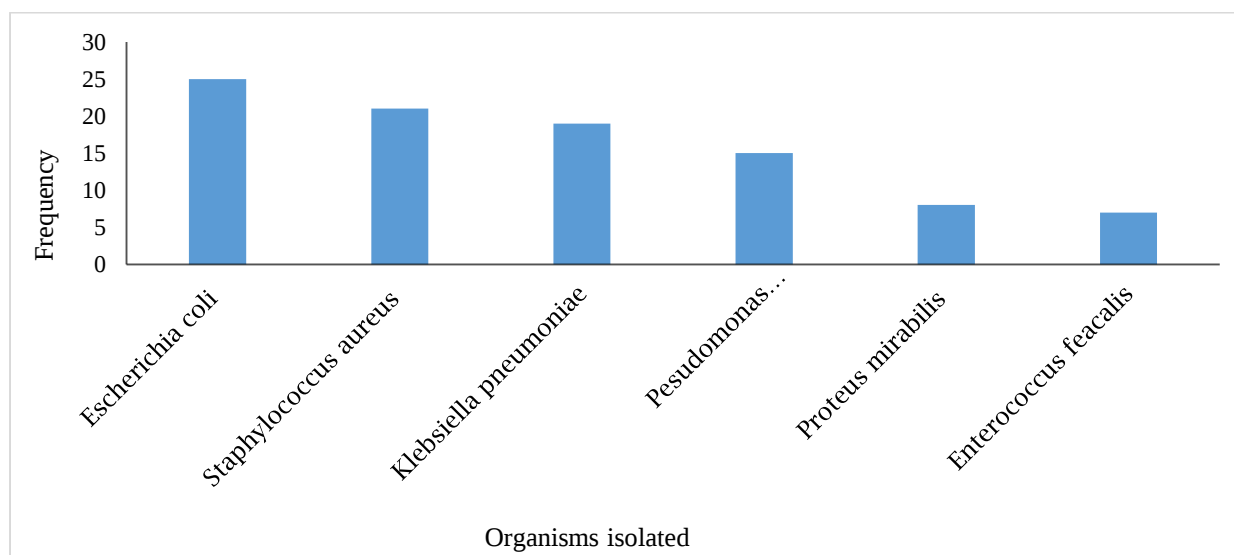


Figure 1: Prevalence of bacterial isolates from breast wound lesions of oncology patients attending the National Hospital Cancer Center.

Distribution of antibiotic resistance among the most prevalent bacteria isolated from the infected breast cancer wound samples collected.

The observable resistance pattern of the organisms, as revealed by the Kirby-Bauer disc diffusion test, showed that the fluoroquinolones (such as levofloxacin, ciprofloxacin) and carbapenems (such as meropenem) were the most effective antibiotics against Gram-positive and negative isolates, while the cephalosporins, such as cefuroxime and ceftriaxone, had the least activity for Gram-negative and positive isolates. This is represented in Figure 2 and 3. This is outlined as follows for *Escherichia coli*

(Ciprofloxacin-40%, Levofloxacin- 32%, Nalidixic acid 48% and Meropenem 28.0%); *Klebsiella pneumoniae* (Ciprofloxacin-36.8%, Levofloxacin-15.8%, Nalidixic acid 26.3% and Meropenem 15.8%) and *Pseudomonas aeruginosa* (Ciprofloxacin-13.3%, Levofloxacin- 33.3% and Nalidixic acid 6.6% and Meropenem 6.6%) while for the most prevalent Gram-positive isolates *Staphylococcus aureus* (Ciprofloxacin- 19.0% and Levofloxacin- 14.3% and Meropenem 9.5%); *Enterococcus faecalis* (Ciprofloxacin- 20.0%, Levofloxacin- 30.0% and Meropenem 20.0%) and *Staphylococcus epidermidis* (Ciprofloxacin-16.7%, Levofloxacin- 0.0% and Meropenem 16.7%). This is represented in Figures 2 and 3.

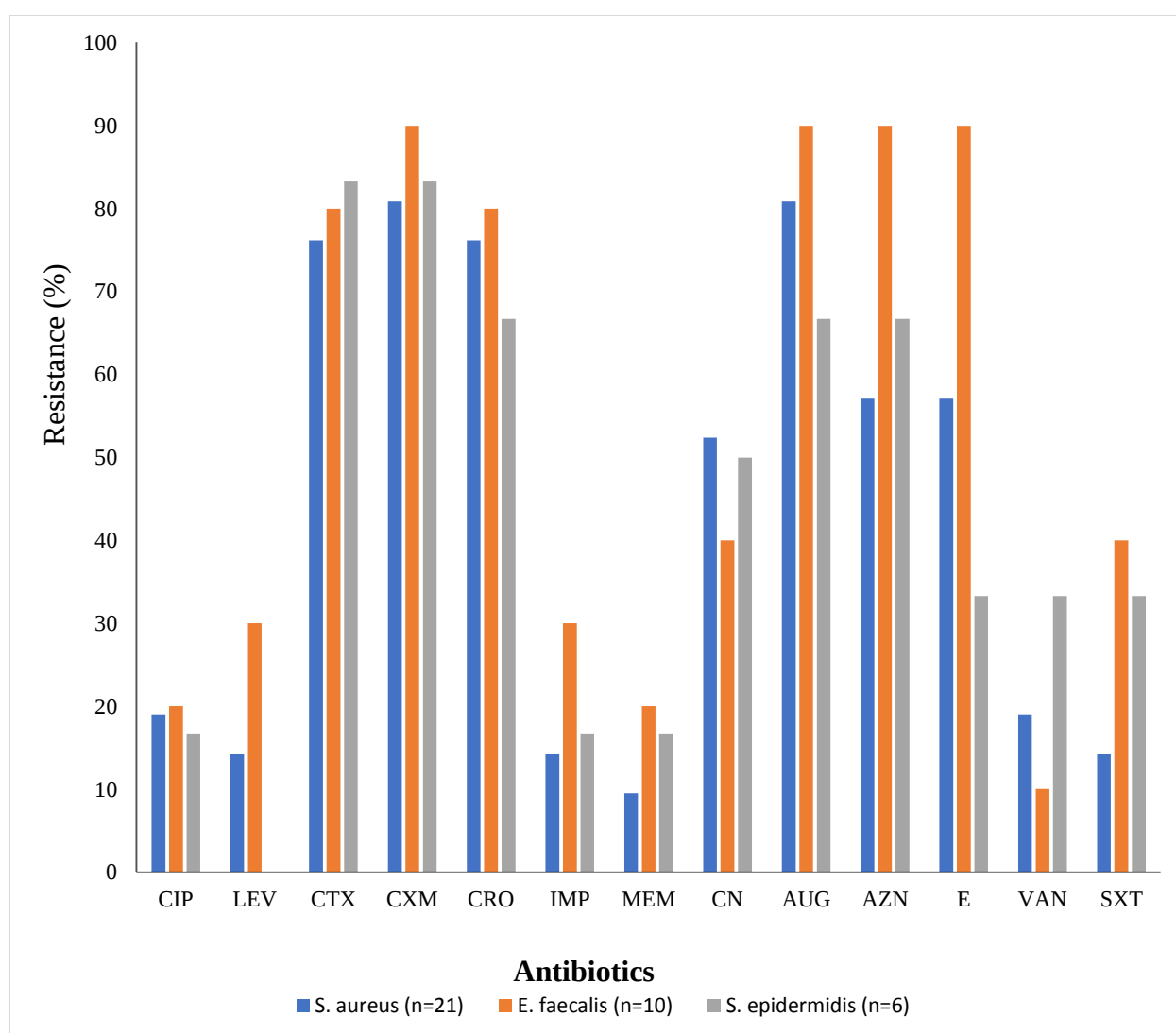


Figure 2: Anti-biogram of the most prevalently isolated Gram-positive pathogens from the oncology samples screened.

NB: Ciprofloxacin = CIP, Levofloxacin = LEV, Cefotaxime= CTX, Cefuroxime = CXM, Ceftriaxone = CRO, Imipenem=IMP, Meropenem = MEM, Gentamycin = GN, Amoxicillin/Clavulanate = AUG, Azithromycin = AZN, Erythromycin = E, Vancomycin = VAN, Trimethoprim-Sulfamethazole= SXT.

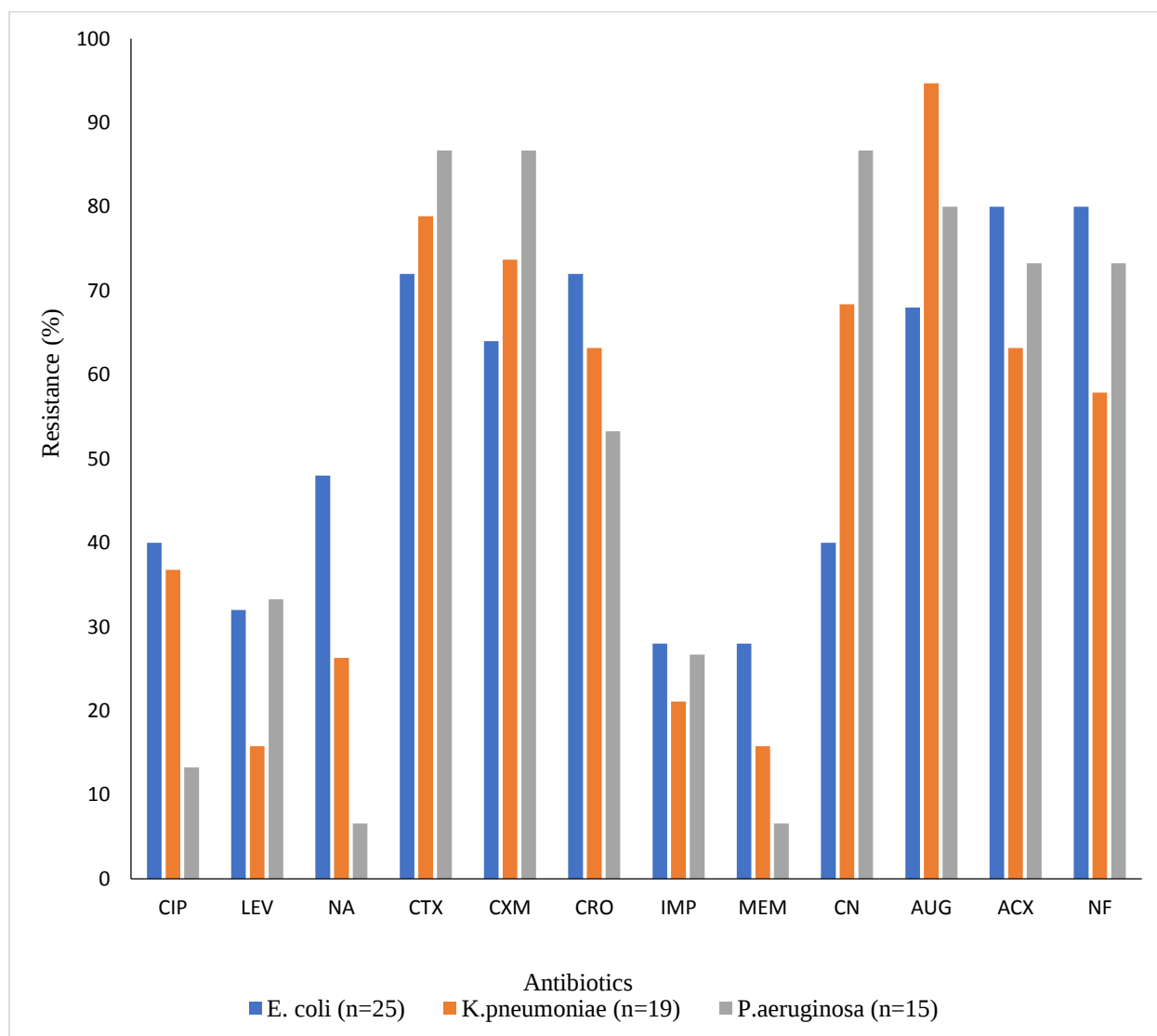


Figure 3: Anti-biogram of the most prevalent isolated Gram-negative pathogens from oncology samples screened.

NB: Ciprofloxacin = Cip, Levofloxacin = Lev, Nalidixic acid = Na, Cefotaxime = Ctx, Cefuroxime = Cxm, Ceftriaxone = Cro, Imipenem= Imp, Meropenem = mem, Gentamycin = Cn, Amoxicillin/clavulanate = Aug, Ampiclox = Acx, Nitrofurantoin = Nf.

DISCUSSION

Fungating wounds in breast cancer patients pose significant challenges to healthcare management, as these wounds are highly susceptible to infection, which can complicate cancer treatment and impede healing. Infections in fungating wounds not only delay the wound healing process but also increase the risk of systemic infection, which can lead to severe complications such as bacteremia and sepsis (Li *et al.*, 2021; Amitabha *et al.*, 2023). This exacerbates the overall morbidity of breast cancer patients and diminishes their quality of life. Furthermore, infections contribute to higher healthcare costs and prolonged hospital stays, ultimately reducing patient survival rates. Given these complications, managing infection

in these wounds is critical to successful treatment. This includes regular monitoring for signs of infection, effective wound care, and timely antibiotic treatment based on antimicrobial susceptibility testing.

The study of bacterial isolates from infected breast cancer wound samples revealed that *Escherichia coli* was the most prevalent pathogen, accounting for 20.3% of isolates, followed by *Staphylococcus aureus* (17%), *Klebsiella pneumoniae* (15.4%), and *Pseudomonas aeruginosa* (12.2%). These findings are consistent with other studies that have highlighted similar bacterial profiles in chronic and fungating wounds (Amitabha *et al.*, 2023; Bhat *et al.*, 2021).

These organisms are frequently found in healthcare-associated infections due to their ability to colonize and persist in both the hospital environment and in immunocompromised patients, such as those undergoing cancer treatments. 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).

The pathogenicity of the most commonly isolated bacteria is largely attributed to their virulence factors, which enable them to survive and thrive in wound environments. *Staphylococcus aureus*, for example, produces various virulence factors such as coagulase, catalase, clumping factor A, and leukocidins, all of which help the bacterium evade the host's immune system and maintain chronic infection (Dissemond, 2009). Similarly, *Pseudomonas aeruginosa* produces elastase, an enzyme that contributes to tissue degradation and promotes infection persistence in chronic wounds (Amitabha *et al.*, 2023). These virulence factors play a significant role in the chronicity of infections in fungating cancer wounds, which complicates treatment and prolongs healing (Bessa *et al.*, 2015). Furthermore, the continuous presence of these bacteria can lead to persistent inflammation, further damaging tissue and delaying recovery.

Antibiotic resistance is a major concern in the treatment of fungating wound infections, and this study found high levels of resistance among the bacterial isolates to commonly used antibiotics. For example, *Staphylococcus aureus* demonstrated significant resistance to many of the antibiotics tested, with susceptibility rates falling below 50% for several agents. This resistance pattern is a growing concern, as it limits treatment options and may contribute to treatment failure (Nwobodo *et al.*, 2023; Salam *et al.*, 2023).

Additionally, *Pseudomonas aeruginosa* exhibited resistance to carbapenems and third-generation cephalosporins, which are often used as last-resort treatments for severe infections (Singh *et al.*, 2024). Resistance to these critical antibiotics is alarming, particularly as these drugs are essential in treating multidrug-resistant infections in immunocompromised patients. The resistance patterns of the bacterial isolates in the study showed a trend consistent with the growing global concern over antibiotic resistance (Amitabha *et al.*, 2023). However, the bacteria remained susceptible to certain antibiotics, including ciprofloxacin,

cefloxacin, streptomycin, and erythromycin, which have previously been shown to be effective against these pathogens (Singh, 2024). The moderate resistance to gentamicin and chloramphenicol observed in the isolates might be linked to the complex pharmacokinetics and the route of administration of these aminoglycosides, which can affect their clinical efficacy (Ehinmidu, 2003; Nwobodo *et al.*, 2023). This highlights the importance of antibiotic stewardship in preventing further development of resistance. Overuse and misuse of antibiotics, especially in hospital settings, exacerbate resistance and compromise treatment effectiveness, making it crucial for healthcare providers to use antibiotics judiciously and based on susceptibility testing. Infection in fungating 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 co-existing 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).

In conclusion, the management of fungating wounds in breast cancer patients is a critical aspect of cancer care, as infections can severely hinder healing and compromise treatment outcomes. The bacterial pathogens commonly found in these wounds, such as *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *S. aureus*, are often highly antibiotic-resistant, posing significant challenges to effective treatment. Given the complexity of these infections, healthcare providers need to employ a comprehensive approach that includes proper wound care, infection prevention, and antibiotic stewardship.

Regular antimicrobial susceptibility testing is crucial for guiding appropriate treatment decisions and minimizing the risk of resistance. Additionally, ongoing research into novel therapies, including the development of new antibiotics and alternative treatment strategies, is essential to address the growing threat of resistant infections in breast cancer patients with fungating wounds.

CONCLUSION

Based on the study's results, it is therefore recommended that fluoroquinolones and quinolones, such as Ciprofloxacin, Streptomycin, and Pefloxacin, may be the best choice of antimicrobials for treating fungating wound infections. Antimicrobial susceptibility patterns are important for clinicians in selecting empiric antimicrobial therapy. A nationwide survey should be undertaken to inform the rational formulation of public healthcare policies and to provide useful information for global surveillance of this pathogenic bacterium. Although this study did not investigate the impact of hygiene on the development of wound infection, we suggest that educating cancer patients on personal hygiene will help enhance wound healing and improve patient management. Rapid and reliable methods for antibiotic susceptibility testing are important for instituting appropriate therapy. The variety of organisms observed in this study underscores the need to obtain culture specimens from infected wounds for

microbiological evaluation and antibiotic susceptibility testing, enabling adapted chemotherapy to be prescribed.

CONFLICT OF INTEREST

The authors declare that no conflicting interests exist.

Authors' contribution

This study was carried out in collaboration among the authors. Dibua, M.E.U. designed and supervised the study. Oka, C. U., Unah, V. U., Oka, U.E., Tiri, T. J., and Aruah, C. conceptualized the study, collected the samples, and conducted the laboratory analysis. Oka, C.U., and Nweke, R. N. prepared and edited the manuscript. All the authors edited the manuscript and approved publication.

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