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Research Article:

Antibiotic resistance profile and detection of extended-spectrum β-lactamase producing bacteria in patients with breast Cancer

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Abstract

Background: Breast cancer patients face increased risk for bacterial infections due to immunocompromised status and treatment-related factors. The human gut microbiota has emerged as a critical modulator of breast cancer development through multiple interconnected pathways, with significant compositional differences in gut microbial communities between breast cancer patients and healthy individuals. The evolution of antimicrobial resistance has become crucial in cancer care, especially regarding bacteria that produce extended-spectrum β-lactamases (ESBLs).

Aims: This study aimed to characterize bacterial isolates from stool samples of breast cancer patients using 16S rRNA gene sequencing and evaluate antimicrobial resistance patterns and extended-spectrum β-lactamase (ESBL) production

Methodology: Fifty bacterial isolates were collected from stool specimens of breast cancer patients at Nanakali Hospital, Erbil, Kurdistan region-Iraq, from September to December 2024. Bacterial identification was employed by conventional biochemical methods followed by 16S rRNA gene sequencing. Antimicrobial susceptibility testing used disc diffusion against 12 antibiotics based on CLSI guidelines. ESBL production was detected through phenotypic screening and multiplex PCR targeting blaTEM, blaSHV, and blaCTX-M genes.

Results: *Escherichia coli* was most prevalent (42%), followed by *Salmonella enterica* (14%), *Enterobacter cloacae* (10%) and *Klebsiella pneumoniae* (10%). General susceptibility was observed against carbapenems and netilmicin, while complete resistance was observed against ticarcillin-clavulanic acid and tigecycline across all the isolates. ESBL production was detected in 56% of the isolates, with *K. pneumoniae* showing highest rates (75%). All the ESBL isolates harbored ≥1 ESBL gene; blaTEM was the predominant single genotype, and blaTEM + blaCTX-M was the most common combination, particularly in *E. coli* (47.61%).

Conclusion: High antimicrobial resistance burden exists among bacterial isolates from breast cancer patients, with notable ESBL prevalence and concerning resistance patterns. The universal presence of ESBL genes in all isolates provides important clinical management insights. While carbapenem susceptibility remains intact, high resistance rates to common antibiotics underscore the need for comprehensive antimicrobial stewardship in oncology settings.

Keywords: *Bacterial isolation, Molecular identification, Extended-spectrum beta-lactamase, Antibiotic resistance, Breast cancer*

1. Introduction

Breast cancer remains the most frequently diagnosed malignancy globally, accounting for over 2.3 million new cases annually and representing 11.7% of all cancer cases worldwide [1]. Despite advances in therapeutic interventions, mortality rates remain substantially high, with complex etiology involving genetics, hormonal influences, environmental exposures, and increasingly recognized microbial components [2].

The human gut microbiota has emerged as a critical modulator of breast cancer development through multiple interconnected pathways. Recent investigations reveal compositional differences in gut microbial communities between breast cancer patients and healthy individuals, with notable reductions in microbial diversity and altered bacterial taxa abundance in cancer patients [3]. These microbial dysbiosis contribute to breast carcinogenesis through β-glucuronidase enzyme production that increases circulating estrogen levels, promotion of chronic inflammatory states, and immune surveillance system modulation [4]. The gut-breast cancer relationship extends beyond local effects to encompass systemic impacts on cancer progression and therapeutic responses. Gut microorganisms influence breast cancer through the gut-mammary axis, wherein microbial metabolites and bacterial translocation affect mammary tissue homeostasis and tumor microenvironment composition [5]. Specific bacterial species demonstrate protective or carcinogenic effects, with certain strains associated with improved chemotherapy and immunotherapy outcomes [6].

The evolution of antimicrobial resistance has become crucial in cancer care at the same time as microbiota-cancer interactions, especially when it comes to bacteria that produce extended-spectrum β-lactamases (ESBLs). Gram-negative bacteria are able to develop enzymes known as ESBLs, which give them resistance to broad-spectrum β-lactam antibiotics, such as third- and fourth-generation cephalosporins, while still making them susceptible to carbapenems [7]. ESBL-producing Enterobacteriaceae prevalence has increased dramatically worldwide with E. coli and Klebsiella pneumoniae being common producers causing clinical management challenges due to treatment failures and increased morbidity [8].

The utilization of 16S rRNA gene sequencing provides powerful tools for bacterial identification and phylogenetic analysis, enabling precise taxonomic classification and detection of previously uncharacterized species. This molecular approach, combined with phenotypic antimicrobial susceptibility testing and ESBL detection methods, offers valuable insights into microbial landscapes associated with breast cancer [9].

Given the complex interplay between gut microbiota alterations, antimicrobial resistance patterns, and breast cancer pathogenesis, comprehensive molecular characterization of bacterial isolates from breast cancer patients is essential for understanding disease mechanisms and optimizing clinical management strategies.

2. Methods and Materials

2.1. Isolation and Identification of Bacteria

Fifty stool samples were taken from breast cancer patients undergoing chemotherapy at Nanakali Hospital in Erbil, Iraq, between September and December of 2024. The women's ages ranged from 33 to 70. Every sample was cultivated and subcultured on appropriate general and selective media, such as nutrition agar, blood agar, and MacConkey agar. Their biochemical profile was then tested utilizing clinical microbiology laboratory procedures for final culture-based identification. This was followed by confirmatory 16S rRNA-based molecular identification with PCR, which was then sequenced and compared to NCBI database.

2.2. Amplification of 16S rRNA Gene

Pure cultures cultivated in LB medium were used to extract genomic DNA from isolates using the GeneAll® ExgeneTM Cell SV micro kit (Songpa-gu, Seoul, Korea). The bacterial cells were grown to log phase at 37°C for 24 hours. The bacterial cells were employed immediately to harvest genomic DNA. A NanoDrop spectrophotometer was used to measure concentrations and purities. DNA samples having A260/A280 ratios between 1.8 and 2.0 and A260/A230 ratios more than 2.0 were deemed suitable for PCR amplification. A thermal cycler (AlphaMAX, UK) was used to amplify partial 16S rRNA gene sequences, using a forward primer: 5′-CACCTTCCGATACGGCTACC-3′ and a reverse primer: 5′-GTTGACTGCCGGTGACAAAC-3′ [10, 11]. The PCR mixture was made up of 20 µL of master mix (AMPLIQON, Denmark), 2.0 µL of each primer, 3.0 µL of extracted genomic DNA, and enough PCR-grade water to make a total of 40 µL. A total of 35 cycles of denaturation at 95°C for 30 seconds, annealing at 59°C for 45 seconds, extension at 72°C for 60 seconds, and a final elongation step at 72°C for 10 minutes comprised the PCR protocol. The initial denaturation lasted 5 minutes at 95°C. 1.5% agarose gel electrophoresis was used to identify the PCR products. An electrophoretic examination utilizing a 0.8% agarose gel was used to evaluate the quality of the extracted DNA. The 16S rRNA gene was predicted to have an amplicon size of 372 bp.

2.3. Sequencing and Alignment of Bacterial 16S rRNA Gene Amplicons

For every isolate, a single 16S rRNA fragment of the expected size was amplified. The Gel Purification Kit (Promega, USA) was used to sequence the gel-purified amplicons. The sequencing data collection program version 1.0.1 was used to gather the sequencing data. The 16S rRNA sequences were taxonomically identified using BLAST-N analysis (http://www.ncbi.nlm.nih.gov/BLAST/). As the sequences directly matched to the GenBank database, each bacterial isolate was assigned to a

unique Operational Taxonomic Unit (OTU). With the aid of Finch TV software, chromatograms of the 16S rRNA gene sequences were modified and base calls confirmed.

2.4. Antimicrobial Susceptibility Testing

The disc diffusion technique (Kirby-Bauer) was used to test the antimicrobial susceptibility to 12 antibiotics on Mueller-Hinton agar (MHA) plates in accordance with the recommendations set out by the Clinical and Laboratory Standards Institute (CLSI) [12]. Amoxicillin-Clavulanic acid (20/10 µg), Ceftriaxone (30 µg), Ciprofloxacin (5 µg), Doxycycline (30 µg), Imipenem (10 µg), Levofloxacin (5 µg), Meropenem (10 µg), Nalidixic acid (30 µg), Ticarcillin/Clavulanic acid (75/10 µg), Piperacillin (100 µg), Netilmicin (30 µg), and Tigecycline (15 µg) were the tested antimicrobial agents.

2.5. Detection of Extended-Spectrum Beta-Lactamase (ESBL) Production by Phenotypic Method

Using a modified phenotypic approach in accordance with CLSI recommendations, ESBL production was identified. Mueller-Hinton agar was cultivated with a standardized inoculum of the test bacteria. A disk containing 20/10 µg of amoxicillin and clavulanic acid was put in the center of a cultivated MHA plate. Discs containing 30 µg of ceftazidime, 30 µg of ceftriaxone, 10 µg of imipenem, and 30 µg of aztreonam were positioned 15-20 mm from the center disc. The plates were incubated at 35 ± 2°C for 18 to 24 hours. A ≥5 mm increase in zone diameter for the clavulanic acid combination as opposed to the cephalosporin alone, or a synergistic enhancement (keyhole effect) between the amoxicillin-clavulanic acid disc and surrounding antibiotic discs, were indicators of ESBL formation [13].

2.6. Detection of ESBL Genotypes by Multiplex PCR Amplification

ESBL-positive isolates were subjected to multiplex PCR to check for the presence of the *bla_SHV*, *bla_CTX-M*, and *bla_TEM* genes as shown in (Table 1). Utilizing the PrestoTM Mini gDNA bacterial kit, template DNA was generated from recently cultivated isolates. The PCR mixture contained a 2 µL DNA template (10 ng/µL), a Master Mix that included 0.2% Tween® and 3 mM $MgCl_2$, 20 mM Tris-HCl pH 8.5, 0.4 mM of each dNTP, 0.4 µM of each primer (Table 1), 0.2 units/µL Ampliqon Taq DNA polymerase, and $(NH_4)_2SO_4$. The PCR reaction was set at initial denaturation at 95°C for 10 minutes, 30 cycles of denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 2 minutes, and final extension at 72°C for 10 minutes. Agarose gel electrophoresis was used to identify the PCR amplicons (genes) [14].

Table 1. Primers Used for Multiplex PCR Amplification of Some EBLs Genes.

Target Gene	Primer	Sequence (5'-3')	Amplicon Size	References
bla_TEM	Forward	TCG CCG CAT ACA CTA TTC TCA GAA TGA	445 bp	[15]
	Reverse	ACG CTC ACC GGC TCC AGA TTT AT		
bla_SHV	Forward	ATG CGT TAT ATT CGC CTG TG	747 bp	[16]
	Reverse	TGC TTT GTT ATT CGG GCC AA		
blaCTX-M	Forward	ATG TGC AGY ACC AGT AAR GTK ATG GC*	593 bp	[15]
	Reverse	TGG GTR AAR TAR GTS ACC AGA AYC AGC GG*		

*Y = C/T; R = A/G; K = G/T (degenerate nucleotides for broad-spectrum detection)

2.7. Ethics and Study Population

This study was approved by the Human Research Ethics Committee of the University of Salahaddin-Erbil (approval number: SU2025HREC/36). All participants provided written informed consent prior to sample collection. Patient data was anonymized and coded to ensure confidentiality. Fifty stool samples were collected from breast cancer patients at Nanakali Hospital, Erbil, Kurdistan region-Iraq, between September and December 2024. Patients aged 33-70 years were included.

3. Results and Discussion:

3.1. Prevalence of Bacteria in Breast Cancer Patients

In the current study, a total of 50 breast cancer patients who were admitted to Nanakali Hospital in Erbil, Iraq were examined for intestinal bacteria. Out of the 50 BC patients' stool, 8 genera and 14 species were obtained and the most common isolated bacteria in current study was *Escherichia coli* (n = 21, 42%), followed by *Salmonella enterica* (n = 6, 12%), *Cronobacter sakazakii* (n = 4, 8%), *Klebsiella pneumoniae* (n = 4, 8%), *Salmonella* spp. (n = 3, 6%), *Klebsiella oxytoca* (n = 2, 4%), *Pseudomonas* sp. (n = 2, 4%), *Shigella* spp. (n = 2, 4%), *Citrobacter freundii* (n = 1, 2%), *Enterobacter cloacae* (n = 1, 2%), *Enterobacter* sp. (n = 1, 2%), *Escherichia albertii* (n = 1, 2%), *Escherichia* spp. (n = 1, 2%), and *Pseudomonas baetica* (n = 1, 2%) as shown in Figure 1.

The present study identified *E. coli* as the predominant bacterial isolate (42%), which aligns with global surveillance data indicating *E. coli* as the leading cause of Gram-negative bacterial infections worldwide [17]. This finding is consistent with recent epidemiological studies from both developed and developing countries, where *E. coli* consistently represents 35-45% of clinical isolates [18]. The high prevalence of *E. coli* can be attributed to its versatility as both a commensal organism and opportunistic pathogen

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coupled with its ability to acquire and maintain resistance determinants [19].

The substantial presence of Salmonella species (18%) reflects the continued clinical importance of these pathogens, particularly in gastrointestinal infections and systemic disease [20]. The detection of *C. sakazakii* (8%) is particularly noteworthy given its association with severe neonatal infections and its emerging role as a nosocomial pathogen [21]. The distribution pattern observed in current study mirrors other reports from clinical laboratories, where Enterobacteriaceae family members continue to dominate antimicrobial resistance surveillance programs [22].

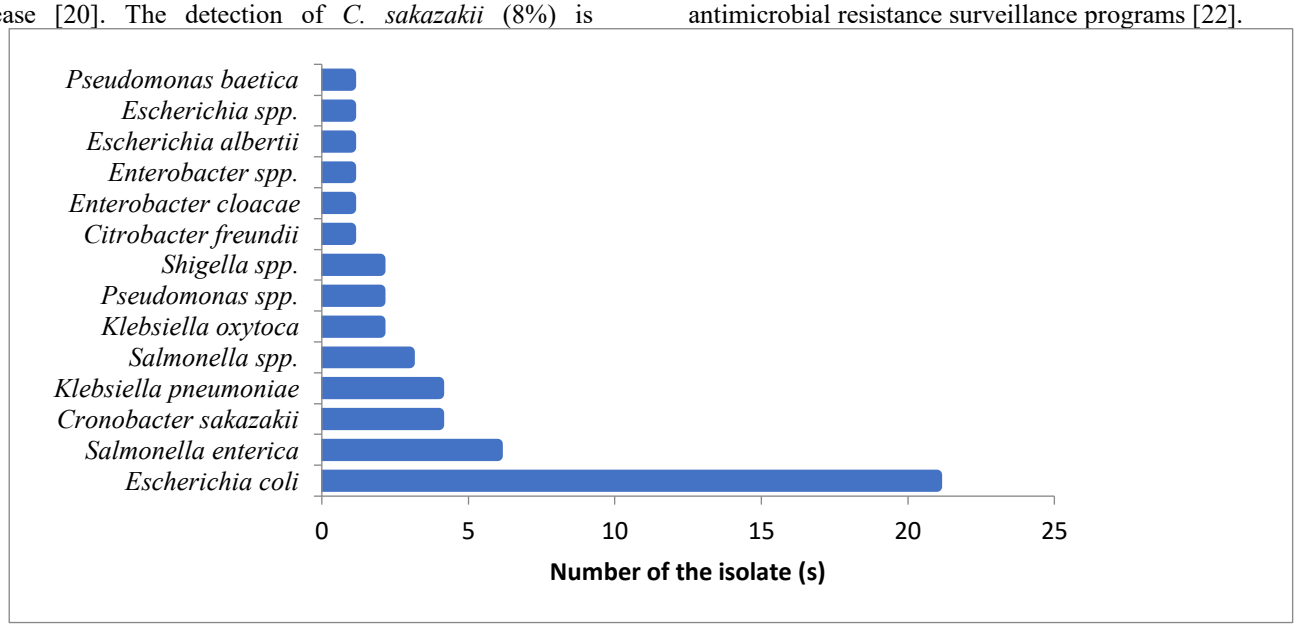

Figure 1: Number of isolated bacteria from breast cancer patient stool

3.2. Antimicrobial Susceptibility Patterns

The antimicrobial susceptibility testing revealed variable resistance patterns for all bacterial isolates against 12 different antimicrobial agents as shown in Table 2. The results showed that all isolated bacteria (100%) were sensitive to imipenem (IPM), meropenem (MEM), and netilmicin (NET). Conversely, complete resistance was observed for ticarcillin-clavulanic acid (TCC) and tigecycline (TI). Among the fluoroquinolones, levofloxacin showed the highest activity with 95.2% susceptibility in *E. coli* isolates, while ciprofloxacin demonstrated good effectiveness ranging from 75-100% across different species. Notably, *E. coli* showed moderate resistance to ciprofloxacin (19% resistance), whereas *Pseudomonas* species and other isolates maintained 100% susceptibility.

The complete resistance to ticarcillin-clavulanic acid and tigecycline across all isolates represents a concerning trend that limits therapeutic options for clinicians. While tigecycline resistance has been increasingly reported in Enterobacteriaceae, particularly among *E. coli* and *Klebsiella* species, the universal resistance observed in this study exceeds rates reported in most surveillance programs [26]. This finding may reflect local prescribing patterns or specific resistance mechanisms prevalent in the study population.

The variable fluoroquinolone susceptibility, with levofloxacin showing superior activity (95.2% susceptibility in *E. coli*) compared to ciprofloxacin, aligns with pharmacokinetic and pharmacodynamic studies demonstrating enhanced efficacy of newer fluoroquinolones [27]. The moderate ciprofloxacin resistance in *E. coli* (19%) falls within the range reported by global surveillance networks, though it represents a clinical concern given the widespread use of fluoroquinolones in empirical therapy [28].

The complete susceptibility to carbapenems (imipenem and meropenem) observed in present study provides reassurance regarding the continued efficacy of these last-resort antibiotics against ESBL-producing organisms [23]. This finding is crucial for clinical decision-making, as carbapenems remain the treatment of choice for serious infections caused by ESBL-producing bacteria [24]. However, the emergence of carbapenem-resistant Enterobacteriaceae in global surveillance research necessitates continued monitoring of their efficacy [25].

Table 2. Percentage of antibiotic resistance patterns of all isolated bacteria.

Antibiotic	Symbol	*E. coli* (n=21)	*K. pneumoniae* (n=4)	*Salmonella* spp. (n=9)	*C. sakazakii* (n=4)	*K. oxytoca* (n=2)	*Pseudomonas* spp. (n=3)	Others (n=7)
Amoxicillin-Clavulanic acid	AMC	76.2	100	77.8	100	100.0	66.7	71.4
Imipenem	IPM	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Meropenem	MEM	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ciprofloxacin	CIP	19.0	25	11.1	25.0	0.0	0.0	0.0
Doxycycline	DOX	33.3	25	44.4	25.0	0.0	0.0	14.3
Ceftriaxone	CTR	38.1	25.0	44.4	25.0	0.0	0.0	14.3
Levofloxacin	LE	4.8	0.0	22.2	0.0	0.0	0.0	0.0
Nalidixic acid	NA	33.3	25	33.3	50.0	0.0	66.7	28.6
Ticarcillin-Clavulanic acid	TCC	100	100	100	100	100	100	100
Piperacillin	PI	95.2	100	88.9	75.0	100	100	71.4
Netilmicin	NET	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tigecycline	TI	100	100	100	100	100.0	100	100

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3.3. ESBL Production and Screening

Extended-spectrum β-lactamase production was assessed using phenotypic screening methods. Of the 50 isolates tested, 28 isolates were positive for ESBL production, representing an overall prevalence of 56% . *Citrobacter freundii*, *Enterobacter cloacae*, *Escherichia albertii*, *Pseudomonas baetica*, and *Shigella* sp. showed 100% ESBL positivity rates, indicating that all single isolates from these species were ESBL producers. Among the isolated bacterial species, *K. pneumoniae* demonstrated the highest ESBL production rate of 75% (3/4 isolates), followed by *Salmonella enterica* 66.6% (4/6 isolates), and *Cronobacter sakazakii* 50% (2/4 isolates). *E. coli*, despite being the most abundant species, showed a moderate ESBL production rate of 47.61% (10/21 isolates) as shown in Figure 2

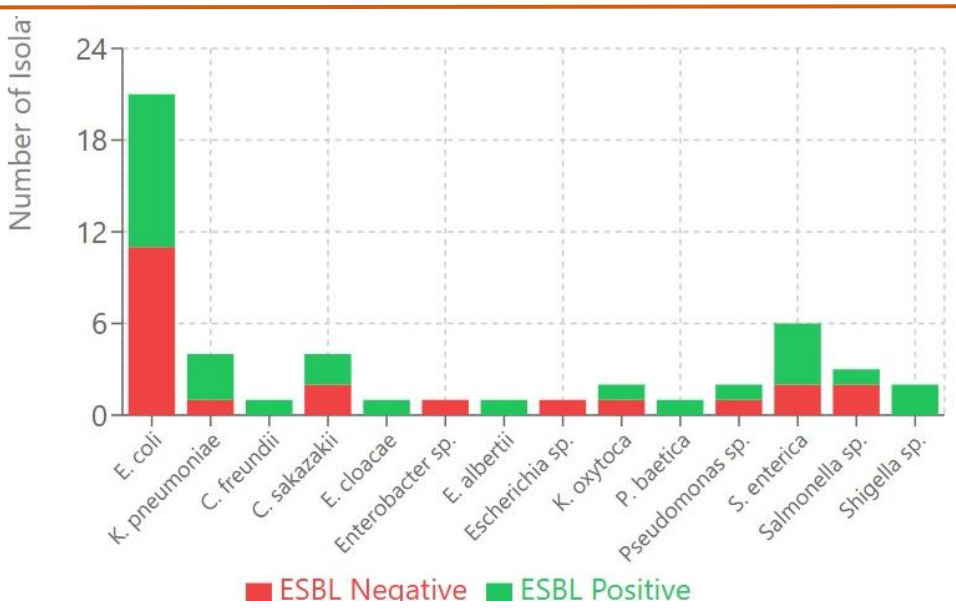

Figure 2: ESBL producer and non-producer isolates

The ESBL production rate observed in this study is similar to a study conducted by Sintondji et al. [29], while much higher than typical global studies reported at 25-40% [30]. This high rate may be due to local spread of resistant bacteria or weakened immune system as a result of chemotherapy may make the breast cancer patients more liable to carry resistant microorganisms. The high rate (75%) of ESBL production by *K. pneumoniae* is consistent with the worldwide detection of the species as the major carrier of ESBL genes in hospital wards [31]. Moreover, ESBL is widely spread in Gram-negative bacteria particularly in *K. pneumoniae* and *E. coli*. Studies with similar results support these findings. *S. enterica* showed 66.6% ESBL production, which is concerning as Salmonella can spread through food, these results are similar to results obtained by Ahmad and Mustafa [32].

3.4. Molecular Characterization of ESBL Genes

Multiplex PCR technique was used to detect the presence of three major ESBL genes: blaTEM (445bp), blaSHV (747bp), and blaCTX-M (593bp) in all the 50 isolates. The molecular analysis revealed that all isolates harbored at least one ESBL gene and multiple gene combinations were detected in some of the isolates (Figure 3).

The blaTEM gene showed the highest prevalence as a single genotype detected in 8 isolates of *E. coli* (38%), all 4 (100%) isolates of *K. pneumoniae*, 3 isolates of *C. sakazakii*, 2 isolates of *Salmonella* sp., 1 isolate of *Shigella* sp., and other isolates from various species. Notably, neither blaSHV nor blaCTX-M genes were detected as single genotypes in any of the tested isolates.

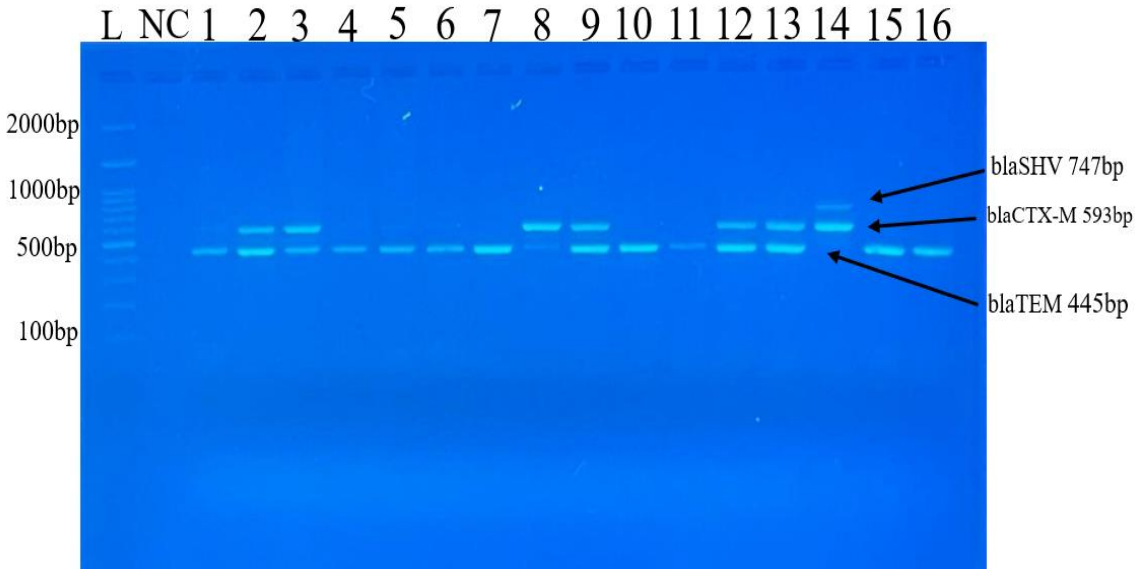

Figure 3. PCR product of *bla*SHV, *bla*CTX-M and *bla*TEM Lane L: ladder (100 bp). Lane NC: Negative control. Lanes 1-16: Positive samples of studied bacteria.

The most prevalent combination was blaTEM + blaCTX-M, found in 10 isolates of *E. coli* (47.61%), 1 isolate each of *C. sakazakii* and *K. oxytoca*, 3 isolates of *Salmonella enterica*, 4 isolates of *Salmonella* spp., 2 isolates of *Shigella*, and 1 isolate from other categories. The blaTEM + blaSHV combination was detected in 2 isolates of *E. coli* (9.52%) and in 1 isolate of *K. oxytoca* (Figure 4).

The most important finding was that all 50 bacteria carried at least one of the three ESBL genes, even though only 56% actually produced ESBL enzymes. This 100% gene presence is unusual since most studies find 15-25% of bacteria lack these genes [33]. This could mean that the detection methods were very sensitive, or there was widespread gene sharing in this hospital.

The *bla*TEM gene was the most common single gene type, found in many different bacteria species. This gene family spreads easily between bacteria [34]. All *K. pneumoniae* bacteria carried this gene, suggesting it spread between related bacterial strains.

The combination of *bla*TEM + *bla*CTX-M genes was found in 47.61% of *E. coli* bacteria. This combination is important because CTX-M genes are now the most common ESBL type worldwide, with *bla*CTX-M-15 being particularly widespread [35]. When bacteria have both genes, they become resistant to almost all penicillin and cephalosporin antibiotics. Some bacteria (4.76% of *E. coli*) carried three different ESBL genes together. The bacteria with multiple genes usually resist more antibiotics and can pass genes to other bacteria more easily [34]. Another study revealed that 81% of the ESBL-producing *E. coli* strains that were gathered had blaTEM, 16.2% had blaSHV, and 32.4% had blaCTX-M genes. Similarly, the isolates of *K. pneumoniae* had 41.1% blaCTX-M, 35.2% blaSHV, and 64.7% blaTEM genes [36].

The detection of same gene combinations in different bacteria, including *E. coli*, *Salmonella* and *Shigella* may suggest gene exchange among the species. This makes controlling antibiotic resistance much harder because genes can spread throughout the entire bacterial community, not just within single species [37].

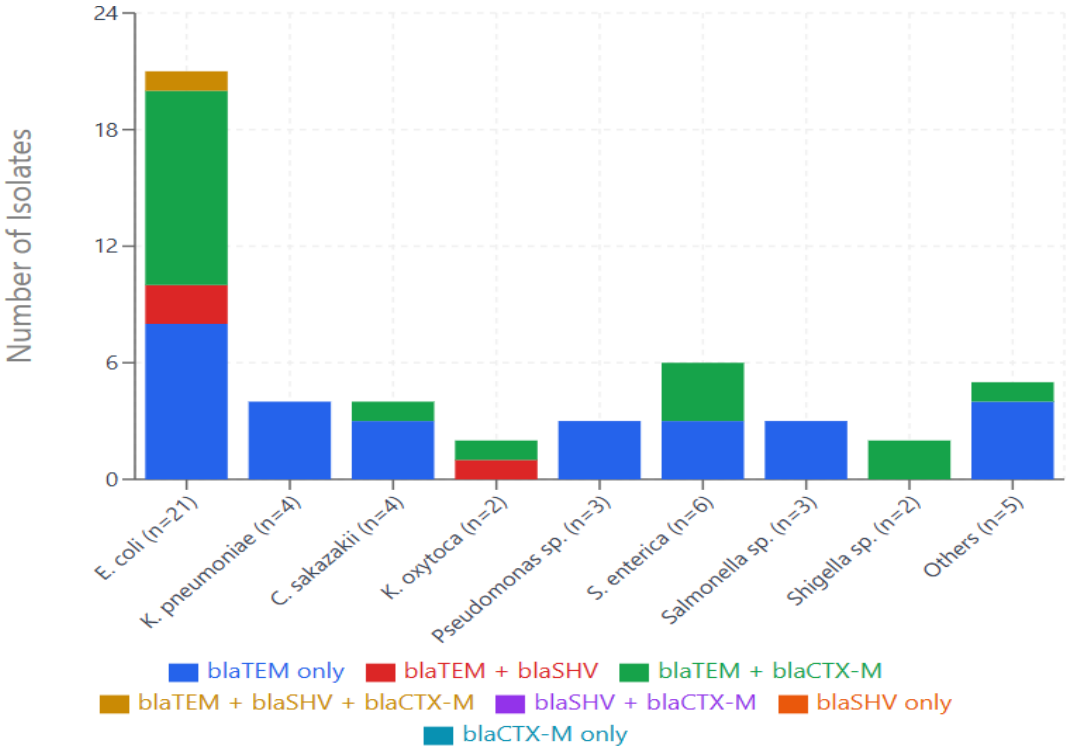

Figure 4. Distribution of ESBL genes among bacterial isolates detected by multiplex PCR

According to results of present study most of the isolated bacteria were drug resistant bacteria belonging to family Enterobacteriaceae and these may be related to several factors. The breast cancer patients after using chemotherapy and radiotherapy become immunosuppressed and also disrupt the balance of normal gut microbiota allowing resistant bacteria such as *E. coli* to dominate and this makes breast cancer patients more sensitive to bacterial infections. Also, the intensive and prolonged use of different and strong antibiotics often prescribed as prophylactic or therapeutic agents against other secondary infections, increases the likelihood of selecting resistant bacterial strains. Moreover, prolonged hospital stays and invasive medical procedures such as the use of intravenous catheters, raise the risk of

acquiring hospital-associated resistant strains, including ESBL producing bacteria.

4. Conclusion

This study demonstrates high antimicrobial resistance burden among clinical bacterial isolates from breast cancer patients, with high ESBL prevalence and concerning resistance patterns to commonly used antibiotics. The universal presence of ESBL genes and strong correlation between genotype and phenotype provide important insights for clinical management and infection control strategies. The maintained carbapenem susceptibility offers therapeutic hope. Indeed, the presence of chronic inflammation related to cancer or its treatment creates a favorable environment for the proliferation of resistant bacteria. These findings underscore the importance of continued surveillance and the development of novel therapeutic strategies to address the growing challenge of antimicrobial resistance.

4.1. Contributorship Statement

Conceptualization: Y.O.A., K.K.M., Data Collection: Y.O.A., Data Analysis: Y.O.A., K.K.M., Writing—Original Draft: Y.O.A., Writing—Review and Editing: Y.O.A., K.K.M., Supervision: K.K.M.

4.2. Conflict of Interest

None

4.3. Acknowledgements

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