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Molecular Characterization of *Staphylococcus aureus* in Street Vended Meat and Its Health Implication in Gwagwalada Market and Its Environs

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Abstract

The possible health hazard associated with ready-to-eat (RTE) meat sold in Gwagwalada Abuja and its environs was evaluated by determining the prevalence of *Staphylococcus* species and investigating the toxigenic potential of *Staphylococcus* and antibiotics profiles of these zoonotic bacterial isolates. A total of 100 RTE meat samples were purchased based on the availability of the ready-to-eat (RTE) meat vended in the markets. Purposive sampling technique was used to select the four meat sampling spots. The samples collected were transported to the laboratory for microbiological analysis. The samples were pre-enriched in peptone water at 37˚C for 24 hours before being streaked on mannitol salt agar plates and incubated for 24 hours. The staphylococcal isolates were identified at the species level by sequencing the *sod*A and 16S rDNA genes. The genes coding for *TSST*-1 (*tsst*), *ETA* (*eta*), *ETB* (*etb*), *and ETD* (*etd*), *SEA* (*sea*), *SEB* (*seb*) were examined using PCR. Phenotypic determination of resistance to 17 antimicrobial agents was carried out using the disc diffusion method, while genes coding for resistance to erythromycin (*erm*A, *erm*B), tetracyclines *tet* (M), *tet* (K), and *ESBL* (*TEM*, *SHV*) genes were determined by PCR amplification using their primers. Twenty-four (48%) of the RTE meat samples contained *Staphylococci aureus*. Eighteen (75%), Ten (41.7%) and Two (8.3%) of the identified *Staphylococcus* species harbored the highest virulence gene staphylococcal enterotoxins (SEs) *seb*, *sea*, *and sec* respectively, *tsst*-1 (25%), *eta* (29%), *etb* (0%), *and etd* (12.5%).

Resistance genes detected in the *Staphylococcus* species were: *tet*M (20.8%), *tet*K (8.3%), *erm*B (29%), *erm*A (54.2%), *bla*TEM (29%) and *bla*SHV (8.3%). Fort-eight per cent of the RTE meat vended in the study area was contaminated with *Staphylococci* and is of public health importance.

Keywords

Ready-to-Eat Food, Toxigenic Potential, Resistant, Pathogenic Strains

1. Introduction

Vended ready-to-eat (RTE) Meat is particularly appealing to the population looking for easy meals since they give easily healthy proteins and nutrients are accessible. Thus, RTE food production is increasing because of changes in the way of living and heightened customer demand for convenience foods. Understanding and reducing the risks to public health from foodborne illnesses requires research on *Staphylococcus aureus* (*S. aureus*) in street food. Studying the presence and traits of *S. aureus* in street food aids in identifying the sources and degree of contamination. *S. aureus* is a common foodborne pathogen that can cause serious illnesses and devastation to society [1]. Meat borne zoonoses of bacterial origin especially of *Staphylococcus* species in meat and meat products are ubiquitous in distribution and cause most cases of food poisoning [1]. Meat borne zoonoses have been shown to constitute about 90% of all foodborne outbreaks in the world [2]. The World Health Organization defines foodborne diseases (FBD) as infectious or toxic disorders that can be transmitted by food or water. More than 250 FBDs have been documented. Bacteria are the primary cause of FBD in most countries, accounting for almost two-thirds of all known outbreaks. Bacteria that cause foodborne illness create toxins through consumption or intoxication. Improving food safety in Nigeria is a top issue. According to Havelaar *et al.* [3], foodborne disease causes an estimated 600 million illnesses and 420,000 premature deaths each year. Rural residents bear most of this burden, accounting for around 75% of foodborne illness deaths (compared to 41% of the global population). This is particularly true in Africa, where the health system lacks the ability to diagnose and treat foodborne illnesses [4] and the per-capita burden of foodborne illness is over 27 times higher than in Europe or North America (3). Foodborne infections also have an economic burden of approximately $20 billion USD per year [5].

In Nigeria, public health is heavily focused on food safety. Food safety is the guarantee that, when prepared and/or consumed as intended, food will not injure the consumer [6]. Developing successful interventions to stop and manage outbreaks requires this knowledge. RTE meals are defined as food intended by the producer or manufacturer for direct human consumption without the need for cooking or other processing effectively to eliminate or reduce to an acceptable level micro-organism of concern. RTE meals made and/or sold by street vendors

are recognized as possible carriers of microbiological foodborne diseases if they are handled improperly during processing and preparation. The variety of foods included in RTE meals has changed over the years, reflecting advancements in technology as well as shifts in cultural practices that have defined various eras [7]. Because they offer easy and healthy options, ready-to-eat (RTE) foods sold are especially alluring to customers looking for quick meals. In underdeveloped nations such as Nigeria, RTE vendors have been linked to low literacy rates, resulting in a lack of awareness about appropriate hygiene and food handling techniques [8]. Furthermore, some RTE foods are cooked, kept, and served in unsanitary settings, and vending is frequently done outside, exposing the foods to outdoor contaminants and biological hazards, all of which can be sources of food contamination [8]. The most significant health risk linked to street food is microbiological contamination, although other potential health risks have been identified as the use of illegal chemical additives, pesticide residues, parasite transmission, and environmental contamination [9]. These health issues are caused by a number of factors, including a lack of basic infrastructure and services (such as potable water supplies), a general lack of factual knowledge regarding the microbiological status of street foods, a lack of resources for laboratory analysis and inspection, a lack of knowledge among street vendors regarding basic food safety procedures, and a lack of public awareness regarding the risks posed by specific street foods [2]. Furthermore, because of their diversity, mobility, and transient nature, street food selling enterprises are too many to manage effectively [6]. The safety of street meals is a big problem, especially in crowded streets and public spaces, because food can become contaminated at any stage of the food chain, from origin to consumption [10]. Street vended meat is a common business in many parts of Nigeria. In major cities and small towns, ready-to-eat meats (RTE) are very popular street foods. From noon till late at night, customers visit the RTE vendors' booths. There is a significant chance of contamination because the products are prepared in a lot of unsanitary circumstances. Records of occasional gastroenteritis instances and food infection symptoms following the eating of unwholesome meat and its preparation demonstrated that the products do, in fact, pose a risk to food safety. Several foods borne zoonoses of meat origin have been documented today in the world. Some of these diseases reported include salmonellosis, *E. coli* gastroenteritis, campylobacteriosis [2].

One of the main causes of foodborne illness is *Staphylococcus aureus*. The absorption of *Staphylococcal* enterotoxins that are already present in the food causes *Staphylococcal* food poisoning. Dack and his colleagues originally investigated staphylococcal enterotoxins in 1930 [11]. By releasing enterotoxins into the food, *S. aureus* causes food poisoning. It can also cause toxic shock syndrome by releasing super antigens into the bloodstream [12]. In addition to being extremely heat resistant, *Staphylococcal* enterotoxins are believed to be more heat resistant in food than in a growth medium used in a laboratory [13]. Ingesting food containing heat-stable *Staphylococcal* enterotoxins (se) can result in *Staphylococcal* food poisoning, which manifests as a quick onset of nausea, vomiting, cramping in the

abdomen, and diarrhea [14]. The poisons remain active even after the bacteria are eliminated by heating to a standard cooking temperature [15]. Analysis of *S. aureus* strains implicated in *Staphylococcal* food poisoning served as the foundation for research on *Staphylococcal* enterotoxins (*se*). The peptide sequence of the first *Staphylococcal* enterotoxins discovered was accessible before the nucleotide sequence. According to Bergdoll and Robbins [16], the classic antigenic *Staphylococcal* enterotoxins are *sea*, *seb*, *sec*1, *sec*2, *sec*3, *sed*, and *see*. In addition, *S. aureus* that produces enterotoxins, is the most hazardous and detrimental to human health. According to Payne and Wood [17], around 50% of this organism's strains can produce enterotoxins linked to food poisoning. Antibiotic resistance, invasiveness, and toxins are the main causes of *S. aureus's* pathogenicity. One of the main causes of nosocomial and community-acquired infections is *S. aureus*. It appears as a normal human flora and colonize the skin, but it can turn pathogenic and cause anything from minor skin infections and abscesses to potentially fatal conditions like septicemia, mastitis, phlebitis, urinary tract infections, osteomyelitis, pneumonia, meningitis, and endocarditis [2].

The public health challenges created by the increasing rate of antimicrobial resistance reported in meat associated with bacteria is also a source of concern. The development and spread of AMR are ascribed to several factors, such as the overuse and abuse of antimicrobial medications, inadequate sanitation, substandard disease prevention and control practices, a lack of knowledge, a lack of public awareness, a lack of legislation, and a lack of innovation and development in drug resources [6]. But, according to Marshall and Levy [18], the main factor contributing to genesis, dissemination, or accelerated development of AMR is the abuse or misuse of antimicrobial medications. One of the main causes of the AMR issue has been identified as the use of antibiotics in food animal production [2] [19]. The resistance of these organisms to antimicrobials agents is becoming a worldwide issue, making effective chemotherapy difficult to attain when there are infections.

2. Materials and Methods

2.1. Study Area

The study was carried out in Gwagwalada Abuja FCT; Abuja is the capital city of Nigeria located in the centre of the country within the Federal Capital Territory (FCT). It is a planned city and was built mainly in the 1980s, replacing the country's most populous city of Lagos as the capital on 12 December 1991. In the Federal Capital Territory, Gwagwalada town is the third-largest urban center and one of the biggest satellite towns [20]. It is home to one of the oldest councils in the Federal Capital Territory (FCT), Abuja, and is among the most densely populated districts of the FCT [20]. Within the Federal Capital Territory, Gwagwalada town is roughly 55 kilometers from the Federal Capital City (FCC). According to Balogun [21], it is located between latitudes 08055'N and 09000'N and longitudes 07000'E and 07005'E (**Figure 1**).

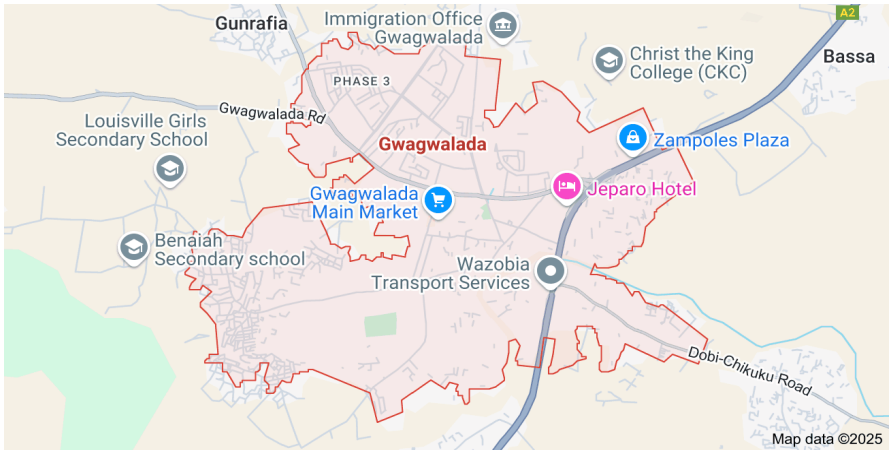


Figure 1. Map of Gwagwalada Town indicating the location of sample collection.

2.2. Study Design

A cross-sectional sampling technique was employed to collect samples of street-sold meat from several locations inside the Gwagwalada market and the neighboring districts. The samples were collected both in the morning and evenings over a specified period of Five months to ensure a representative image of the microbiological quality of the meat being sold. A stratified random selection strategy was employed to ensure the inclusion of a variety of meat varieties (e.g., beef, goat, and chicken) and vendor types (e.g., roadside vendors, mobile vendors, and fixed stalls). From each of the market's geographically distinct zones or clusters, vendors were selected at random. A total of 100 RTE meat samples were purchased, and each sample was transported to the Peak Medical laboratory Gwagwalada in a separate sterile bag inside a cooler in a cold chain process to avoid degradation and other changes in microbial contamination. The samples consisted of roasted beef (suya), boiled chicken and boiled goat meat. The specific locations where the samples were collected are Gwagwalada Motor Park (GMP), Gwagwalada Main Market (GMM) and Gwagwalada Cafeteria and Restaurants (GCR). The survey was conducted over a period of five months (Table 1). *Staphylococcus aureus* was isolated, identified, and molecularly characterized in the Iquaba Molecular laboratory south Africa. PCR and conventional microbiological methods were used to identify virulence genes and antibiotic resistance profiles. The geographical limitation is that since the study was limited to Gwagwalada market and its environs, the findings might not be generalizable to other markets in Nigeria or other regions with different environmental and economic conditions.

2.3. Isolation and Identification of *Staphylococcus*

This was carried out according to the method described by Cheesbrough [22]. A loopful of the pre-enriched sample was collected and streaked on salt agar plates and incubated at 37˚C for 24 hrs. The plates were observed for round, white/yellowish convex colonies. On each plate, a colony of the suspected *Staphylococcus* organism was further purified on plain nutrient agar plates by streaking and

Table 1. Distribution of sampling areas, type of RTE and number of meat samples collected in the study area.

Sampling areas	RTE meat types	Samples collected
Gwagwalada-motor park (GMP)	Roasted beef suya	10
	Boiled chicken	10
	Boiled goat	10
Gwagwalada cafeteria and restaurants (GCR)	Roasted beef suya	10
	Boiled chicken	10
	Boiled goat	10
Gwagwalada main market (GMM)	Roasted beef suya	15
	Boiled chicken	15
	Boiled goat	10
Total		100

incubating at 37˚C for 24 hrs. The suspected colonies that tended to be white/yellowish circular and convex colonies were Gram stained using crystal violet as the primary stain, lugols iodine as the mordant, acetone as the decolorizer and safaranin as the counter stain. Stained slides were examined using 100× oil immersion for Gram positive cocci in bunches. Presumptive *Staphylococcus* colonies were subjected to catalase test as described by Cheesbrough [23]. A drop of 3% H_2O_2 was placed on a glass slide, and a colony of the test organism was picked up and emulsified in the H_2O_2 drop. Observation was made for immediate vigorous bubbling, which is indicative of a positive catalase test. Confirmed that *Staphylococcus* species were stocked on nutrient agar slants supplemented with sodium chloride for subsequent analysis. Isolates were further screened using STAPH Latex Reagent (Liofilchem, Italy) according to the manufacturer's instruction. Presumptive *S. aureus* isolates that were stored on nutrient agar slants supplemented with sodium chloride were transported to Inquaba Laboratory, South Africa, for molecular characterization of isolates.

2.4. Characterization of *Staphylococci* from Ready-to-Eat Meat Samples DNA Extraction and PCR Amplifications

DNA was extracted using the protocol stated by [24] with minor modifications. Briefly, Single colonies grown on medium were transferred to 1.5 ml of liquid medium and cultures were grown on a shaker for 48 h at 28˚C. After this period, cultures were centrifuged at 4600 g for 5 min. The resulting pellets were resuspended in 520 µl of TE buffer (10 mMTris-HCl, 1 mM EDTA, pH 8.0). Fifteen microliters of 20% SDS and 3 µl of Proteinase K (20 mg/ml) were then added. The mixture was incubated for 1 hour at 37˚C, then 100 µl of 5 M NaCl and 80 µL of a 10% CTAB solution in 0.7 M NaCl were added and votexed. The suspension was incubated for 10 min at 65˚C and kept on ice for 15 min. An equal volume of

chloroform: isoamyl alcohol (24:1) was added, followed by incubation on ice for 5 min and centrifugation at 7200 g for 20 min. The aqueous phase was then transferred to a new tube and isopropanol (1:0.6) was added and DNA precipitated at –20˚C for 16 h. DNA was collected by centrifugation at 13,000 g for 10 min, washed with 500 µl of 70% ethanol, air-dried at room temperature for approximately three hours and finally dissolved in 50 µl of TE buffer. PCR amplification was performed on an Eppendorf thermocycler (Roche Co., Mannheim, Germany) by application of a set of primers of 5′-GCCAGTTGAGGACGTATTCT-3′ and 5′-CCATTTCAG TACCTTCTGGTAA-3′, which can amplify a 412 bp fragment of *tuf* gene (30) as previously described by Hendolin [25]. A polymerase activation step (95˚C for 15 min) was followed by 35 cycles of denaturation (95˚C for 1 min, 30 s), annealing (55˚C for 1 min), and extension (72˚C for 1 min), with a final extension step (72˚C for 7 min). The PCR reaction mixture was analyzed by electrophoresis through a 2% high-resolution agarose gel (Promega, Madison, WI) in 1 × Tris-borate-EDTA buffer (pH 8.3). The sizes of the amplification products were estimated by comparison with a 100-bp molecular size ladder (Thermo Scientific Fermentas). Gels were stained with ethidium bromide and viewed under UV light using a transilluminator (BXT-26.M, Uvitec, Cambridge, UK). Each profile was visually compared with those obtained from the staphylococcal reference strains (these include positive controls (samples known to amplify), negative controls (no template or a blank sample from the laboratory), and extraction controls (checking the efficiency of DNA/RNA extraction)). Identification of the staphylococcal isolates at the species level was done by sequencing the *sod*A and 16*S tuf* genes (Table 2).

2.5. Antimicrobial Susceptibility Testing

The resistance profile of the *Staphylococcus* isolates was evaluated by disk diffusion method according to Clinical and Laboratory Standards Institute [26] guidelines [27] using following antibiotic disks: Ciprofloxacin (5 ug), Vancomycin (30 ug), Fusidic acid (10 ug), Clindamycin (10 ug), Streptomycin (30 ug), Tetracycline (30 ug), Sulphamethoxazole \trimethoprim (25 ug), Erythromycin (30 ug), Mupirocin (5 ug), Gentamicin (10 ug), Chloramphenicol (10 ug), Cefoxitin (30 ug), Cefotaxime (10 ug), Oxacillin (10 ug), Teicoplanin, Levofloxacin (10 ug) and Linezolid (10 ug). A single colony of each of the test organisms was picked with a sterile wire loop and inoculated into 3 ml of sterile nutrient broth. The inoculated nutrient broth was incubated at 37˚C for 1 hr. The broth culture was poured aseptically into the iso-sensitest agar. The excess broth was drained into a discarded pot containing isol^{R} disinfectant. The inoculated plates were then incubated at 37˚C for 30 minutes to enable the surface to dry.

2.6. Molecular Analysis of Antibiotics and Virulent Genes

Molecular investigations of virulence an antibiotic resistance gene was by simple PCR on the extracted DNA using gene specific primers. Primer sequences were

Table 2. The primer sequence and PCR profile used in amplifying each fragment

Gene	Primer name	Primer sequence	References
Staph *tuf* gene	TStaG422	5′-GGC CGT GTT GAA CGT GGT CAA ATC A-3′	[28]
	TStag765	5′-TIA CCA TTT CAG TAC CTT CTG GTA A-3	[24]
Soda	*sod*AF	CCITAYICITAYGAYGCIYTIGARCC	[24]
	*sod*AR	ARRTARTAIGCRTGYTCCCAIACRTC	
Tsst	*Tsst*-F	TGCAAAAGCATCTACAAACGA	[29]
	Tsst-R	TGTGGATCCGTCATTCATTG	
Eta	*eta*F	ACTGTAGGAGCTAGTGCATTTGT	[29]
	*eta*R	TGGATACTTTTGTCTATCTTTTTCATCAAC	
Etb	*Etb*-F	CAGATAAAGAGCTTTATACACACATTAC	[29]
	Etb-R	AGTGAACTTATCTTTCTATTGAAAAACACTC	
Etd	*Etd*-F	CGCAAATACATATGAAGAATCTGA	[29]
	Etd-R	TGTCACCTTGTTGCAAATCTATAG	
Sea	GSEAR-1	GGTTATCAATGTGCGGGTGG	[30]
	GSEAR-2	CGGCACTTTTTTCTCTTCGG	
Seb	GSEBF-1	GTATGGTGGTGTAACTGAGC	[30]
	GSEBR-1	CCAAATAGTGACGAGTTAGG	
Sec	GSECR-1	AGATGAAGTAGTTGATGTGTATGG	[30]
	GSEBR-2	CACACTTTTAGAATCAACCG	
*erm*B	*erm*BF	CCGAACACTAGGGTTGCTC	[30]
	*erm*BR	ATCTGGAACATCTGTGGTATG	
*erm*A	*erm*AF	TAACATCAGTACGGATATTG	[31]
	ermAR	AGTCTACACTTGGCTTAGG	
*tet*K	*tet*K-1	TTAGGTGAAGGGTTAGGTCC	[32]
	*tet*K-2	GCAAACTCATTCCAGAAGCA	
*tet*M	*tet*M-1	GTCCGTCTGAACTTTGCGGA	[32]
	*tet*M-2	GCGGCACTTCGATGTGAATG	
*bla*TEM	*Tem*F	GTCGCCGCATACACTATTCTCA	[33]
	*Tem*R	CGCTCGTCGTTTGGTATGG	
*bla*SHV	*shv*F	GCCTTGACCGCTGGGAAAC	[34]
	*shv*R	GGCGTATCCCGCAGATA	

as earlier documented by [35]. Reaction cocktail used for all PCR per primer set included (Reagent Volume µl)-5× PCR SYBR green buffer (2.5), $MgCl_2$ (0.75), 10 pM DNTP (0.25), 10 pM of each forward and backwards primer (0.25), 8000 U of taq DNA polymerase (0.06) and made up to 10.5 with sterile distilled water to

which 2 µl template was added. Buffer control was also added to eliminate any probability of false amplification. Table 2 below shows the primer sequence and PCR profile used in amplifying each fragment. PCR was carried out in a GeneAmp 9700 PCR System Thermalcycler (Applied Biosystem Inc., USA) using the appropriate profile as designed for each primer pair.

2.7. Integrity

The integrity of the amplified gene fragment was checked on a 1.5% Agarose gel ran to confirm amplification. The buffer (1XTAE buffer) was prepared and subsequently used to prepare 1.5% agarose gel. The suspension was boiled in a microwave for 5 minutes. The molten agarose was allowed to cool to 60˚C and stained with 3 µl of 0.5 g/ml ethidium bromide (which absorbs invisible UV light and transmits the energy as visible orange light). A comb was inserted into the slots of the casting tray and the molten agarose was poured into the tray. The gel was allowed to solidify for 20 minutes to form the wells. The 1XTAE buffer was poured into the gel tank to barely submerge the gel. Two microliter (2 l) of 10× blue gel loading dye (which gives colour and density to the samples to make it easy to load into the wells and monitor the progress of the gel) was added to 4 µl of each PCR product and loaded into the wells after the 100 bp DNA ladder was loaded into well 1. The gel was electrophoresed at 120 V for 45 minutes visualized by ultraviolet trans-illumination and photographed. The sizes of the PCR products were estimated by comparison with the mobility of a 100 bp molecular weight ladder that was ran alongside experimental samples in the gel.

2.8. Data Presentation and Analysis

Descriptive statistics, such as percentages, frequency tables, and graphical representations, were used to methodically arrange and present the data gathered for this study to make trend interpretation and visualization easier. To investigate the connections between the variables, inferential statistical studies were performed. In particular, the statistical significance of the correlation between the kind and location of RTE meat product collection and the presence of the detected zoonotic bacteria was evaluated using the binomial chi-square test. Using chi-square testing, the relationship between the various kinds of RTE meat and the detected levels of antibiotic resistance in the bacterial isolates was also examined. A significance threshold of $p < 0.05$ was applied to all statistical analyses, which were performed at a 95% confidence level. For the statistical analysis, SPSS version 29.0.1.0 was used.

3. Result

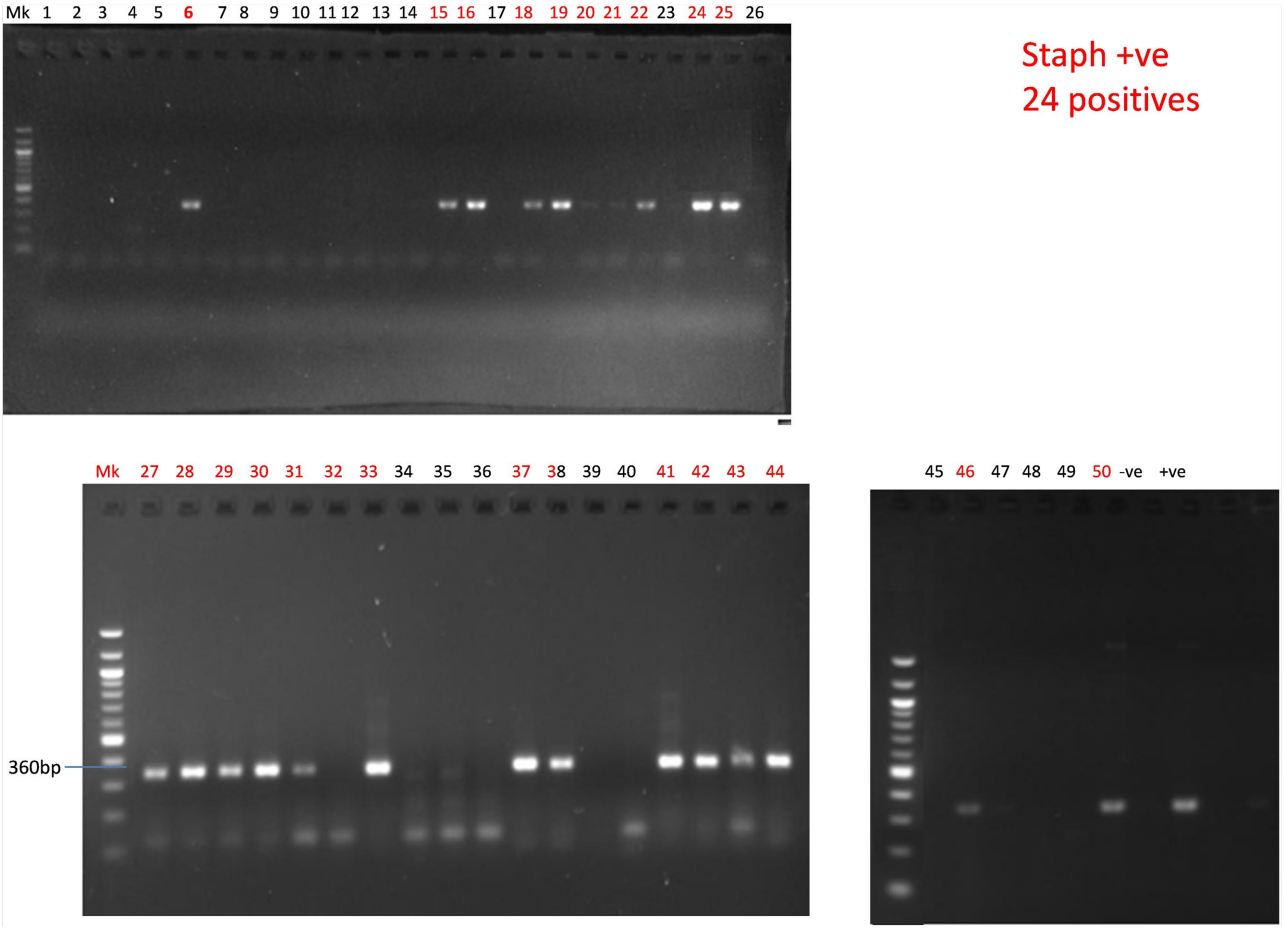
3.1. Prevalence of *Staphylococci* in Ready-to-Eat Meat in Gwagwalada and Its Environ

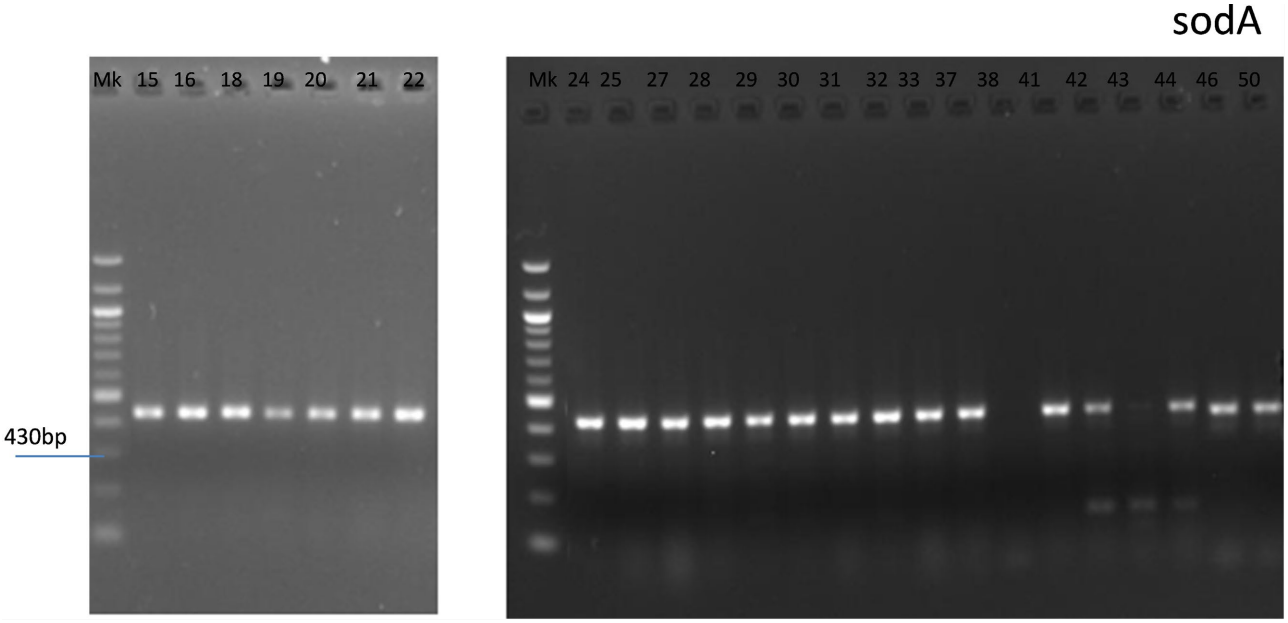
Out of the 100 samples processed, 53 (53%) grew on salt agar and produced round white/yellowish convex colonies. Out of the 53 presumptive *Staphylococcus* iso-

lates, 40 were confirmed as *Staphylococcus* biochemically. This gave an overall prevalence of 40% for *Staphylococcus*. The prevalence of *Staphylococcus* strain with respect to meat types was 54% for suya, 31% for roasted chicken meat and 33% for roasted goat meat (Table 3). There was no significant association ($P > 0.05$) between staphylococcal contamination and type of RTE meat (The chi-square statistics are 3.2. The *p*-value is .51). They simply mean that the study did not have sufficient evidence to reject the null hypothesis (*i.e.*, the hypothesis of no difference) this may be due to sample size limitations. Based on the sequencing result of *tuf* gene and *sod*A gene, 50 isolates were identified to species level, and 24 (48%) belonged to *S. aureus* (Figure 2).

Table 3. Prevalence of *Staphylococci* in ready-to-eat meat according to meat type in FCT.

RTE meat type	No analyzed	No (%) that grows on salt agar	No (%) confirmed as *Staphylococcus*
Suya	35	24 (69)	19 (54)
Chicken meat	35	16 (46)	11 (31)
Goat meat	30	13 (43)	10 (33)
Total	100	53 (53)	40 (40)





Note bands at the 360 bp and 430 bp, MK = molecular weight marker (100 bp ladder), tve: control, buf = buffer (−ve)

Figure 2. Gel pictures show positive amplification of *soda* and *tuf* genes of the isolates.

3.2. Distribution of *Staphylococcus* in RTE Meat according to Location of Samples

The highest percentage of *Staphylococcus* isolation was from the RTE meat samples obtained from Gwagwalada main market (45%), followed by Gwagwalada main park (30%), and Gwagwalada cafeteria and restaurants (25%) (**Table 4**). There was no significant ($p < 0.05$) association between meat contamination by *Staphylococcus* species and sample collection location.

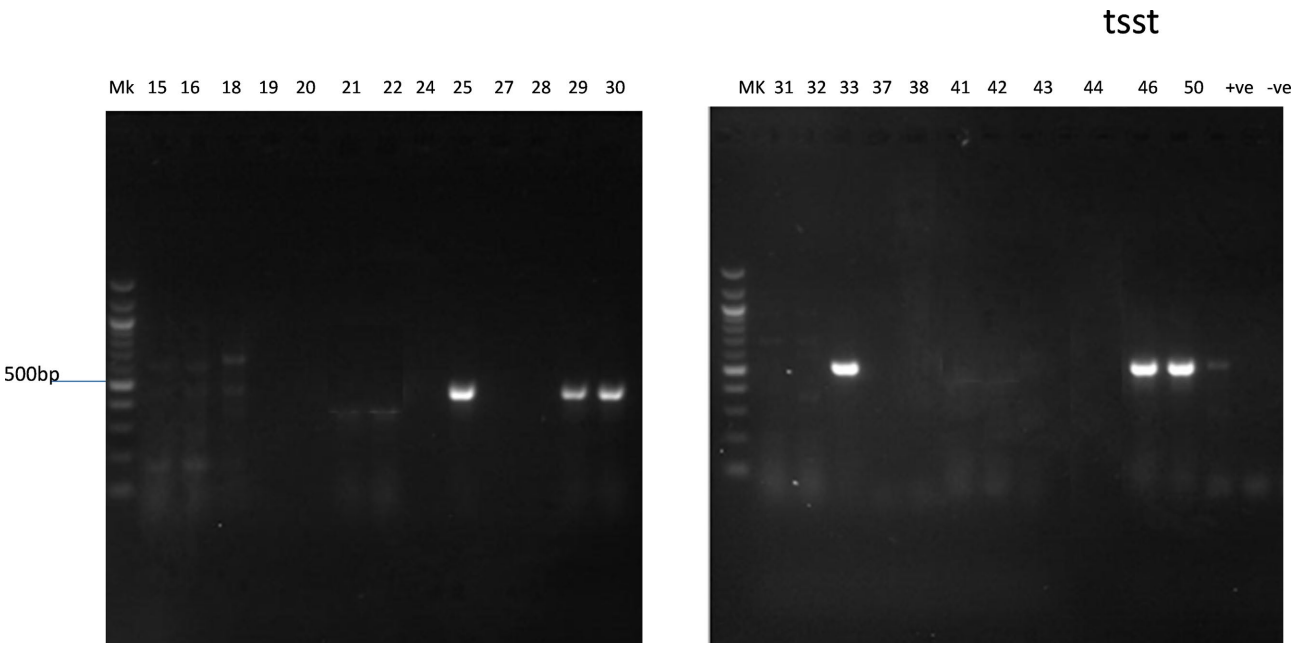
Table 4. Prevalence of *Staphylococci* in ready-to-eat meat according to location in Gwagwalada and its environs.

Location	No analyzed	No (%) confirmed as *Staphylococcus*
Gwagwalada main market (GMM)	40	18 (45)
Gwagwalada motor park (GMP)	30	12 (30)
Gwagwalada cafeteria and restaurants (GCR)	30	10 (25)
Total	100	40 (40)

3.3. Toxigenic Potential of *Staphylococci* Isolated from RTE Meat

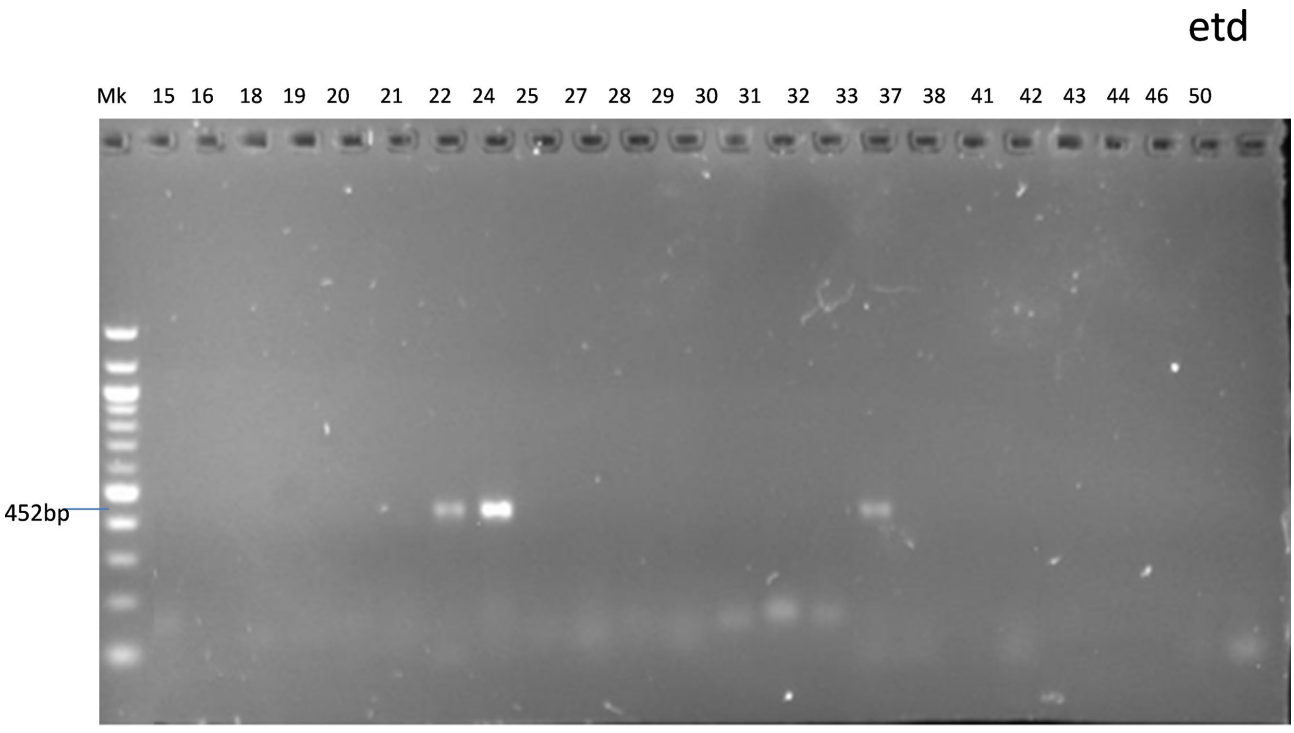
Seven (29%) and three (12.5%) of the 24 *Staphylococcus* strain harbored the *eta* and *etd* gene which codes for exfoliative toxin (*ETA*) production. The seven *eta* positive strains were all *S. aureus* and were isolated from suya and chicken sample respectively. Seven (25%) of the species harbored genes coding for toxic shock syndrome-1 (*TSST*-1), Panton Valentine leukocidin and enterotoxin production. 18 (75%), 10 (41.7%) and 2 (8.3%) of the 24 strains harbors *Seb*, *sea* and *sec* re-

spectively which codes for staphylococcal enterotoxins A, B and C respectively (Table 6, Figures 3-6).



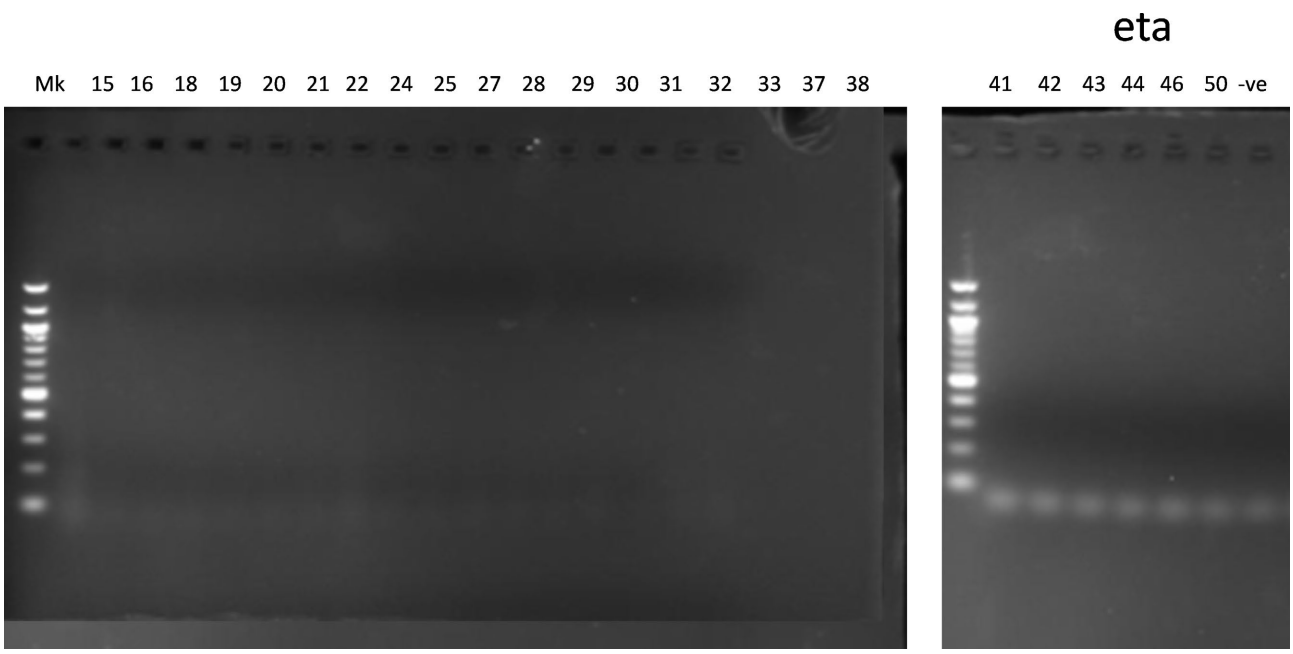
Note bands at the 500 bp, MK = molecular weight marker (100 bp ladder), tve: control, buf = buffer (−ve).

Figure 3. Gel pictures show positive amplification of *tsst* genes of the isolates.



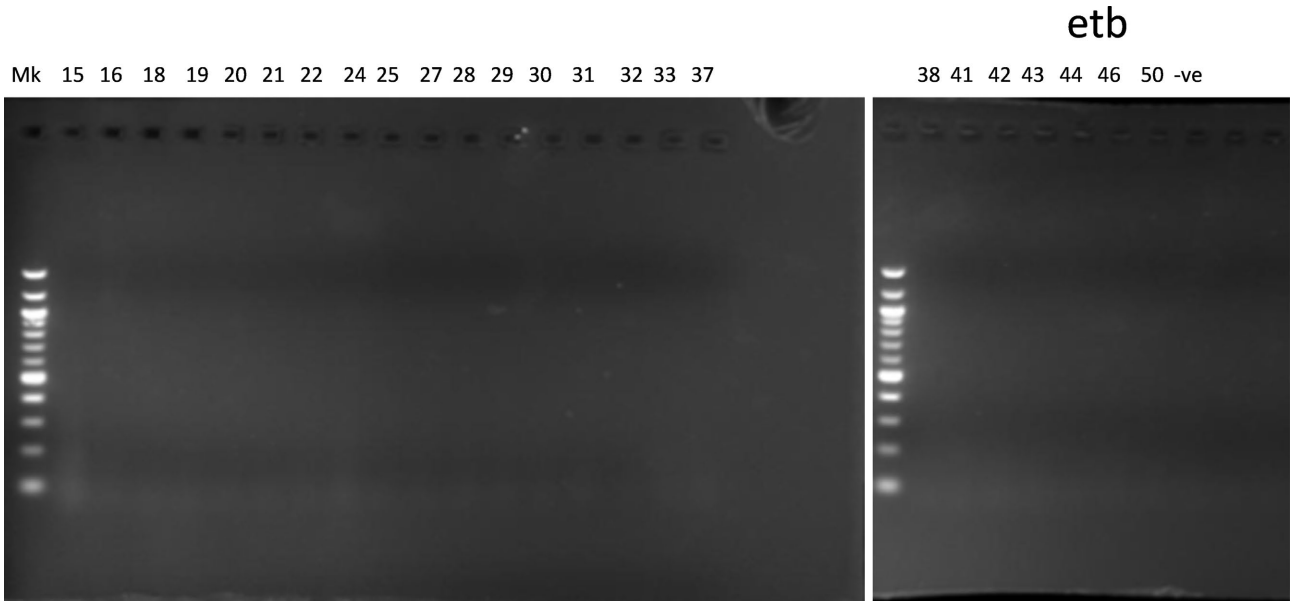
Note bands at the 452 bp, MK = molecular weight marker (100 bp ladder), tve: control, buf-buffer (−ve).

Figure 4. Gel pictures show positive amplification of *etd* genes of the isolates.



Note bands at the 190 bp, MK = molecular weight marker (100 bp ladder), tve: control, buf = buffer (−ve).

Figure 5. Gel pictures show positive amplification of *eta* genes of the isolates.



Note bands at the 612 bp, MK = molecular weight marker (100 bp ladder), tve: control, buf = buffer (−ve).

Figure 6. Gel pictures show positive amplification of *etb* genes of the isolates.

3.4. Antimicrobial Resistance Profile of *Staphylococci*

The resistance profile of staphylococcal species is presented in Table 5. Seventy per cent of the isolates were resistant to Erythromycin while 66%, 64%, 56% were resistant to Cefoxitin, Fusidic acid, and Cefotaxime. None of the isolates were resistant to Teicoplanin, Ciprofloxacin, levofloxacin and Chloramphenicol. Two of the strains were resistant to Linezolid, Gentamycin and streptomycin. Out of the

24 staphylococcal isolates identified, 7(29.2%) were multi-drug resistant, being resistant to three or more classes of antimicrobials. The resistance genes detected in the *Staphylococcus* species were *TEM* (29%), *SHV* (8.3%), *tet*K (8.3%), *tet*M (20.8%), *erm*A (54.2%) and *erm*B (29%) (Table 6, Figures 7-10).

Table 5. Antimicrobial resistance profile of *Staphylococcus* species isolated from RTE meat in Gwagwalada and its environs (n-50).

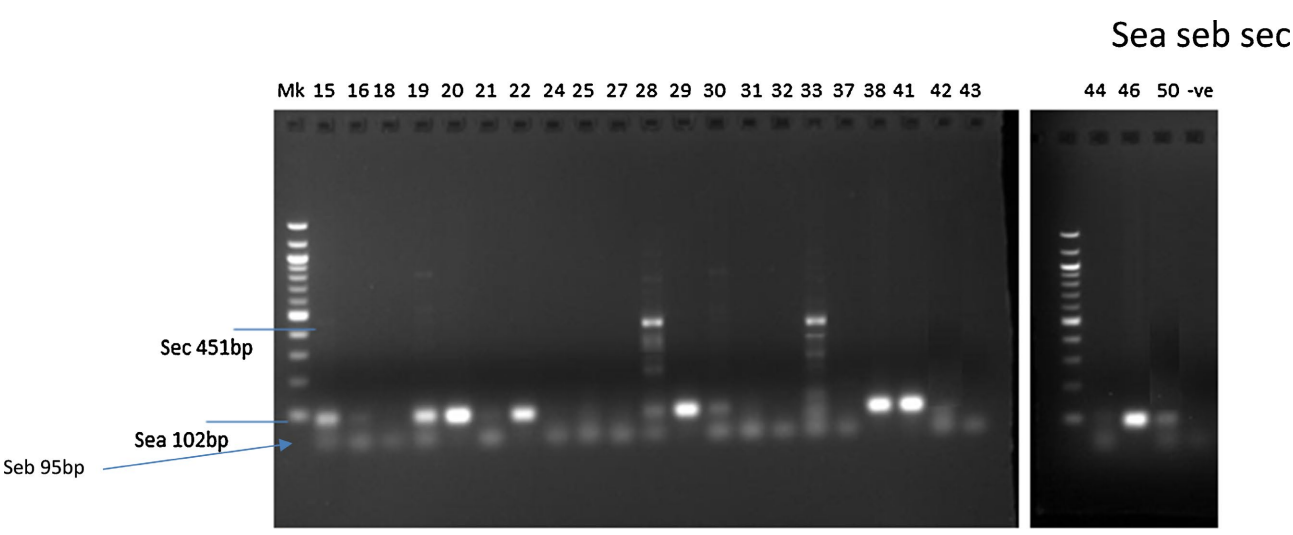
Antimicrobial agents (Disc potency ug)	Class	No (%) of isolates exhibiting resistance
Fusidic acid (10)	Steroids like antibiotics	32 (64)
Cefoxitin (30)	Cephalosporins	33 (66)
Cefotaxime (10)		28 (56)
Oxacillin (10)	Penicillin beta lactam	8 (16)
Tetracycline (30)	Tetracycline	18 (36)
Clindamycin (10)	Lincomycin	15 (30)
Erythromycin (30)	Macrolides	35 (70)
Vancomycin (30)	Glycopeptides	20 (40)
Teicoplanin (5 ug)		23 (46)
Mupirocin (5)	Macrolides	8 (16)
Sulphamethoxazole/trimethoprim (25)	Sulphonamides	9 (18)
Gentamicin (10)	Aminoglycosides	2 (4)
Streptomycin (30)		2 (4)
Chloramphenicol (10)	Amphenicol	0 (0)
Ciprofloxacin (5)	Fluoroquinolones	0 (0)
Levofloxacin (10)		0 (0)
Linezolid (10)	Oxazolidinone	2 (4)

Table 6. Antimicrobial resistance genes and virulent genes detected among the staphylococcal isolates recovered from RTE meat in Gwagwalada and its environ.

Strain ID	*Staphylococcus* species	Virulent gene detected	Resistance genes detected
S_{18}	*Staphylococcus aureus*	*Seb*	*erm*A, *tet*M
S21	*Staphylococcus aureus*	*Seb*	*Erm*A, *TEM*, *tet*M
S25	*Staphylococcus aureus*	*Tsst, sea, seb*	*erm*B
S29	*Staphylococcus aureus*	*Tsst, sea*	*Erm*A
S32	*Staphylococcus aureus*	*Seb*	*Erm*B, *TEM*
S37	*Staphylococcus aureus*	*Seb*	*TEM*
S38	*Staphylococcus aureus*	*Sea*	

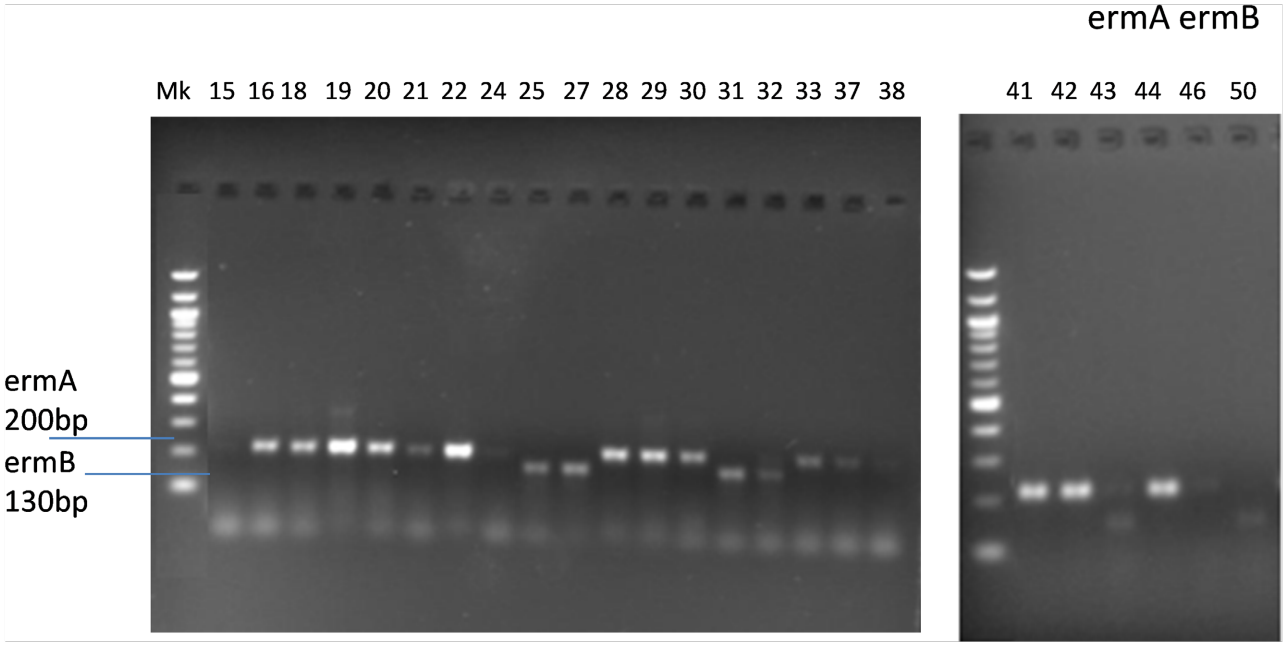
Continued

S42	*Staphylococcus aureus*	*Eta, seb*	*Erm*B, *SHV*
S43	*Staphylococcus aureus*	*Eta, seb*	
S46	*Staphylococcus aureus*	*Tsst, eta*	*TEM*
S50	*Staphylococcus aureus*	*Tsst, eta, seb*	*SHV*
G15	*Staphylococcus aureus*	*Sea, seb*	*tet*M
G20	*Staphylococcus aureus*	*Sea*	*erm*A, *tet*M
G22	*Staphylococcus aureus*	*Etd, sea, seb*	*erm*A
G28	*Staphylococcus aureus*	*Sea,seb, sec*	*erm*A,
G31	*Staphylococcus aureus*	*Seb*	*Erm*B, *TEM*
G33	*Staphylococcus aureus*	*Tsst, sea, seb, sec*	*Erm*A, *tet*K
G41	*Staphylococcus aureus*	*Eta, sea*	*erm*B
G44	*Staphylococcus aureus*	*Eta, seb*	*erm*B
C16	*Staphylococcus aureus*	*Sea, seb*	*Erm*A, *TEM, tet*K
C19	*Staphylococcus aureus*	*Sea, seb*	*Erm*A, *tet*K
C24	*Staphylococcus aureus*	*Etd, seb*	*TEM*
C27	*Staphylococcus aureus*	*Seb*	*Erm*B
C30	*Staphylococcus aureus*	*Tsst, sea, seb*	*Erm*A,



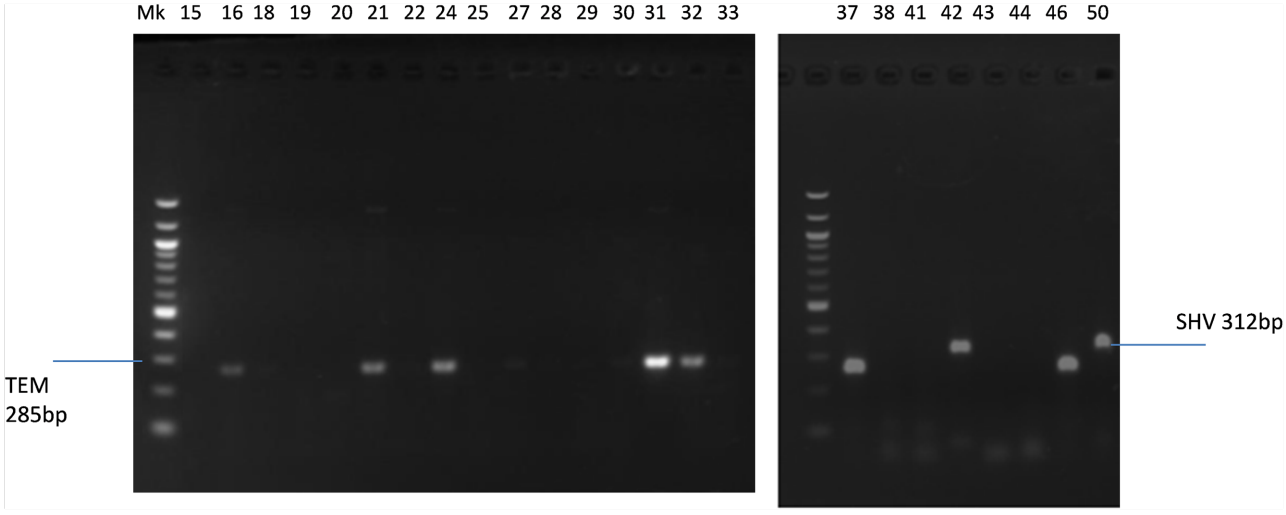
Note bands at the 102 bp, 415 bp and 95 bp, MK = molecular weight marker (100 bp ladder), tve: control, buf = buffer (−ve).

Figure 7. Gel pictures show positive amplification of *sea*, *seb*, *sec* genes of the isolates.



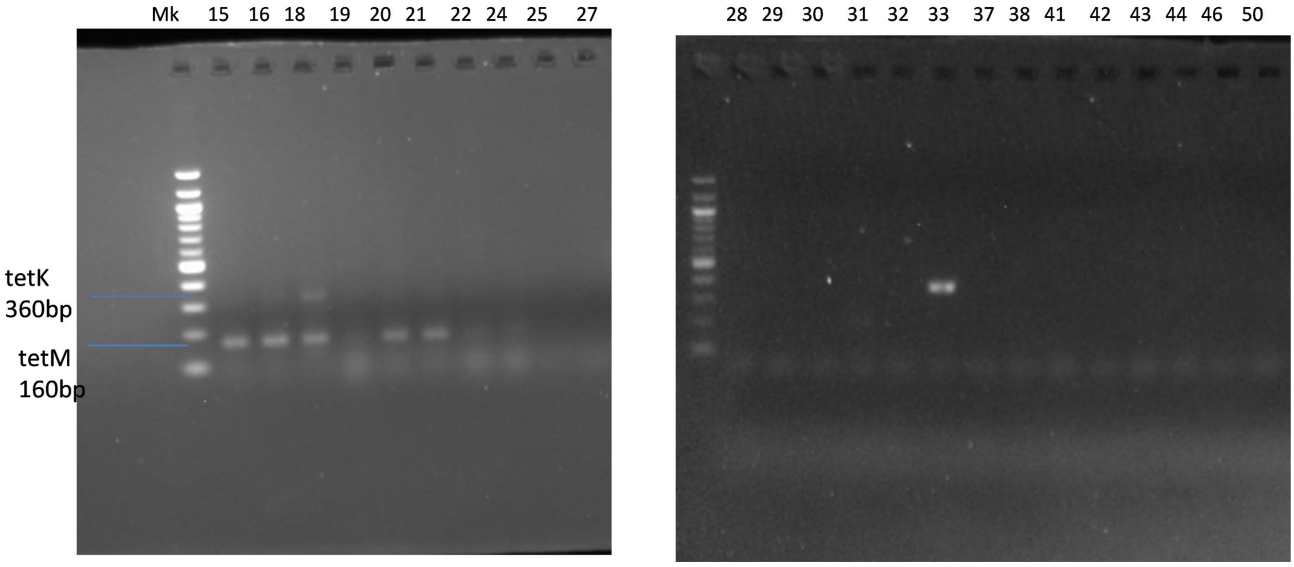
Note bands at the 200 bp and 130 bp, MK = molecular weight marker (100 bp ladder), tve: control, buf = buffer (−ve).

Figure 8. Gel pictures show positive amplification of *erm*A and *erm*B genes of the isolates.



Note bands at the 200 bp and 130 bp, MK = molecular weight marker (100 bp ladder), tve: control, buf = buffer (−ve).

Figure 9. Gel pictures show positive amplification of *TEM* and *SHV* genes of the isolates.



Note bands at the 200 bp and 130 bp, MK = molecular weight marker (100 bp ladder), tve: control, buf = buffer (−ve).

Figure 10. Gel pictures show positive amplification of *tet*K and *tet*M genes of the isolates.

4. Discussion

RTE food prepared or sold by street vendors is recognized as a potential vehicle of microbiological foodborne pathogens if they are mishandled during processing, preparation, and transportation. It may have acute health effects on many affected consumers [36]. The detection of *Staphylococcus* species in this study showed that there may have been post-processing contamination of the street-vended ready-to-eat meat by the handlers in Gwagwalada and its environs. This organism has been identified as a major causal bacterium in human and animal diseases and remains a global public health problem [36].

The overall prevalence of 40% of *Staphylococcus* observed in this study is a little bit higher than that recorded by Adejisi *et al.* [37], Achi and Madubuike, [38] and Ndahi *et al.* [39] of 31.1%, 32.1%. And 33.7% while Akagha *et al.* [40] and Salihu *et al.* [41] reported a higher prevalence of 71.6% and 69.9% of *Staphylococcus aureus* than the one observed in this study. The prevalence of *Staphylococcus aureus* obtained from this study could be attributed to Many factors in the study area, such as lack of basic infrastructure and services (e.g., potable water supplies), water is widely used almost in all street food vending operations, and it is frequently used for drinking purposes, washing of food ingredients, incorporated into food as a component, processing of meat and in the washing of hands, equipment, utensils, and containers. Water is susceptible to being contaminated with microbiological hazards. Also, poor knowledge of street vendors in basic food safety measures, and inadequate public awareness of risks posed by certain street meat. Unhygienic practices during food preparation, handling, and transport. Vendor poor personal hygiene can also contribute to the samples being contaminated by *Staphylococcus aureus*. Food handlers carrying enterotoxin-producing *S. aureus* on their skin or noses are likely to contaminate food through direct contact [42].

Animal foods (meat and meat products) also contain low-acid components, which are frequently contaminated with pathogens before they are prepared into foods and cooked [43]. Bacteria may release toxins into these products that are not destroyed by cooking and may cause food poisoning [43]. Moreover, meat products support quicker bacterial growth compared to high moisture content, and the problems are intensified in tropical countries due to high ambient temperature and humidity [43]; this could be where foods are converted into excellent culture media for bacterial growth to be a possible reason for the surviving of the pathogens in the meat. If food handlers suffer from specific diseases, microbiological hazards are present on their skin or in their respiratory tract, intestine, and feces may contaminate foods [2]. Moreover, cross-contamination can occur by food handlers after handling raw materials.

In this study, the high prevalence of *Staphylococcus* recorded in Gwagwalada main market (GMM) and Gwagwalada Motor Park (GMP) compared to Gwagwalada cafeteria and restaurants (GCR) may be an indication of poor hygienic practices carried out in the two vending areas. The two locations are open areas where busy daily markets and transportation operations expose the RTE meat to contamination from the dusty environment. Poor environmental sanitation of the street food vending location is another significant contributory factor to the likelihood of microbiological contamination. Unhealthy surroundings (e.g., proximity of drains and public discharge sites), insufficient facilities for waste disposal, and inappropriate accumulations of garbage, dirty plates or utensils that attract insects, flies, rodents, or birds, the design, infrastructure, and maintenance of vending units, utensils, and equipment are also essential issues for microbiological safety of street foods. Staphylococcal food poisoning (SFP) is one of the most common foodborne diseases and is caused by the ingestion of food contaminated by one or more staphylococcal enterotoxins (SEs) produced by *S. aureus* strains [3]. SFP symptoms—including nausea, vomiting, abdominal cramps and pain, myalgia, diarrhea, giddiness, and headache—typically have a rapid onset, occurring 2 - 8 h following ingestion of the toxin-contaminated food [44]. Approximately 20% - 60% of humans are permanent or intermittent carriers of *S. aureus*, which harbor SE genes in one- to two-thirds of cases [45].

A major clinical finding is the high level of resistance to macrolides (erythromycin) and cephalosporins (cefoxitin and cefotaxime), with 70% and 66% of isolates displaying resistance, respectively. The treatment of staphylococcal infections is made more difficult by resistance to erythromycin, a popular antibiotic used to treat a variety of diseases. This frequently necessitates the use of more toxic or powerful substitutes. Similarly, the effectiveness of conventional treatment procedures is seriously called into question by resistance to cephalosporins, which are commonly administered as first-line empirical therapies for Gram-positive infections. In addition to reducing the range of available treatments, the development of this resistance raises the possibility of transferable resistance mechanisms that could infect additional harmful bacteria and exacerbate the issue of

multi-drug resistance.

Particularly concerning is the high resistance to Fusidic acid (64%), a steroid-like antibiotic usually used to treat *Staphylococcus aureus* infections, especially those affecting the skin and soft tissues in hospital settings. This tendency is a serious worry for infection control and patient outcomes because there aren't many options in hospital care settings. Although resistance to oxacillin and penicillin is relatively low (16%), any level of resistance is clinically significant because both antibiotics are essential beta-lactams. The identification of drug resistance suggests that certain local strains of Staphylococcus bacteria may have or be developing mechanisms like altered penicillin-binding proteins or beta-lactamase production, which could result in more widespread resistance phenotypes like MRSA (Methicillin-resistant *Staphylococcus aureus*). The modest resistance seen to glycopeptides, which are prescribed as last-resort therapy for severe Gram-positive infections, is especially concerning. These glycopeptides are vancomycin (40%) and teicoplanin (46%). Resistance at these levels is a concerning trend since it impairs the efficacy of vital medications used in life-threatening circumstances, reducing the number of available treatment options and raising uncertainty about the results. Since lincomycin is commonly used to treat Gram-positive pathogens, the 30% resistance to the antibiotic further complicates treatment. Although resistance to fluoroquinolones (ciprofloxacin and levofloxacin) and aminoglycosides (gentamicin and streptomycin) are still low at 4%, these results should be regarded cautiously. Additionally, no resistance to chloramphenicol was found. Even in these agents that are now effective, continued abuse or overuse may result in increased resistance.

In conclusion, this study's high rates of resistance to important antibiotic classes point to a major public health concern. They emphasize how urgently strong antimicrobial stewardship, surveillance initiatives, and the creation of novel treatment approaches are needed. The risk is further increased by the potential for horizontal gene transfer to spread resistance genes, especially in hospital and community settings. In addition to putting individual patient care at risk, this circumstance may jeopardize larger public health initiatives, particularly when it comes to foodborne staphylococcal infections, for which there may already be few available treatments [46]. The findings of this study have important ramifications for public health and food safety. RTE meat can act as a conduit for the spread of resistant *Staphylococcus* strains to people, particularly if it is handled, stored, or prepared incorrectly. These strains are more likely to cause longer lasting or more serious infections because they are resistant to several different classes of antibiotics. Additionally, the fact that these bacteria can carry multiple resistance genes raises the possibility that contaminated RTE meat could serve as a reservoir for the community's spread of AMR, potentially harming immunocompromised people, the elderly, and children [47].

Twenty antibiotics representing ten different classes of antibiotics were tested for antimicrobial resistance in various meat types from different African countries

by some researchers. The results showed that ampicillin (50%), clindamycin (33.8%), doxycycline (27%), and ofloxacin (57%), although ofloxacin represented a small sample size, had the highest levels of resistance. Antibiotic resistance to beta-lactams is generally high. Studies on tetracycline resistance differ slightly from one another [48]. Because of its high virulence and resistance to antibiotics, *S. aureus* has been identified as a dangerous pathogen. A hypervirulent, multi-drug-resistant superbug may be on the rise, as evidenced by the relative ease with which strains *S. aureus* exchange genetic material encoding antibiotic resistance and virulence determinants with other species [18]. Unless the mutation rate of resistance to the drug is extremely high, as in the case of streptomycin (aminoglycoside) and erythromycin (macrolide), *S. aureus* is unlikely to exhibit a change in sensitivity to a drug given for a single brief course [49]. In general, *S. aureus* is less susceptible to erythromycin than pneumococci or hemolytic streptococci, and in vitro, resistance to the antibiotic has been shown to develop quickly, particularly in *Staphylococci* [50]. *In vivo*, it was observed that resistance is more likely to arise with prolonged use of erythromycin but is typically not a significant clinical issue with short courses of treatment. It is concerning that these *Staphylococcus aureus* strains have multiple antimicrobial resistance genes. Numerous isolates had resistance genes, including *erm*A, *erm*B (*macrolide-lincosamide-streptogramin B resistance*), *tet*M, *tet*K (*tetracycline resistance*), *TEM* (*beta-lactam resistance*), *and SHV* (*extended-spectrum beta-lactamase resistance*). These resistance genes demonstrate the pathogens' capacity to withstand common antibiotics, which makes treatment options more challenging in the event of an infection. For example, *tet*M *and tet*K contribute to tetracycline resistance, which is commonly used in both human and veterinary medicine, while *erm*A *and erm*B confer resistance to macrolides [49]. A troubling pattern of multidrug resistance was observed in the isolates. Several strains, including *S*21, *G*15, *and G*33, exhibited resistance to several antibiotic classes, including beta-lactams, macrolides, and tetracyclines. This multi-drug resistance implies that there is a greater risk of *Staphylococcus aureus* contamination of RTE meat because these bacteria can spread resistant infections in people, particularly in susceptible groups like the elderly, young children, and people with weakened immune systems [45].

Numerous virulent genes were present in the *Staphylococcus aureus* strains that were identified from the RTE meat samples. It was common to find common virulent genes *like the sea* (*Staphylococcal enterotoxin A*), *seb* (*Staphylococcal enterotoxin B*), *eta* (*Exfoliative toxin A*), *tsst* (*Toxic shock syndrome*), *and sec* (*Staphylococcal enterotoxin C*). These virulent characteristics increase *S. aureus's* capacity for pathogenicity and raise the risk of dangerous foodborne diseases like toxic shock syndrome and food poisoning. This implies that these dangerous pathogens may be present in the RTE meat in the research area. Numerous virulent genes were present in many of the strains. For instance, *strain G*33 *carried tsst*, *sea*, *seb*, *and sec*, *whereas strain S*25 *had tsst*, *sea*, *and seb*. Consuming contaminated RTE meat increases the risk to the public's health because the presence of multiple vir-

ulent genes in a single strain suggests a higher pathogenic potential and may lead to more severe clinical outcomes [50]. The public health implications of theses Multiple virulence genes found in *Staphylococcus aureus* strains from ready-to-eat (RTE) meat raise the possibility that these items include extremely harmful organism. Serious disorders like food poisoning, toxic shock syndrome, and skin infections are directly associated with virulent genes like *sea*, *seb*, *sec* (enterotoxin), *tsst* (toxic shock syndrome toxin), and *eta* (exfoliative toxin). Because virulence factors and illness severity are correlated, strains that carry many toxins, such as strain G33 (*tsst*, *sea*, *seb*, *sec*), are more likely to cause severe and potentially fatal infections. This implies that infections brought on by these multi-toxin strains may have more serious consequences. Public health systems would have to deal with the burden of extensive healthcare treatments if outbreaks were connected to such strains. There may be a higher chance of problems or mortality for vulnerable groups, such as children, the elderly, and those with impaired immune systems. Toxic shock syndrome: *TSST*-1 can cause toxic shock syndrome (TSS), a severe disease that can lead to multiorgan system dysfunction, fever, rash, and hypotension. *S. aureus* can cause Staphylococcal food poisoning (SFP), which is associated with the *tsst* gene. *TSST*-1 producing *S. aureus* has been detected in more than 60% of individuals with Kawasaki syndrome, the main cause of acquired heart disease in children. PTSAgs, such as *TSST*-1, may theoretically cause autoimmune disease by activating autoreactive T-cell clones. The *tsst* gene-associated *Staphylococcus aureus* Pathogenicity Island (SaPI) can induce tissue damage. SaPI can cause immunological suppression. The SaPI can stimulate the release of inflammatory cytokines. The tst gene is a type of superantigen (SAg) that is resistant to desiccation, acids, proteolysis, and heat. SAgs can cause excessive cytokine release and T-cell activation, which can impair immune system function.

The genes *sea*, *seb*, and *sec* produce staphylococcal enterotoxins (SEs), which can cause food poisoning and diseases in humans and animals. *SEA* is one of the most well-studied SEs and is closely related to clinical isolates. *SEB* is a common cause of food poisoning in humans. SEC: There are three varieties of SECs: SEC1, SEC2, and SEC3. *SEA* and *SEB* are called superantigens because they can attach to antigen-presenting cells and activate enormous numbers of T lymphocytes. *S. aureus* carriage may relate to autoimmune disorders such as lupus erythematosus. Enterotoxin and antibiotic resistance are strongly linked. Rheumatoid arthritis and Wegener's granulomatosis. The *eta*, *etb*, and *etd* genes in *Staphylococcus aureus* encode exfoliative toxins (ETs) that play a role in staphylococcal skin infections.

The possibility of human exposure to both virulent and resistant strains is highlighted by the fact that these isolates were found in RTE meat, which is frequently eaten not processed well or half done. Food product contamination by virulent and antimicrobial-resistant bacteria can contribute to the community's spread of resistance genes and infections, resulting in a vicious cycle that is challenging to break [50]. The results highlight how crucial it is to keep an eye on and regulate the quality of RTE meat products in Gwagwalada and comparable settings. Im-

proved food safety procedures, such as the correct handling, storage, and cooking of meat products, are desperately needed given the prevalence of virulent and resistant strains of *S. aureus*. This will lower the risk of foodborne illness and the spread of antibiotic resistance. To identify and manage *Staphylococcus aureus* contamination in RTE meat, food safety laws and surveillance systems should be strengthened. Putting in place routine testing for antibiotic resistance in foodborne pathogens, such as *S. aureus*, to guide public health initiatives. Raising awareness of the dangers of eating undercooked or incorrectly handled meat, particularly considering virulent pathogens and antibiotic resistance. Lowering the selection pressure for resistant strains by promoting the prudent use of antibiotics in both human and veterinary medicine. In conclusion, *Staphylococcus aureus* strains isolated from RTE meat in Gwagwalada exhibit both virulent genes and antimicrobial resistance, highlighting a significant public health concern. To reduce the risk of foodborne illnesses and the spread of antibiotic resistance, more efficient surveillance and preventative actions are required. Stricter oversight, regulation, and control of food production and distribution are necessary considering the discovery of these virulent strains in RTE beef, which suggests weaknesses in food safety procedures. It is essential to continuously monitor foodborne pathogens and their virulent profiles. To prevent severe outbreaks and safeguard customers from contaminated RTE meat products, there is an urgent need for improved food safety procedures, public health surveillance, and awareness, as evidenced by the association between virulent genes and illness severity. To safeguard the public's health, these findings might call for changes to meat production, packaging, and sale policies.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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