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Research Paper

Molecular Characterization and Antibiotic Resistance of Foodborne Pathogens in Street-Vended Ready-to-Eat Meat Sold in South Africa

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ABSTRACT

The consumption of food contaminated with microbial populations remains a key route of foodborne infection in developing countries and creates a serious public health burden. This study aimed at identifying foodborne pathogens and their antibiotic resistance profiles in ready-to-eat meat sold in public eateries in the Johannesburg area. A total of 115 samples were examined for the incidence of bacteria pathogens and their antibiotic resistance profiles against commonly used antibiotics (ampicillin, tetracycline, chloramphenicol, erythromycin, ciprofloxacin, streptomycin, and sulphonamides) using the molecular and the disc diffusion methods. Fifteen bacteria species were detected in the samples. *Staphylococcus aureus* had the highest prevalence (25%), and 53.33% of the isolates exhibited multidrug resistance to the antibiotics tested. Among the isolated bacteria, *S. aureus* was resistant to at least six antimicrobial agents, whereas 100% of *S. aureus, Enterococcus faecalis,* and *Planomicrobium glaciei* were resistant to streptomycin, ciprofloxacin, and chloramphenicol, respectively. This study revealed that a wide diversity of bacteria species contaminate meat sold on the street, which indicates that consumers of ready-to-eat meat sold in public eateries are at risk of food poisoning. Hence, strict intervention strategies should be put in place by government agencies to reduce the menace of food poisoning in the country.

HIGHLIGHTS

- RTE meats were analyzed for microbial contamination.
- Foodborne pathogens were detected in the meat samples.
- Antibiotic resistance profiles were tested.
- RTE meats contain foodborne pathogens, and isolates exhibited antibiotic resistance.
- Consumers of RTE meat in the Johannesburg CBD are at risk of food poisoning.

Key words: Antibiotic resistance; Meat; Molecular characterization; Pathogens; Public eateries; Ready-to-eat

In developing countries such as South Africa, street food is a common part of city lifestyle due to high unemployment and limited work opportunities *(18, 63)*. Street foods are appreciated not only for their singular flavors, suitability, and their expression of cultural and social tradition, they also provide a significant portion of the population's nutritional needs, especially the low-income earners *(48)*.

Several studies have shown that street foods are frequently stored at inappropriate temperatures, excessively handled by food retailers, and sold in very dirty environments *(9)*. Therefore, the Food and Agriculture Organization and other institutions have raised concerns about these foods and their possible link to outbreaks of foodborne infections in developing countries *(51, 52)*. These infections, which are linked to a wide range of illnesses, are caused by bacterial, viral, parasitic, or chemical contamination of foods. Although nearly half of foodborne illnesses are caused by viruses, most hospitalizations and deaths associated with them are due to bacterial agents *(47)*. However, data on foodborne diseases in Africa are limited *(57)*.

It has has been reported that there are risks associated with the consumption of contaminated street-vended foods that have high levels of coliform and pathogenic bacteria, such as *Salmonella* species, *Staphylococcus aureus, Escherichia coli, Bacillus cereus,* and *Clostridium perfringens (24)*. In addition, consumption of food containing the above organisms could contribute to the occurrence of multidrug

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resistance in consumers, hence posing a real risk to the health of the general population *(29)*.

Antibiotic resistance of infectious agents is one of the greatest challenges to public health worldwide *(64)*. Foods of animal origin are considered to be key reservoirs of antibiotic-resistant bacteria owing to the use of antibiotics in the food production industry *(27, 28, 58)*. Antibiotic-resistant bacteria may reach humans indirectly along the food chain through consumption of contaminated food or food-derived products *(15, 23)*. Food contamination with antibiotic-resistant bacteria can be a major threat to public health because the antibiotic resistance determinants can be transferred to other pathogenic bacteria, potentially compromising the treatment of severe bacterial infections *(2, 27, 54)*. Furthermore, antibiotic resistance has been given low priority and limited attention in most developing countries, such as South Africa *(64)*.

The worldwide emergence of antimicrobial-resistant strains has aroused public health concern. In Nigeria, Seoul (South Korea), and Cotonou (Benin), pathogens isolated from street-vended food and from commercial and cooked foods showed resistance to various antibiotics *(49, 53, 56)*. *E. coli* is highly capable of acquiring and transferring antimicrobial resistance genes *(50)*. *S. aureus* pathogens isolated from street-vended food in Cotonou, Benin, were resistant to a wide range of antibiotics *(60)*. Also, the emergence of ciprofloxacin-resistant *Salmonella* spp. was reported in Europe in travelers returning from North East and East Africa *(3, 20)*.

Multidrug resistance of *Salmonella* spp., as well as *S. aureus,* is frequently found, at rates that have increased considerably in recent years *(42)*. The multidrug resistance of *Salmonella* spp. to ciprofloxacin *(61)* and *S. aureus* to methicillin and vancomycin *(12)* has also been reported in African countries. *S. aureus* is known to cause a number of pathological conditions in humans and animals, ranging from mild skin infections, bacteremia, systemic diseases, and osteomyelitis to the more complicated toxic shock syndrome and staphylococcal food poisoning *(45)*. The emergence of multidrug-resistant *S. aureus* poses therapeutic challenges to public health workers, veterinary professionals, and dairy cattle producers *(30)*.

Ready-to-eat (RTE) meats sold in the street are exposed to contamination, which occurs mostly during handling, processing, and preparation, when pathogens could multiply exponentially under favorable conditions, leading to foodborne infections. Therefore, this study was aimed at identifying the foodborne pathogens associated with RTE meat sold in public eateries in the Johannesburg area and the antibiotic resistance profiles of those pathogens.

MATERIALS AND METHODS

Study design. A cross-sectional descriptive study was conducted on three streets: Plein Street (MTN Taxi rank), Bree Street (corner of Plein Street), and Hancock Street (corner of Claim Street) in the central business district of Johannesburg, South Africa, from December 2015 to April 2016. This was done to assess and evaluate the hygienic quality of street-vended meat and the antibiotic resistance profiles of the bacterial isolates. The study areas were selected because of their strategic locations and high population densities.

Sample collection. Meat samples ($n = 115$), including chicken ($n = 24$), chicken gizzard ($n = 21$), beef head meat ($n = 24$), beef intestines ($n = 22$), and wors ($n = 24$), were randomly purchased twice a week for 12 consecutive weeks in the three streets. Meat samples were collected between 12:00 noon and 2:00 p.m., after which all samples were immediately placed in a cooler box and transported to the Microbiology Laboratory at the Animal Health Department, Mafikeng Campus, North-West University. The samples were kept in a deep freezer at −18°C for 1 day to prevent further multiplication of the intrinsic bacteria in the meat before analysis.

Microbial analysis and isolation of bacteria pathogen. Microbial analysis and isolation were done as described by the American Public Health Association *(5)*. Twenty-five grams of each sample was added to 225 mL of nutrient broth as the first enrichment, and the inoculum was incubated aerobically at 37°C for 24 h in a shaker incubator (model FSIESP024, serial no. L21842, LABCOM Technologies Ltd., Maraisburg, South Africa) for enrichment. From the homogenized inoculum, 1 mL was pipetted and added to a test tube containing 9 mL of sterile nutrient broth (Biolab, Merck NT laboratory supplier, Modderfontein, South Africa) and homogenized using a vortex mixer (model SO200-230 V-EU, Labnet International, Edison, NJ) at 260 rpm for 2 min. The diluted inoculum was then used as base solution to make serial dilutions (10^{-2}, 10^{-3}, and 10^{-4}). One milliliter of the 10^{-4} dilution was pipetted from test tubes and plated on sterile nutrient agar and MacConkey agar (Acumedia LAB, Neogen culture media, Heywood, Lancashire, UK), using an L-shaped sterile glass rod, for the surface spread method. The inoculated plates of each agar (an uninoculated plate for each agar served as the control) were then incubated aerobically at 37°C for 48 h in a room incubator (model Su 131h, ECR Manufacturing, Johannesburg, South Africa). The absence of bacterial growth on the control plate confirmed the sterility of the environment in which samples were cultured. Isolated bacteria were subcultured on fresh nutrient agar, and the plates were aerobically incubated at 37°C for 24 h. The bacterial isolates were subcultured three times to obtain pure cultures of the isolates.

Identification of bacterial isolate. Identification of bacteria was based on morphological, biochemical, and molecular characterization of the isolates.

Morphological examination. The colony surface and pigmentation, appearance, shape, elevation, edge, etc., of each isolate, as described in *Bergey's Manual of Systematic Bacteriology,* were observed after 24 h of incubation *(31)*.

Biochemical tests. The series of biochemical tests performed on the bacterial isolates were the Gram stain, catalase reaction test, oxidase test, Voges-Proskauer test, and the indole test.

Gram staining. Gram staining was performed on each isolate using standard methods *(26, 55)* as follows. A single colony was picked up from a pure culture with a sterile wire loop and smeared on the slide with few drops of water, and the colony was heat fixed on the slide by gentle heating for 20 s. After this, the slide was flooded with crystal violet stain (Unilab, Merck NT laboratory supplier) for 1 min, and the stain was washed off with sterile water. This was followed by the addition of a few drops of iodine

solution (UnivAR, Merck NT laboratory supplier) to the slide; the iodine solution was left for another minute before it was washed off with sterile water. Next, a few drops of 95% ethanol were applied on the smear to decolorize the slide, and then the slide was washed again with sterile water. Finally, a few drops of safranin solution (Saarachem, Merck Chemicals Ltd., Wadeville, South Africa) were placed on the slide as a counterstain for 1 min; after the slide was washed with sterile water, blotted, and air dried, it was examined under a light microscope (model 3108001956, Axiostar plus, Carl Zeiss, Gottingen, Germany) at 400× using immersion oil *(26, 55)*.

Catalase test. A catalase test was performed to differentiate bacteria that produce the enzyme catalase, such as *Staphylococci* and *Enterobacteriaceae,* from non–catalase producing bacteria, such as streptococci. The catalase test was performed as described by Mosupye and Von Holy *(37)*. From a pure culture, a single colony of bacteria was collected with a sterile wire loop and smeared on the glass slide, and then two to three drops of hydrogen peroxide were added on top of the bacteria. The presence of the enzyme in the bacterial isolate was evident with rapid elaboration of gas (oxygen) bubbles, whereas the lack of catalase was evident by a lack of or weak bubble production from a culture not more than 24 h old *(16)*.

Oxidase test. The oxidase test was performed to identify bacteria that produce cytochrome oxidase. A single pure colony from a 24-h culture was smeared over Microbact oxidase strips (MBO266A, Oxoid Ltd., Basingstoke, England). The result was read after 5 to 10 s; a positive result was indicated by an intense blue coloration. A delayed positive result appeared within 10 to 60 s, and a negative result was observed by the absence of coloration or by coloration after 1 min.

Voges-Proskauer test. Voges-Proskauer test was performed as described by Marr et al. *(33)*. Bacterial cultures were inoculated into methyl red Voges-Proskauer broth (Hardy Diagnostics, Merck KGaA, Darmstadt, Germany) and were incubated at 37°C overnight. After incubation, 20 drops of Barritt's reagents A and B (HiMedia Laboratories, Pvt. Ltd., Mumbai, India) were added to the inoculum and shaken carefully. The presence or appearance of red color within 15 to 20 min was an indication of a positive result.

Indole test. This test was performed using Kovac's reagent (Pro-LAB Diagnostics, IVD, Bromborough, Wirral, UK). Tryptone broth (Oxoid Ltd., Basingstoke, Hampshire, England) was inoculated with the test organism and incubated at 37°C for 24 to 28 h. After 24 h, three to five drops of Kovac's reagents were added; a red color ring at the upper layer of the liquid was an indication of indole positive, whereas yellow or no color was an indication of indole negative.

Molecular identification of bacterial isolates. The identification of bacterial isolates in the present study was based on 16S rDNA gene sequence analysis. Numerous methodologies were applied to investigate molecular characterization of isolated bacteria, such as DNA extraction, PCR, and sequencing analysis *(25, 32)*.

Extraction of genomic DNA. The extraction of genomic DNA was performed as described by Ngoma et al. *(43)*. Pure bacteria isolated from the nutrient agar (after aerobic subculture for 24 h) were inoculated into 5 mL of nutrient broth and incubated aerobically at 37°C for 24 h. The inoculum was then transferred into a 15-mL centrifuge tube (Nunc polypropylene conical tubes, Thermo Scientific, City, South Africa) and centrifuged (Z200A, 44070016, Hermie Labortechnik, Wehingen, Germany) at 15,000 rpm for 10 min. Next, pellets were collected and DNA was extracted using ZR fungal-bacterial DNA MiniPrep kit (D6005, Zymo Research Corp., Irvine, CA; supplied by BioLab, Stellenbosch, South Africa) as follows: pellets were suspended in 750 µL of lysis solution, disrupted with a gene disruptor (model SID258, Inqaba Biotec, Pretoria, South Africa), and vortexed at 1,000 rpm for 14 min, followed by centrifugation at 10,000 rpm for 1 min. Four hundred microliters of the upper aqueous phase was aliquoted into a new Zymo-Spin IV spin filter (Zymo Research Corp.) and was centrifuged at 7,000 rpm for 1 min; 1,200 µL of genomic lysis buffer was added to the filtrate, and 800 µL of the mixture was transferred to a new collection tube (Zymo-Spin IIC column, Zymo Research Corp.) and centrifuged at 10,000 rpm for 1 min. The filtered DNA was prewashed by the addition of 200 µL of DNA prewash buffer and then centrifuged at 10,000 rpm for 1 min. The process was followed by the addition of 500 µL of DNA wash buffer to the Zymo-Spin IIC column and centrifugation at 10,000 rpm for 1 min. Finally, 100 µL of DNA elution buffer was added to elute the DNA into a sterile 1.5-mL microcentrifuge tube by centrifuging at 10,000 rpm for 30 s.

Amplification of 16S rDNA. PCR amplification of the 16S rDNA gene was performed using a DNA Engine DYAD Peltier thermal cycler (Bio-Rad, Hercules, CA). A reaction volume of 25 µL containing 12 µL of PCR master mix, 1 µL of template DNA, 10 µL of nuclease-free water, and 1 µL each of forward and reverse oligonucleotide primers was prepared and mixed in PCR tubes *(43, 44)*. Amplification of the 16S rDNA gene was performed according to Ngoma et al. *(43)*. PCR was conducted using universal primers, forward 27F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and reverse 1492R (5′-TGA CTG ACT GAG ACG TTG CGA-3′). These primers were commercially synthesized by Inqaba Biotec. The thermocycling (T100 thermal cycler, Bio-Rad, Singapore) conditions were as follows: an initial denaturation step at 95°C for 5 min followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 61°C for 30 s, and extension at 72°C for 5 min, and by a single and final extension step at 72°C for 7 min in a Peltier thermal cycler (Bio-Rad, Johannesburg, South Africa).

Agarose gel electrophoresis. Electrophoresis of PCR products was performed as described by Ngoma et al. *(43)*. The PCR amplicons were analyzed by electrophoresis on 1% (w/v) agarose gel at electrophoretic conditions of 90 min at 80 V and 250 mA; the gel was visualized under UV light at 420-mm wavelength using a Chemi DOCMP imaging system (Bio-Rad). The presence of a single bright band (DNA bands) at the expected size (1,500 bp) for each sample indicated a successful amplification. The PCR products were sent to Inqaba Biotech for sequencing.

Antimicrobial test. The antimicrobial test was carried out as described by Bauer et al. *(10)*. Pure colonies of isolated bacteria were inoculated in 5 mL of peptone water and grown at 37°C under aerobic conditions for 24 h. After the incubation period, a sterile cotton swab was dipped into the suspension and streaked over the entire surface of Mueller-Hinton agar plates (LAB M, Neogen Company). Inoculated plates were allowed to dry for 5 min, followed by the application of antibiotic discs and incubation for 24 h at 37°C under aerobic atmosphere. The diameters of zones of inhibition were measured with a caliper and were interpreted as

TABLE 1. *Microbial profile of pathogenic bacteria isolated from meat samples*

Sample identity	Sequence ID	Bacterial identity	Accession no. of meat isolates	Accession no. of matched organism at GenBank	% identity with similar organism at GenBank
Wo12 D4	Seq3	*Staphylococcus aureus*	MG711859	KX447584	99
BI29D2 l1	Seq5	*S. aureus*	MG711860	KX447584	97
GIZ 20 D1	Seq6	*S. aureus*	MG711861	KX447584	97
BHM2 D2	Seq8	*Planomicrobium glaciei*	MG711862	HF545326	97
CH4 D3	Seq9	*Bacillus cereus*	MG711863	KY078799	98
BHM 23M	Seq11	*Macrococcus caseolyticus*	MG711864	MG543841	99
Wo D3	Seq12	*Staphylococcus vitulinus*	MG711865	NR_024670	87
Wo 8D1	Seq13	*B. cereus*	MG711866	MG027671	99
Wo 8D2	Seq41	*Bacillus thuringiensis*	NA[a]	EU161995	82
GIZ 15D1	Seq16	*Planococcus antarcticus*	MG711867	KF318398	99
Wo 23 D3	Seq17	*P. antarcticus*	MG711868	KF318398	99
CH 22 D1	Seq18	*M. caseolyticus*	MG711869	MG543838	95
CH 22 D1	Seq19	*Enterococcus faecalis*	MG711870	HG799973	96
CH 22 D1	Seq20	*Enterococcus faecium*	MG711871	KR858813	96
BI 19D3	Seq21	*M. caseolyticus*	MG711872	KJ638988	90
GIZ 3D3L1	Seq22	*Macrococcus* spp.	MG711873	GU322006	98
BI 04D2	Seq23	*M. caseolyticus*	MG711874	MG543841	99
CH5 D3	Seq24	*Staphylococcus equorum*	MG711875	DQ232735	97
CH 11D2	Seq25	*M. caseolyticus*	MG711876	MG543840	89
GIZ 30DM L1	Seq26	*M. caseolyticus*	MG711877	MG543840	98
Wo 32D3 l1	Seq27	*M. caseolyticus*	MG711878	MG543841	99
BHM 1D3	Seq28	*Macrococcus* spp.	MG711879	MF101694	89
BHM 23D3	Seq29	*P. antarcticus*	MG711880	KF318398	96
BHM 23 D3	Seq30	*S. aureus*	MG711881	KX447584	97
BHM 17D3	Seq32	*M. caseolyticus*	MG711882	MG543840	97
CH 3M	Seq33	*Lysinibacillus* spp.	MG711883	HG931343	98
CH22 D2	Seq34	*Kurthia* spp.	MG711884	CP013217	99
CH5 D3	Seq36	*Kurthia* sp.	MG711885	CP013217	98
Wo5 D4	Seq37	*Lysinibacillus* sp.	MG711886	KT029127	96
Wo10 D4	Seq40	*S. vitulinus*	MG711887	JQ684235	99
CH25 ML1	Seq1	*S. aureus*	MG711860	KX447584	95
BI3 D1	Seq2	*S. vitulinus*	MG711865	KM378591	97
BHM1 D4	Seq4	*S. aureus*	MG711861	KX447584	97
Wo17 D1	Seq7	*Bacillus* sp.	NA	LT548958	81
Wo3 D1	Seq10	*M. caseolyticus*	MG711877	MG543840	97
CH20 M	Seq14	*B. cereus*	MG711863	KU977289	78
BHM29 D4L1	Seq15	*E. faecium*	MG711871	CP012440	96
CH20 M	Seq31	*Bacillus* sp.	NA	JF701942	81
GIZ3 D1L1	Seq35	*Citrobacter* sp.	NA	KT150059	83
CH0 D4	Seq38	*B. cereus*	MG711866	KP235535	82
GIZ16 D4	Seq39	*E. faecium*	MG711871	FJ915708	74

[a] NA, not assigned accession number at GenBank.

recommended in the Clinical and Laboratory Standard Institute guidelines *(17–19)*. Antimicrobial disks impregnated with ampicillin (10 μg), gentamicin (10 μg), tetracycline (30 μg), sulphonamides (300 mg), streptomycin (10 μg), ciprofloxacin (5 mg), chloramphenicol (30 mg), and erythromycin (5 mg) used in this study were obtained from Mast Group Ltd. (Bootle, UK). Results were used to classify isolates as resistant or nonresistant to a particular antibiotic.

Statistical analysis. Data generated were analyzed using simple descriptive statistical analysis, such as a frequency bar chart and a pie chart, to evaluate the percentage of occurrence of isolated bacteria and frequencies of isolate resistance to antibiotics, using Microsoft Office Excel 2010 Software and Statistical Package for the Social Sciences version 20.0 (SPSS Inc., Chicago, IL). Cross tabulation was used to summarize bacterial counts according to the resistance categories. Chi-square was also used to determine the variations at the 5% significance level among the isolates.

RESULTS

Identification of bacterial pathogens in meat. Fifteen bacterial isolates belonging to nine different genera (*Macrococcus caseolyticus, Enterococcus faecium, Enterococcus faecalis, Citrobacter* species, *Bacillus* species, *B. cereus, Bacillus thuringiensis, Bacillus subtilis, Lysinibacillus* sp., *S. aureus, Staphylococcus vitulinus, Staphylococcus equorum, Kurthia* species, *Planomicrobium glaciei,* and *Planococcus antarcticus*) were identified based on ampli-

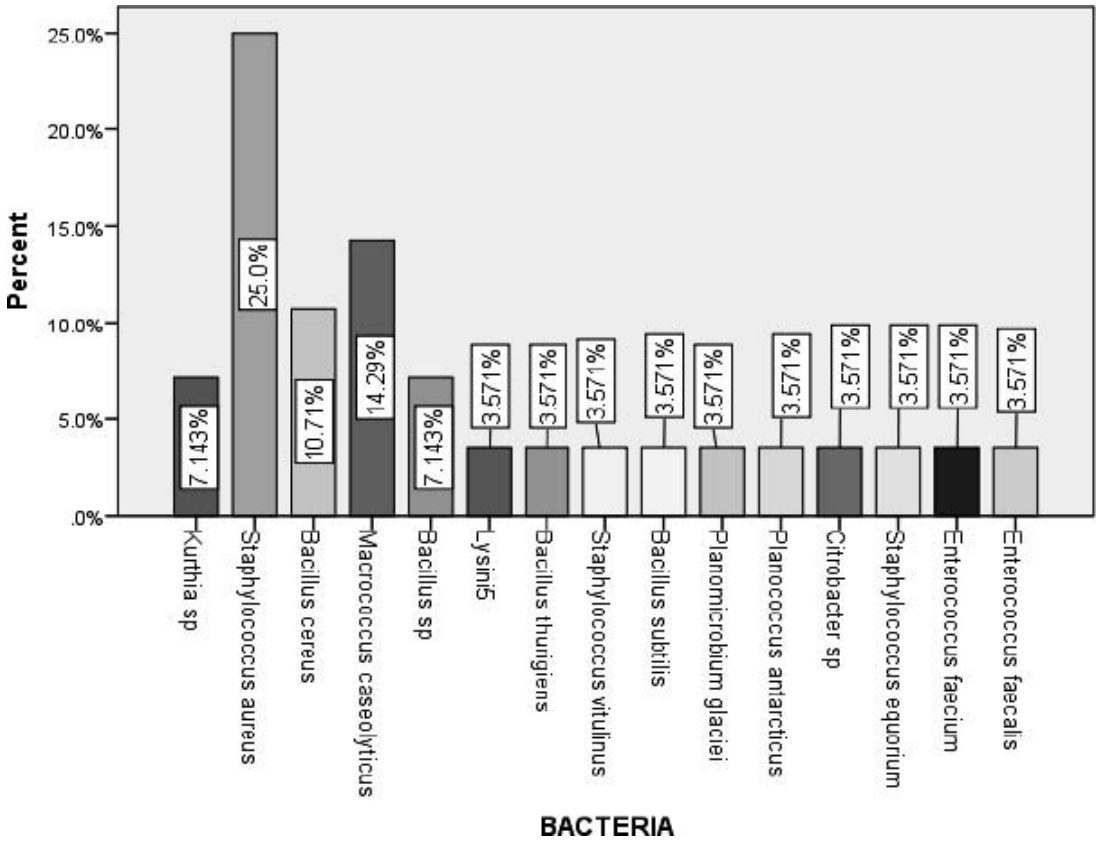

FIGURE 1. *The percentage of occurrence of bacteria isolates in meat samples. Lysini5,* Lysinibacillus *spp.*

fication of the 16S rDNA gene (Table 1). Of the isolates, 25% were confirmed to be *S. aureus,* followed by *M. caseolyticus* (14.3%), *B. cereus* (10.7%), *Kurthia* spp. (7.1%), and *Bacillus* spp. (7.14%), whereas the remaining 10 bacterial isolates had a prevalence of 3.6% each (Fig. 1).

Antibiotic resistance profile. The antimicrobial test for the samples collected around MTN Taxi rank showed that 30% of *B. cereus* and 14% each of *Lysinibacillus* spp., *B. subtilis, P. glaciei, P. antarcticus,* and *E. faecium* were resistant to ampicillin (Fig. 2). It was also observed that 16% of *S. aureus* and *B. cereus* were resistant to tetracycline. *Lysinibacillus* spp., *B. subtilis, P. antarcticus,* and *E. faecium* also showed resistance to tetracycline (17% each) (Fig. 2), whereas 33% each of *P. glaciei* and *Lysinibacillus* spp., and 34% of *S. aureus,* were resistant to sulphonamides. Furthermore, 100% of *P. glaciei* were resistant to chloramphenicol, whereas 25% each of *B. subtilis, B. cereus, P. antarcticus,* and *P. glaciei* showed resistance to erythromycin.

Furthermore, in the samples collected around Bree Street (Fig. 3), 19% of *Kurthia* sp., 18% of *M. caseolyticus,* and 9% each of *S. aureus, B. cereus, Bacillus* sp., *B. thuringiensis, E. faecalis, S. equorum,* and *S. vitulinus* were resistant to ampicillin, whereas 29% of *Kurthia* sp., 15% of *Bacillus* spp., and 14% each of *M. caseolyticus, S. aureus, S. equorum,* and *S. vitilinus* were resistant to tetracycline. The results also revealed that 50% each of both *S. aureus* and *M. caseolyticus* were resistant to sulphonamides, whereas 100% of *E. faecalis* exhibited resistance to ciprofloxacin. Of the *Bacillus* spp., *M. caseolyticus, B. thuringiensis, S. aureus,* and *E. faecalis* isolated in this study, 20% were resistant to erythromycin. Resistance to chloramphenicol was also observed in this study (Fig. 3).

The results of the antimicrobial test for the samples collected around Hancock Street (Fig. 4) showed that 20% each of *S. aureus, Citrobacter* spp., and *Bacillus* spp., and 40% of *M. caseolyticus,* were resistant to ampicillin. *S. aureus* (50%), *M. caseolyticus* (33%), and *Bacillus* species (17%) were resistant to tetracycline, whereas 50% of *Bacillus* spp. and *S. aureus* were resistant to sulphonamides and 100% of *S. aureus* to streptomycin. The result of this study also revealed that 50% of *S. aureus,* 25% of *Bacillus* sp., and 25% of *M. caseolyticus* were resistant to chloramphenicol, and 50% each of *Citrobacter* spp. and *S. aureus* were resistant to erythromycin.

Multiple antibiotic resistances. The bacteria isolates showed different levels of phenotypic resistance to different antibiotics tested. Most of these bacterial isolates showed resistance to more than two of the antibiotics tested. For instance, *S. aureus* showed coresistance to ampicillin, tetracycline, sulphonamides, streptomycin, chloramphenicol, and ciprofloxacin. *B. cereus* also exhibited multiple resistances to ampicillin, tetracycline, and erythromycin, whereas *Bacillus* spp. showed resistance to ampicillin, tetracycline, sulphonamides, chloramphenicol, and ciprofloxacin (Table 2).

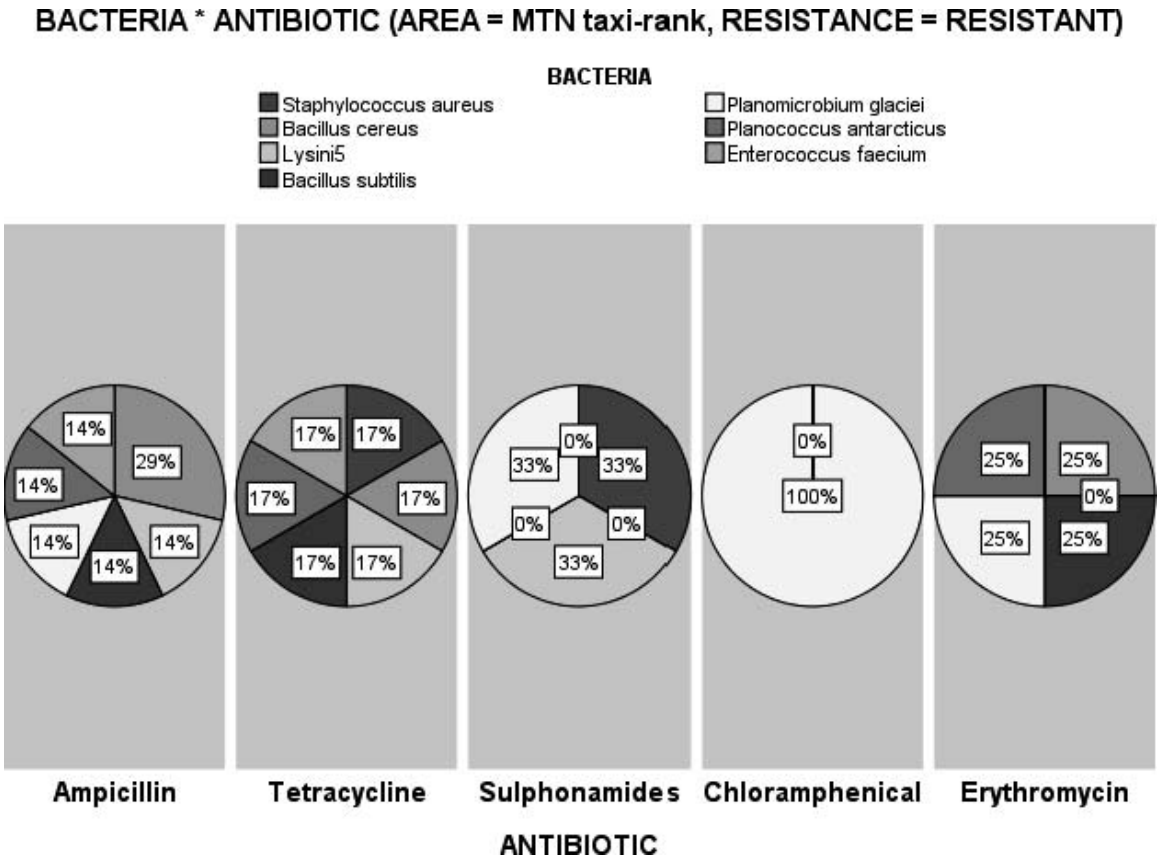

FIGURE 2. *Antibiotic resistance of bacteria isolated in meat samples from MTN Taxi rank. Lysini5,* Liysinibacillus *spp.*

DISCUSSION

Despite the fact that street-vended food is cheap and readily available, there is concern about the quality and safety of such vended foods *(38, 63)*. Most of these foods are prepared in unhygienic circumstances and environments, and most vendors lack an adequate understanding of food safety regulations *(35)*.

Staphylococcal food poisoning is an illness caused by a toxin or poison released by staphylococcus bacteria *(6)*. It is a foodborne intoxication that develops in people who ingest food that has been improperly stored or cooked (such as salad, ice cream, ham, processed meats, chicken, pastries, and hollandaise sauce), in which *S. aureus* has grown. Thus, among the three *Staphylococcus* species isolated in this study, *S. aureus* is the main one implicated in food poisoning.

S. vitulinus and *S. equorum* have been reported in bovine, caprine, and ovine milk and dairy products *(11),* whereas *S. equorum* subsp. *equorum* has been isolated from healthy horses *(59)* and from the milk of a cow with mastitis, as well as from healthy goats *(36)*. However, *S. equorum* subsp. *equorum* strains have been also been reported from human clinical materials *(4)*.

The percentage of *M. caseolyticus* (14.29%) recovered from the meat samples corroborates the findings of Baba et al. *(7)*; *M. caseolyticus* was also found to occur in various animal products and parts, such as cow's milk, bovine organs, and from swabs of chicken skin *(62)*. Although *Micrococcus* spp. are generally regarded as harmless saprophytes that inhabit or contaminate the skin, mucosa, and perhaps the oropharynx, they can be opportunistic pathogens for the immunocompromised *(8)*. They have been associated with various infections, including bacteremia, continuous ambulatory peritoneal dialysis peritonitis, and infections associated with ventricular shunts and central venous catheters *(34)*.

Among the different *Bacillus* species (*cereus, subtilis,* and *thuringiensis*) isolated from the meat samples, *B. cereus* has been implicated in food spoilage and foodborne illnesses *(21). B. cereus* is widespread in nature and is readily found in soil, where it adopts a saprophytic life cycle, germinating, growing, and sporulating *(17, 62)*. The incidence of *B. cereus* has also been reported in a study of the microbial quality of street foods in Botswana and other countries *(40). B. cereus* is recognized worldwide as a foodborne bacterium that causes infections that account for a significant proportion of reported foodborne diseases with known etiology *(39)*. Its presence in different food types emphasizes the public health importance of this organism, especially because this bacterium causes not only food poisoning, which is usually self-limiting, but also gastrointestinal infections, which are life-threatening, particularly in immunocompromised patients *(41)*.

The antibiotic tests performed on the selected isolates revealed varying degrees of resistance to different antibiotics. Most of the bacterial isolates were found to be resistant to ampicillin, tetracycline, and chloramphenicol. The multidrug resistance exhibited by the organisms might be due to cross-contamination from the environment. Foods

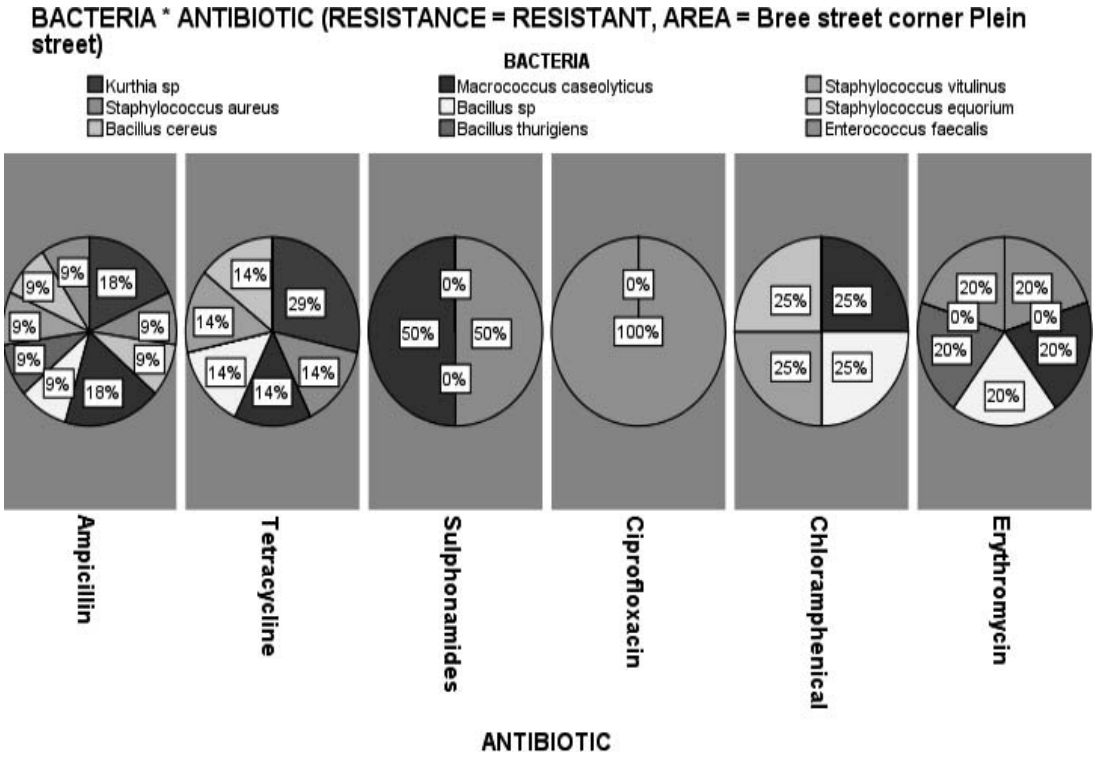

FIGURE 3. *Antibiotic resistance of bacteria isolated in meat samples from Bree Street.*

contaminated with multidrug-resistant microorganisms could be potential vehicles for the transmission of foodborne infections among consumers and could cause serious public health problems. Darwish and co-workers *(22)* also reported a high resistance of *S. aureus* to tetracycline; however, *S. aureus* was resistant to at least six antimicrobial agents in this report. *S. aureus* isolated from meat and poultry has been reported to have multidrug resistance to ampicillin and erythromycin *(1, 65)*.

It was also observed that *M. caseolyticus* strains were resistant to at least five antimicrobial agents, namely, ampicillin, tetracycline, sulphonamides, chloramphenicol,

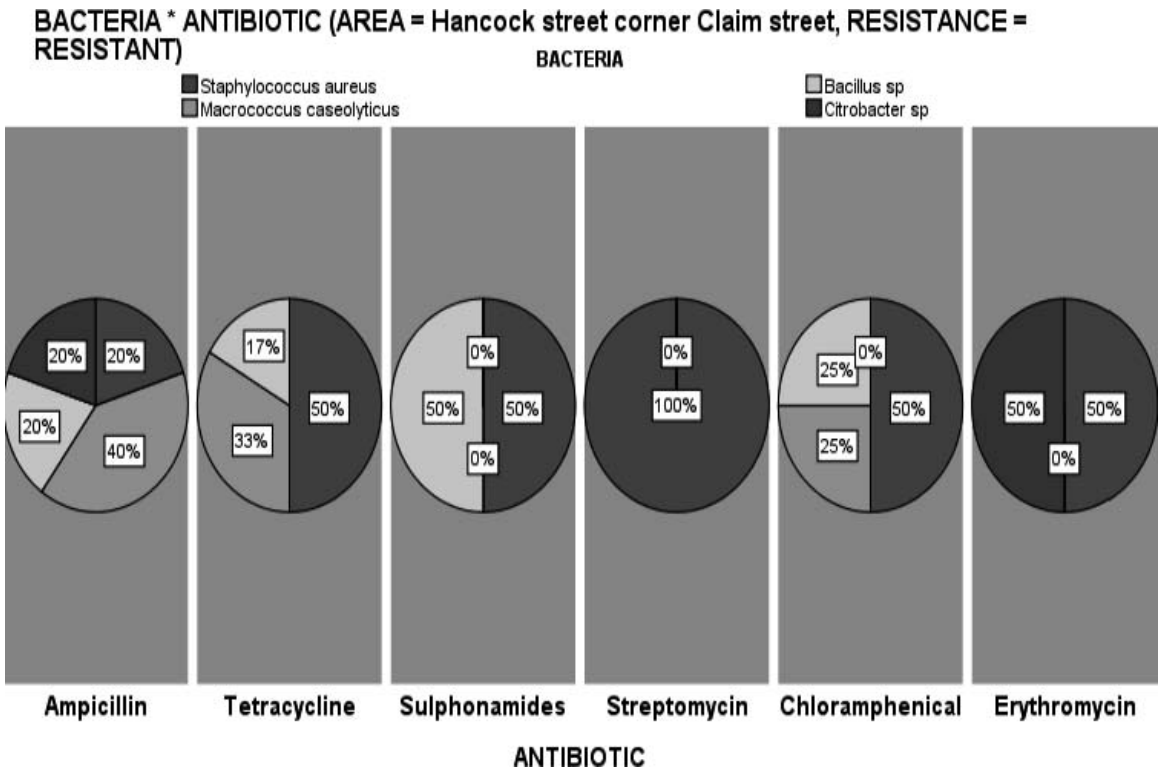

FIGURE 4. *Antibiotic resistance of bacteria isolated in meat samples from Hancock Street.*

TABLE 2. *Multidrug resistance profile of bacteria isolates*

Antibiotic resistance profile of:							
Kurthia spp.	*Bacillus thuringiensis*	*Bacillus cereus*	*Staphylococcus vitulinus*	*Lysinibacillus* spp.	*Bacillus* spp.	*Macrococcus caseolyticus*	*Staphylococcus aureus*
Ampicillin Tetracycline	Ampicillin Ciprofloxacin	Ampicillin Tetracycline Erythromycin	Ampicillin Tetracycline Chloramphenicol	Ampicillin Tetracycline Sulphonamides	Ampicillin Tetracycline Sulphonamides Chloramphenicol Ciprofloxacin	Ampicillin Tetracycline Sulphonamides Chloramphenicol Ciprofloxacin	Ampicillin Tetracycline Sulphonamides Streptomycin Chloramphenicol Ciprofloxacin

and ciprofloxacin. This result is also in line with previous studies that found that *M. caseolyticus* was resistant to ampicillin, erythromycin, and tetracycline *(7, 25)*.

Furthermore, several studies have reported that *B. cereus* isolated from meat or chicken was resistant to different antibiotics, such as ampicillin and tetracycline. Noor Uddin et al. *(45)* reported that *Bacillus* spp. were resistant to ampicillin and erythromycin, whereas Turnbull et al. *(61)* reported the resistance of *B. cereus* isolated from clinical and environmental sources to ampicillin and tetracycline. The findings of these authors complement our report that *B. cereus* was resistant to at least three antimicrobial agents: ampicillin, tetracycline, and erythromycin.

In addition, other *Bacillus* spp. isolated from the different meat samples were found to be resistant to at least five antimicrobial agents (e.g., ampicillin, tetracycline, sulphonamides, chloramphenicol, and ciprofloxacin); this confirms the findings of Sadashiv and Kaliwal *(54),* who also reported the resistance of *Bacillus* spp. to ampicillin, tetracycline, and chloramphenicol.

The emergence of drug resistance is one of the most serious health problems in developing countries *(13, 46)*. Recurrent usage of antibiotics in medicine and in food of animal origin production has increased the prevalence of bacterial strains that are resistant to different antimicrobial agents *(14, 46)*.

In conclusion, this study revealed that the high prevalence of foodborne pathogens and antibiotic-resistant strains of bacteria in RTE meat is a potential threat to public health. This calls for intervention from appropriate governmental agencies and stakeholders to ensure food security and safety of the masses in South Africa. The relatively high multidrug resistance exhibited by these bacteria is also of concern: it will be difficult for infected consumers to be treated, and infected consumers may need to pay more to buy more sensitive or imported antibiotics to be healed. Strict intervention strategies should be put in place by government agencies to educate and enforce the correct administration of antibiotics to livestock by farmers to avoid misuse and abuse of antibiotics.

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