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The Utility of MALDI-TOF-Mass Spectrometry, Analytical Profile Index (API) and Conventional-PCR for the Detection of Foodborne Pathogens from Meat

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Abstract The rich nutrient composition of meat and its sufficient water activity supports the growth of both spoilage and pathogenic bacteria. The microbial quality of 150 raw meat samples (50 chicken, 50 pork and 50 beef) were evaluated for foodborne pathogens using Analytical Profile Index (API Staph-Ident kit, API-20E strips and API-20A), MALDI-TOF-MS, and conventional PCR. Thirty isolates (30) were identified using biochemical tests and Confirmatory biochemical test (API). All 30 isolates were confirmed by *16S rDNA* PCR and sequencing. Detected isolates are *Macrococcus caseolyticus* 57%, *Enterococcus faecalis* 23%, *Bacillus cereus* 3%, *Enterococcus mundtii* 3%, *Escherichia coli* 3%, *Citrobacter freundii* 3%, and *Clostridium subterminale* 3%. Seven (23%) isolates were correctly identified using MALDI-TOF MS, whilst 18 (60%) isolates were misidentified, and 5 isolates were not detected (No peaks). Seventy three percent 73% (22) of the organisms showed high degree of resistance to tetracycline, followed by sulphonamide with 13% (4/30), and ciprofloxacin 3% (1/30). However, *Bacillus cereus*, *Citrobacter freundii* were not resistant to any of the antibiotics. All the isolates were susceptible to streptomycin. Furthermore, some bacteria exhibited multiple antibiotic resistances with tetracycline. Due to the presence and potential hazard of pathogens in meat samples, the detection of these pathogens in different types of meat is vital to maintain public health.

Keywords: *MALDI-TOF MS, API, PCR, antimicrobial resistance, meat contamination, food safety*

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1. Introduction

Meat, a rich source of protein, low in carbohydrate content and with sufficient water activity, supports the growth of both spoilage and pathogenic bacteria [1]. Food-borne illness caused by microbial contamination of foods is an important international public health problem and is known to be a major cause of diarrheal diseases especially in developing countries [2]. Different methods have been used to detect microorganisms in food [3].

One of those methods is MALDI-TOF mass spectrometry (MS) which has been used in the analysis of different biomolecules for decades and its application to microbiological diagnosis seems to be promising valuable screening and rapid identification method [4,5,6,7]. The polymerase chain reaction (PCR) has revolutionized quantification and detection of pathogens. This assay is a useful device to conquer time-consuming assays [8] and its technique is rapid, specific, and more sensitive [9]. PCR assays are now extensively used in the detection and identification of microorganisms [10,11]. Therefore, it can be used as a trustworthy means of identification and detection of microorganisms in resource-limited countries [10,11,12].

Veterinary antimicrobial use is a selective force for the appearance and prevalence of antimicrobial resistant bacteria in animal products [13]. Despite obvious benefits, improper use of different classes of antibiotic causes bacterial resistance against infectious diseases [14]. Some antibiotics like Tetracycline, undergo minimal metabolism and they are mainly excreted in urine and faeces in their microbiologically inactive forms. They are widely distributed in the body with the highest concentrations of residue being found in kidney and liver [15]. This tissue

distribution pattern is comparable in all food producing animals [15,16].

This study was aimed at evaluating foodborne pathogens in meat samples using Analytical Profile Index (API), MALDI-TOF MS, and PCR and to assess the microbial and antibiotic profile of the three major types of meat (beef, chicken and pork) sold at the butcheries and supermarkets around city of Mafikeng in North West Province of South Africa.

2. Materials and Methods

2.1. Sampling

A total of 150 raw meat (muscle, liver and kidney) samples consisting of chicken (50 specimen), pork (50 specimen), and beef (50 specimen), were randomly collected in butcheries and supermarkets around Mafikeng, the capital city of the North West province and were packed in properly labelled sterile polyethylene bags and transported under a complete aseptic condition in an icebox to the laboratory. All samples were collected within the recommended dates for consumption. The samples were kept in -20°C freezer until further processing.

2.2. Bacterial Isolation from Samples

2.2.1. Bacterial Culturing

Twenty-five grams (25 g) of each sample was chopped by sterile blade on cutting board and transferred to 225 mL of Nutrient Broth (NB) and mixed homogenously for 5 min, the samples were enriched overnight and incubated at 37°C. After 24 hrs, a sterile wire loop full of broth was streaked onto different media including Mannitol salt agar (HiMedia®, India), MacConkey (Acumedia LAB, Neogen culture media, Heywood, Lancashire, UK), and Nutrient agar (Merck, Wadeville, South Africa) then incubated at 37°C for 24 hrs. The obtained isolates were purified by further sub-culturing and observed for presumptive identification based on their morphological characteristics and various biochemical tests [17]. The Bergey's Manual of Systematic Bacteriology [18] was used to characterize different isolates. Bacterial colonies with dissimilar morphology were selected and purified on nutrient agar for API, DNA extraction and MALDI-TOF MS.

2.2.2. Primary Biochemical Test

Gram-staining, Voges Proskauer, urease, catalase, oxidase, indole, and coagulase test methods were done as previously reported by other authors [17,19,20].

2.3. Confirmatory Biochemical Tests

Isolates that satisfied morphological structures and primary biochemical tests were confirmed using the Analytical Profile Index (API) API Staph kit (BioMe´rieux, Marcy-l'Etoile, France), API-20E bioMérieux Vitek, St. Louis, MO, USA) and API-20A (BioMérieux, Marcy-L'Etoile / France). The test was performed according to the manufacturer's instructions.

2.4. MALDI-TOF for Identification of Bacteria

After purification of the isolates, 5-10 mg of colonies were harvested and submitted to the Department of Microbiology and Plant Pathology (University of Pretoria) for analysis. To identify isolated bacteria, matrix assisted laser desorption/ionisation time-of-flight MALDI-TOF-MS (Bruker Daltonik GmbH, Germany) analysis was used.

2.5. Molecular Characterization of the Isolates

2.5.1. Extraction of Genomic

Pure isolates from the Nutrient agar were inoculated into 20 mL of nutrient broth aerobically at 37°C for 24 hrs while shaking. Total genomic DNA of cultivated isolates was extracted following the manufacture's recommendation (Zymo-Research Fungal/Bacterial Soil Microbe DNA MiniPrep kit,USA). Extracted DNA was eluted with 100 µL of DNA elution buffer into a 1.5 mL microcentrifuge tube and stored at -20°C until PCR was performed. The DNA concentration was determined by using NanoDrop ND-1000 UV spectrophotometer (Thermo-Fisher Scientific Inc., USA) with a wavelength of 260 nm. The extracted DNA was kept in −20°C until PCR was performed.

2.5.2. Amplification of *16S rDNA* Gene and Sequencing

Amplification of the *16S rDNA* gene was carried out by polymerase chain reaction using an Engine DYAD Peltier thermal cycler (Bio-Rad, Johannesburg, South Africa). Reaction volume of 25 µL, containing: 12.5 µL of 10× PCR Master Mix, 1 µL template DNA, 8.5 µL nuclease-free water and 1 µL each of oligonucleotide primer, and nuclease-free water was used as negative control. PCR of endophytic bacteria was done by using the universal primers: forward 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and reverse 1492R (5'-TGA CTG ACT GAG GCT ACC TTG CGA-3'). PCR conditions consisted of an initial denaturation step at 95°C for 5 min followed by 35 cycles of denaturation at 94°C for 30 sec, annealing at 61°C for 30 sec and extension at 72°C for 5 min, followed by single final extension step at 72°C for 7 min and infinite hold at 4°C. Amplified PCR products were resolved in 1% agarose gel discoloured with ethidium bromide (0.1 µg/ml) and visualized with Syngene Ingenius Bioimager (UK) to confirm the expected size of the product.

Purified PCR positive fragments of the *16S rDNA* were sent for sanger sequencing at Inqaba Biotechnical Industrial (Pty) Ltd. (Pretoria, South Africa). The acquired sequences were aligned against GenBank database using nucleotide Basic Local Alignment Search Tool (BLASTn) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) from the National Center for Biotechnology Information (NCBI) database to identify sequences with high similarity.

2.6. Antibiotic Susceptibility Profiles of the Isolates

Fresh overnight cultures were prepared and used for antibiotic sensitivity tests. The discs diffusion method was

conducted on a Mueller Hinton agar (Biolab, supplied by Merck). Six (6) mm filter paper disk impregnated with a known concentration of an antimicrobial compound was placed on a Mueller-Hinton (MH) agar and was incubated aerobically at 37°C for 24 hrs. Antibiotic susceptibility profiles of different species isolated were tested against four different antimicrobial agents and outcomes reported as percentages. Antibiotic susceptibility pattern was determined by Kirby Bauer disc diffusion method [21] using a wide range of commonly used antibiotics including Streptomycin (300µg), Tetracycline (30µg), Sulphonamides (300µg) and Ciprofloxacin (5µg) and they were obtained from Davies's diagnostics (Davies's diagnostics, SA). The inhibition zone was measured in millimeters to interpret Sensitive, Intermediate or Resistance in accordance with the guidelines of the Clinical Laboratory Institute Standards.

3. Results

3.1. The Detection of Isolates Using API, MALDI-TOF MS and PCR

Thirty isolates were identified and confirmed through MALDI-TOF MS, Analytical Profile Index (API) and PCR technique. Preliminary results from primary biochemical tests were confirmed by the Analytical Profile Index strips test for the identification of isolates. The positive isolates at the API-Ident were 98.9% likelihood level. Sequencing *16S rDNA* gene and all 30 isolates showed 96%−100% identity according to BLASTn sequence homology results. All the sequences were deposited to the GenBank database to obtain accession numbers (MG543814 - MG543843) indicated in Table 1. Thirty isolates (30) identified in this study are as follows: *Macrococcus caseolyticus* 57% (17/30), *Enterococcus faecalis* 23% (7/30), *Bacillus cereus* 3% (1/30), *Enterococcus mundtii* 3% (1/30), *Escherichia coli* 3% (1/30), *Citrobacter freundii* 3% (1%) and *Clostridium subterminale* 3% (1/30). Seven (23%) isolates were correctly identified using MALDI-TOF MS, 18 (60%) isolates were misidentified. This technique was unable to provide identification or similarity for five isolates (M5, M60, M102, M16Y and M85) i.e. there were no peaks for such samples.

3.2. Frequency of Occurrence

Seven different genera of bacteria were isolated from the meat sample with *M. caseolyticus* having the highest occurrence of 30%, 17% and 10% found in the liver, muscle and kidney of the meat samples respectively. Followed by 13% occurrence of *E. faecalis* in the liver and 10% for muscle. Seven percent occurrence of *B. cereus* was found in the liver. The remaining four genera had only 3% occurrence each in the different parts of the meat (Table 2).

Table 1. Results for 30 isolates; MALDI-TOF and 16S sequencing (PCR)

Isolates	MALDI-TOF identity	API identification (% assurance)	*16S rDNA* gene (PCR)	Acc. No. in GenBank	Assigned accession numbers
M49	*M. caseolyticus*	*M. caseolyticus*	*M. caseolyticus*	NR074941	MG543814
M44Y	*S. condimenti*	*E. faecalis*	*E. faecalis*	KJ726743	MG543815
M5	NP	*M. caseolyticus*	*M. caseolyticus*	NR074941	MG543816
M105	*Escherichia coli*	*Bacillus cereus*	*Bacillus cereus*	KP100327	MG543817
M77	*Hafnia alvei*	*Bacillus cereus*	*Bacillus cereus*	KJ882426	MG543818
M60	NP	*Clostridium* spp.	*C. subterminale*	NR113027	MG543819
M73	*Hafnia alvei*	*M. caseolyticus*	*M. caseolyticus*	NR074941	MG543820
M70	*Hafnia alvei*	*M. caseolyticus*	*M. caseolyticus*	NR074941	MG543821
M3Y	*Hafnia alvei*	*M. caseolyticus*	*M. caseolyticus*	NR074941	MG543822
M3W	*Hafnia alvei*	*E. faecalis*	*E. faecalis*	KF598930	MG543823
M100	*Proteus vulgaris*	*Enterococcus* spp.	*E. faecalis.*	JF799879	MG543824
M48	*Hafnia alvei*	*E. faecalis*	*E. faecalis*	DQ118026	MG543825
M4W	*C. braakii*	*M. caseolyticus*	*M. caseolyticus*	NR074941	MG543826
M71	*M. caseolyticus*	*M. caseolyticus*	*M. caseolyticus*	NR074941	MG543827
M17	*Hafnia alvei*	*M. caseolyticus*	*M. caseolyticus*	NR074941	MG543828
M16W	*M. caseolyticus*	*M. caseolyticus*	*M. caseolyticus*	NR0749419	MG543829
M44W	*M. caseolyticus*	*M. caseolyticus*	*M. caseolyticus*	AB898316	MG543830
M91	*Hafnia alvei*	*Enterococcus* spp.	*E. faecalis.*	JF799879	MG543831
M86	*K. oxytoca*	*E. faecalis*	*E. faecalis*	NR074941	MG543832
M61Y	*Hafnia alvei*	*E. faecalis*	*E. faecalis*	KP090135	MG543833
M102	NP	*M. caseolyticus*	*M. caseolyticus*	FJ263452	MG543834
M83	*M. caseolyticus*	*M. caseolyticus*	*M. caseolyticus*	NR074941	MG543835
M16Y	NP	*M. caseolyticus*	*M. caseolyticus*	NR074941	MG543836
M46	*M. caseolyticus*	*M. caseolyticus*	*M. caseolyticus*	NR074941	MG543837
M96	*M. caseolyticus*	*M. caseolyticus*	*M. caseolyticus*	NR074941	MG543838
M74	*C. braakii*	*Escherichia coli*	*Escherichia coli*	KF917161	MG543839
M13	*M. caseolyticus*	*M. caseolyticus*	*M. caseolyticus*	NR074941	MG543840
M85	NP	*M. caseolyticus*	*M. caseolyticus*	NR074941	MG543841
M56	*M. caseolyticus*	*C. freundii*	*C. freundii*	KM509080	MG543842
M12	*Escherichia coli*	*E. mundtii*	*E. mundtii*	AB898311	MG543843

NP= No pick.

Table 2. Number of isolates and their percentages isolated from different organs: Liver, muscle, and kidney

Organisms	No. of Isolates (%)	Liver	Muscle	Kidney
Macrococcus caseolyticus	17 (57%)	9 (30%)	5 (17%)	3 (10%)
Enterococcus faecalis	7 (23%)	4 (13%)	3 (10%)	–
Enterococcus mundtii	1 (3%)	1 (3%)	–	–
Clostridium subterminale	1 (3%)	–	–	1 (3%)
Bacillus cereus	2 (7%)	1 (3%)	1 (3%)	–
Escherichia coli	1 (3%)	1 (3%)	–	–
Citrobacter freundii	1 (3%)	1 (3%)	–	–

3.3. Antibiotic Susceptibility

The results in Table 3 show the antimicrobial profile of eight isolates which showed a high resistance rate to tetracycline; *M. caseolyticus* 53.3% (16/30), followed by *E. faecalis* 13% (4/30), *E. mundtii* 3.33% (1/30) then *E. coli* 3.33% (1/30). Ten percent (3) of *M. caseolyticus* and *E. faecalis* 3.33% (1/30) were resistant to sulphonamide. One isolate (*E. faecalis*) was resistant to ciprofloxacin. However, *B. cereus, C. freundii* were not resistant to any of the tested antibiotics. Out of 30, 53.3% was for a non-pathogenic *M. caseolyticus*, while 20% was for the pathogenic, which is an indication that the overall level of resistance was low from the pathogenic bacteria. All the isolates were susceptible to streptomycin.

Table 3. Resistance, susceptible and intermediate resistance patterns of microorganisms in the meat samples to sulphonamides, tetracyclines, ciprofloxacin and streptomycin

S−ID	Microorganisms	Organ/Area	Streptomycin S_{300}	Tetracycline TE_{30}	Sulphonamide $S3_{300}$	Ciprofloxacin CIP_5
M49	*M. caseolyticus*	L^{B1}	S	R	S	S
M44Y	*E. faecalis*	M^{B1}	S	R	S	S
M5	*M. caseolyticus*	L^{B1}	S	R	S	S
M105	*Bacillus cereus*	K^{S}	S	S	S	S
M77	*Bacillus cereus*	L^{B1}	S	I	S	S
M60	*C. subterminale*	M^{B2}	S	S	S	S
M73	*M. caseolyticus*	M^{B2}	S	R	S	S
M70	*M. caseolyticus*	M^{S}	S	R	S	S
M3Y	*M. caseolyticus*	L^{B2}	S	R	S	S
M3W	*E. faecalis*	L^{B2}	S	S	R	S
M100	*E. faecalis.*	L^{S}	S	S	S	S
M48	*E. faecalis*	L^{S}	S	S	S	S
M4	*M. caseolyticus*	M^{B1}	S	R	R	S
M70	*M. caseolyticus*	M^{B2}	S	R	R	S
M17	*M. caseolyticus*	M^{B2}	S	R	R	S
M16	*M. caseolyticus*	K^{B2}	S	R	S	S
M44	*M. caseolyticus*	K^{S}	S	S	S	S
M91	*E. faecalis.*	L^{B1}	S	R	S	R
M86	*E. faecalis*	M^{B2}	S	R	S	S
M61Y	*E. faecalis*	M^{B2}	S	R	S	S
M102	*M. caseolyticus*	K^{B2}	S	R	S	S
M83	*M. caseolyticus*	L^{B2}	S	R	I	S
M16Y	*M. caseolyticus*	M^{S}	S	R	S	S
M46	*M. s caseolyticus*	L^{S}	S	R	S	S
M96	*M. caseolyticus*	L^{B1}	S	R	S	I
M74	*Escherichia coli*	L^{B2}	S	R	S	S
M13	*M. caseolyticus*	M^{B2}	S	R	S	S
M85	*M. caseolyticus*	L^{S}	S	R	S	S
M56	*C. freundii*	K^{B1}	S	S	S	S
M12	*E. mundtii*	L^{B1}	S	R	S	I

S−ID= sample Identity, **R**= Resistance, **I**= Intermediate and **S**= Susceptible, **L**= liver, **M**= muscle, **K**= kidney, ^{B1}= butchery1, ^{B2} =butchery 2, ^{S}= supermarket.

4. Discussion

In this study, biochemical tests, MALDI-TOF and PCR identifications were performed in order to identify organisms. A combination of PCR and sequencing showed accuracy for identification of 30 microbes whereby *M. caseolyticus*, *E. faecalis*, *B. cereus*, *E. mundtii*, *E. coli*, *C freundii*, and *C. subterminale* were detected from meat samples. However, MALDI-TOF only detected 27% (*M. caseolyticus*) isolates which were correctly identified. Previous studies of Bagge et al., [22] and Nucera et al. [23], also reported that PCR was accurate as a diagnostic test for bacterial identification while MALDI-TOF identification was not a reliable and efficient identification method. They indicated that both API and PCR (at the 99.9% likelihood level) were demonstrated to be accurate diagnostic tests for bacterial identification. Therefore, validation of both PCR and API as accurate diagnostic tests suggests that either of them can be used with similar results. The advantage of PCR is that positive products were sequenced and the obtained *16S rDNA* gene sequences were aligned in BLASTn to find their matching sequences on NCBI GenBank database, which insured accurate identification of detected bacteria. The *16S rDNA* gene sequence allows for a superior identification of poorly described and rarely isolated strains [24]. This molecular technique is routinely used in the food industry [20], microbial ecological studies [25], clinical studies [26], for the identification of novel pathogens and uncultured microbes [17].

Despite not being accurate in this study, MALDI-TOF MS has been used detect a large number of bacterial species based on their protein fingerprint mass spectra [7,27]. However, it is unable to differentiate between related species such as *E. coli* from *Shigella* spp. [28]. In some cases, where the MALDI-TOF MS cannot generate the results, it is because the relevant species information is not obtained from the database [28,29,30]. Therefore, the reliability of detection can be attained by placing new reference spectra in the database [31,32,33]. In addition, Abd El-Aziz at al [34] also reported that MALDI-TOF MS is unable to detect all organisms in polymicrobial infections.

The *M. caseolyticus* has been detected in this study with the highest percentage incidence in the meat sample (Liver, kidney and muscle). It has been implicated as a food spoilage causative organism and is also known as indicator of faecal contamination which suggest poor handling and hygiene status [35]. Bacteria such as *M. caseolyticus* originate from animal sources such as cows, in food-processing factory and it can also be isolated from animal skin and meat, and has also been isolated from a skin swab of chicken in Japan [36].

Despite isolating different organisms, pathogenic organisms were also isolated. The relatively high frequency of occurrence of pathogenic organisms; *E. mundtii*, *Bacillus* spp., *Bacillus cereus*, *Enterococcus* spp. and *Escherichia* spp. *E. coli* can be pathogenic both within and outside the gastrointestinal tract. Although the frequency of *E. coli* in the samples was low (3%), its presence in the meat sample (Liver) tested in this study calls for concern as the presence of the organism in foods is linked to feacal contamination. The findings of this study concur with previous studies conducted by Bahadoripour *et al*. [37], Kiranmayi, and Krishnaiah [38] whereby 26% and 11% samples were contaminated with *E. coli* respectively. Moreover, in a study conducted in Ahvaz, South West of Iran, 28% *E. coli* was detected in beef [39]. This is contrast to what was previously reported by Ali et al., [40] in Pakistan who detected *E. coli* (35%) as the first predominant bacterial isolates from hotels, butchery and abattoir in raw meat sold in Pakistan. Additionally, similar study in Nigeria [41] recorded 13.3% occurrence of *E. coli* as the second major bacterial isolates in street-vended-ready-to-eat fish.

The other pathogenic isolate detected in this study is *Enterococcus faecalis* which is the third most generally isolated healthcare pathogen [42]. The frequency of *E. faecalis* was higher in the muscles (13%) followed by liver (10%). While *E. mundtii* carriers was only found in the liver (3%) These species are capable of causing a range of infections such as sepsis, endocarditis, and urinary tract infections in humans [43,44]. So far more than ninety percent (90%) of enterococcal infections are caused by two species: *E. faecium* and *E. faecalis [42]*. Enterococci have been recognised as an important cause of nosocomial infections, the most common cause of this is *E. faecalis* and *E. faecium* [45]. Isolation of these organisms should be taken as a considerable threat to the human health.

B. cereus whose occurrence is 3% for both muscle and liver samples, is an opportunistic pathogen, one of the causes of food poisoning as shown by the emetic syndrome [46]. Foods that are rich in protein have been associated with food-borne outbreaks of diarrhea caused by *B. cereus* [47].

The *C. subterminale* is one of the species of *Clostridium* that is most often isolated from soil [48]. However, study conducted by Miyazaki et al. [49], showed that *C. subterminale* was detected from a person who went for umbilical cord blood transplantation. Therefore, *C. subterminale* should be considered as one of the causal agents of septicaemia. Moreover, in 2011 *C. subterminale* was also detected from an adult with acute lymphoblastic leukemia [50]. The case of *C. subterminale* septicemia in an immunocompetent patient who presented with acute mediastinitis following spontaneous esophageal rupture was also reported by Daganou et al. [51], it was also detected from a patient with esophageal cancer [52].

The resistance shown by a large percentage of the organisms; *M. caseolyticus*, *E. faecalis*, *E. mundtii*, and *E. coli* to tetracycline in this report coincides with previous reports from various studies. In Iran, 69% of isolates from retail chicken and beef were resistant to tetracycline [53]. In Tai'an, China, 78% of the isolates from rabbit showed resistant against tetracycline [54]. The study conducted in South Africa (Eastern Cape); 43.8% *E. coli* isolates obtained from meat samples were resistant to tetracycline [55]. It was also reported that *E. coli* isolated from some broiler chicken also showed a high level of resistance to tetracycline (36 to 97%) in northeast Georgia [56]. The high prevalence of isolates resistant to tetracycline, which is moderately cheaper in the market is of concern because of the limited access and high cost of antibiotics such as quinolones [57]. It was observed in this research work that meat samples from the butcheries exhibited multi drug resistance.

Despite the resistance of other antibiotics from different isolates, all isolates from this study were susceptible

to streptomycin. This is a relief to human beings as streptomycin is well-known as anti-tuberculosis medication [58]. However, results obtained in this study differ from other studies conducted in Addis Ababa, Ethiopia where 58% of streptomycin were found in raw and ready-to-eat meat [59]. The greater the number of resistant bacteria in different meat organs, the higher is the possibility for the transfer of the encoding resistant genes to pathogenic bacteria and their distribution in the environment and foods of animal origin. The acquisition of bacteria resistant to aminoglycoside antibiotics is associated with modification of enzymes such as aminoglycoside adenyltransferases which are responsible for resistant streptomycin antibiotics [60].

5. Conclusion

This study revealed that Analytical Profile Index (API) and PCR are very useful tool for the detection of pathogenic and non-pathogenic bacteria. The relatively high frequency of occurrence of pathogenic organisms; *B. cereus*, *Enterococcus* spp. and *Escherichia* spp. and pathogenic organisms observed in this study are among the causes of food-borne illnesses and danger to public health. It was observed that majority of bacteria were isolated on meat from the butcheries than supermarket. The other objective of this study was to evaluate microbial susceptibility to different antibiotics commonly used in veterinary medicine. The results obtained from this exercise revealed that of eight detected bacterial species showed resistance to antibiotics including tetracycline, sulphonamides and ciprofloxacin. However, *B. cereus*, *C. freundii* were not resistant to any of the antibiotics. Continuous exposure at low doses might induce mutability in both animals and consumers. Due to the presence and potential hazard of pathogens in meat samples, the detection of these pathogens in different kinds of meat is vital to maintain public health. We recommend stricter control of antibiotics use to meat animals and higher hygienic practices at the butcheries from sampled areas.

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Conflicts of Interest

The authors declare no conflicts of interest.

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