Journal of Food and Nutrition Research, 2024, Vol. 12, No. 5, 301-317
Available online at http://pubs.sciepub.com/jfnr/12/5/10
Published by Science and Education Publishing
DOI:10.12691/jfnr-12-5-10

Bacteriological Analysis, Detection, and Comparative Characterisation of Fresh Product-Associated Bacteria As Potential Bacteriophages Hosts

Adelokiki Babawande Williams[1], Awoyemi Olusoji Blessing[1], Aruwa Eleojo Christiana[2,*]

[1]Department of Microbiology, School of Life Sciences, Federal University of Technology, Akure, PMB 704, Ondo State, Nigeria
[2]Department of Biotechnology and Food Science, Faculty of Applied Sciences, Durban University of Technology, PO Box 1334, Durban 4000, South Africa
*Corresponding author: christianaa@dut.ac.za, aruwachristiana@gmail.com

Received April 18, 2024; Revised May 20, 2024; Accepted May 27, 2024

Abstract Foodborne illness poses significant health and economic challenges, costing billions annually. Pathogens in food products arise from various sources (ingredients, poor hygienic practices, environment) and the use of bacteriophages as biocontrol agents may offer promise in reducing bacterial levels in foods without the drawbacks of traditional chemical sanitizers. Thus, the study aimed to isolate bacterial strains from fresh shrimp, turkey, and beef products, and identify strains that are good bacteriophage hosts (using plaque assay), as well as conduct a microbiological safety assessment of the products using standard traditional (total viable bacterial and coliform counts, biochemical characterisation) and molecular (16S rRNA sequencing) methods. It assessed total bacterial and colony counts across the samples, revealing variations between them. Notably, *Paenibacillus pectinilyticus* emerged as the predominant strain, followed by *Raoultella planticola*, *Klebsiella pneumonia*, *Neobacillus niacin*, *Enterobacter asburiae*, and *Serratia grimesii*. Of the seven (7) bacterial strains identified using 16S rRNA molecular characterization, only four showed potential as good phage hosts. As another key finding, emerging pathogens like *S. grimesii*, *E. asburiae* and *R. planticola* were identified from the products for the first time. Again, over 57% of the time probable microbial identities from traditional techniques did not align with molecular identities (99.28-100% identity coverage). Hence, molecular methods cannot be overemphasized in ensuring the accuracy of microbial identities, and detecting emerging pathogens. The presence of emerging pathogens, high coliform and viable bacterial counts further indicated the unsafe nature of these fresh products. The screening for, and identification of food-based bacteriophages may hold promise as alternative bio-control tools against foodborne infections. However, further research is warranted to elucidate their characteristics and efficacy in enhancing food safety.

Keywords: *Food product, Phages, 16S rRNA, Molecular characterization, Paenibacillus pectinilyticus, Raoultella planticola*

Cite This Article: Adelokiki Babawande Williams, Awoyemi Olusoji Blessing, and Aruwa Eleojo Christiana, "Bacteriological Analysis, Detection, and Comparative Characterisation of Fresh Product-Associated Bacteria As Potential Bacteriophages Hosts." *Journal of Food and Nutrition Research*, vol. 12, no. 5 (2024): 301-317. doi: 10.12691/jfnr-12-5-10.

1. Introduction

Foodborne illness (FBI) is a universal problem that impacts public health and global economies [1]. The annual cost linked to FBIs is estimated by the United States Department of Agriculture to be up to $15.5 billion [2]. Given the increasingly intricate need for globalized food supply, it is crucial for the public to have confidence in the safety of the food they consume [3]. Food products can harbour pathogens due to contaminated ingredients, cross-contamination during handling/processing, improper cooking or storage, water contamination, inadequate packaging, and poor hygiene practices [4,5].

Foods and food products are contaminated by bacteria that serve as significant causal agents of foodborne illness, posing substantial risks to public health [6]. Pathogenic species like *Escherichia coli*, *Campylobacter*, *Salmonella*, *Listeria monocytogenes* are commonly implicated [7]. Food safety represents a critical global issue [8], encompassing a spectrum of measures and regulations aimed at guaranteeing that food is devoid of contaminants, hazards, and pathogens that could pose risks to consumer health [9]. Biocontrol in food safety entails the exploitation of naturally existing microorganisms or their derivatives to suppress or manage the proliferation of pathogens or other deleterious microorganisms in food items [10,11]. This strategy capitalizes on the introduction of beneficial microorganisms, such as bacteriophages,

probiotics, or competitive exclusion cultures, which possess the capacity to effectively compete with pathogens for vital nutrients or generate antimicrobial compounds that impede their proliferation [12].

Food safety methods, such as chemical sanitizers and heat treatments may result in microbial resistance and quality degradation challenges, while irradiation encounters consumer acceptance issues [13]. Although hazard analysis and critical control protocols (HACCP) and good manufacturing practices (GMPs) may be complex to implement uniformly, and surveillance systems may suffer from gaps and underreporting, it is integral that a multifaceted approach is used to ensure safety of foods or food products [14]. One of such approaches includes the application of phages. Phages are ubiquitous, abundant, and outnumber bacteria. Phages constitute a part of the normal microflora, and can also be isolated from a range of foods including fruits, vegetables, meat, and dairy [15].

Different studies highlight phage efficacy in reducing contamination levels in foods, including sea foods, meat or poultry products. The Food and Drug Administration (FDA)-cleared phage preparations show promise in reducing bacterial levels on surfaces and directly in foods, presenting a viable alternative to antibiotic treatments [16]. The ability of phage cocktails to target specific bacterial strains and reduce contamination across different food types have also been shown [17]. Using bacteriophages (phages) as a biocontrol method to reduce FBI occurrence offers numerous advantages. Their unparalleled specificity enables precise targeting of pathogenic bacteria while preserving beneficial microbiota, thus ensuring the safety of food products [18]. Generally recognized as safe (GRAS) by regulatory bodies, phages pose no risk to human health, making them a preferable alternative to chemical antimicrobials. By reducing reliance on such chemicals, phages promote the production of minimally processed, and additive-free foods. Their effectiveness in controlling bacterial contamination across various food categories, coupled with their ability to prevent antibiotic resistance development underscores their importance in ensuring food safety [19]. In addition, food hygiene and safety levels assessment before and after purchasing or processing foods and food products from varied environments remain relevant parts of quality checks in the food industry [20]. The methods used in analysing foods to determine whether they meet set quality or microbiological standards have also evolved to include molecular techniques [polymerase chain reaction (PCR) and sequencing] since conventional methods are quite laborious and time consuming [21].

The foregoing, and aforementioned benefits of phages have thus informed the screening of varied fresh food product samples for potential bacteriophages detection. This is in a bid to increase reports on their identifications and potential use and exploration in the food industry for safety applications. In addition to this, the microbiological quality (viable and coliform counts, frequency across samples) the food products were also assessed. As such, the aim of this study was to microbiologically analyse fresh beef, shrimp, and turkey samples, and screening for presence of bacteriophages and their host bacteria associated with the products using conventional and molecular bacterial characterization methods. Both methods were thereafter compared to assess their levels of accuracy in bacterial detection and identification. The investigation utilizes a holistic approach towards understanding the ecology of fresh-product-associated bacteriophages, their potential uses for food safety and implications of food-borne bacterial pathogens to public health.

2. Materials and Methods

2.1. Samples Collection and Bacterial Isolation

The samples of each fresh food product (shrimp, turkey, beef) were purchased from food vendors in Akure main market, Ondo State, Nigeria, and transported in sterile polythene bags to the Microbiology laboratory of the Federal University of Technology, Akure, for subsequent analysis. For analysis, 25 g portion each of shrimp, turkey, and beef was homogenized in 225 mL of buffered peptone water, and used in serially dilution under aseptic conditions to achieve the desired dilution factor. For bacterial isolation/culturing, 1 mL of sample from the 5th dilution was transferred into respective and appropriately labelled sterile Petri dishes. Then MacConkey agar (for coliform count) and nutrient agar (for viable bacterial counts) were poured into respective plates and the mixture swirled gently to achieve even distribution of the inoculum. The plates were incubated at 37°C for 24 h in an inverted position to facilitate bacterial growth. Each sample was cultured in duplicate following the pour plate method as described by Martínez-Laorden *et al.* [22]. Following incubation, enumeration of bacterial and coliform counts was done, and then distinct colonies were picked from incubated plates, sub-cultured on fresh agar to obtain pure strains which were then inoculated into sterile agar slants for preservation and identification [23].

2.2. Conventional Identification of Bacterial Isolates

The isolated bacteria were preliminarily identified based on cultural and morphological characteristics and subjected to biochemical tests. The biochemical tests include Gram staining, catalase, indole, motility, Voges-Proskauer, methyl red, casein, nitrate, citrate, lipase, sugar fermentation tests, and hydrogen sulphide (H_2S) production test. These tests aided in preliminary bacterial identification and characterization according to Fawole and Oso [24] (2004). The names of the probable microorganisms were retrieved using the Automated Biometric Information System (ABIS) online software version 12 for bacterial identification (http://www.tgw1916.net).

2.3. Screening for Phage Host – Plaque Assay

Bacteriophage enrichment was conducted using the modified method of Van Twest and Kropinski [25] (2009). Samples were centrifuged at 6000 rpm for 10 min to remove large particulates, and the supernatant was transferred to sterile 15 mL test tubes. A viral suspension was prepared by filtering the supernatant through a 0.8 µm cellulose filter to remove particulates, followed by

filtration through a 0.45 μm filter to eliminate bacterial cells and cellular debris, ensuring enrichment of bacteriophages. The modified phage assay [26,27] was employed to detect phages in food suspensions. The use of a soft agar layer in this double overlay plaque test ensures proliferation of phage particles, and feeding of bacteria from the underlying solidified agar, thus enhancing precision in the enumeration of plaques. Bacterial cultures were standardized to a 0.5 McFarland standard [optical density (OD) of 0.08 at 600 nm), and dispensed into the overlay TS (7%) agar containing 1 mM $CaCl_2$. This was added to varied phage dilutions. This set-up was poured unto the underlay TS (15%) agar which was also enriched with 1 mM $CaCl_2$, left to allow solidification, followed by incubation of Petri plates at 37°C for 24 h. Plaques within the bacterial lawn, depicted as areas of clearing or halos, indicated phage presence.

2.4. 16S rRNA Molecular Identification of Bacterial Phage Hosts

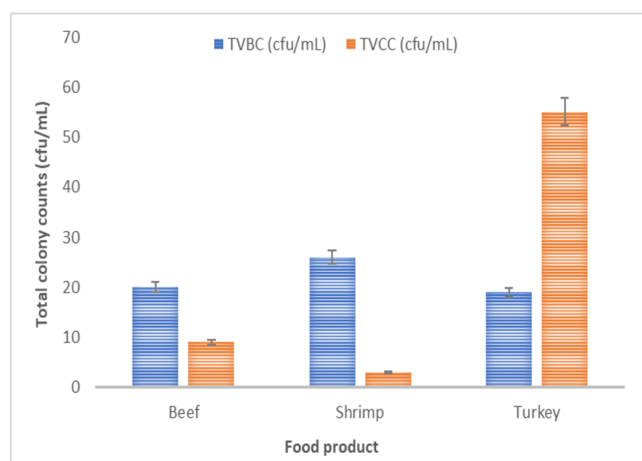
The DNA extraction of bacteria found to be potential phage hosts was performed according to Rijpens and Herman [28]. Single colonies were cultured in liquid medium for 48 h, followed by centrifugation at 4600 ×g for 5 min, and resuspension in tris-EDTA (TE) buffer. Thereafter, proteinase K (3 μl of 20 mg/ml) and sodium dodecyl-sulphate (SDS) (15 μl of 20% SDS) were added to the suspension and incubated for 1 h at 37ºC. This was followed by addition of 5 M NaCl (100 μl) and 10% cetyltrimethylammonium bromide (CTAB) solution (80 μL) in 0.7 M sodium chloride (NaCl), mixture was vortexed and incubated at 65ºC for 10 min, and kept on ice for 15 min. An equal volume of chloroform: isoamyl alcohol (24:1) was added and incubation for 5 min on ice and then centrifuged (7200 ×g for 20 min). The aqueous phase was transferred to a new tube, isopropanol (1:0.6) was added. The DNA was precipitated at –20ºC for 16 h, collected by centrifugation (13000 ×g for 10 min), washed with 70% ethanol (500 μl), airdried at room temperature for 3 h, and finally dissolved in 50 μl of TE buffer.

Polymerase chain reaction (PCR) was conducted using the GeneAmp 9700 PCR System Thermalcycler (Applied Biosystems, USA), specific universal primers (27F 5'AGAGTTTGATCMTGGCTCAG3'; 1525R, 5′AAGGAGGTGATCCAGCC3′) and Taq DNA polymerase (0.3 units) (Promega, USA), with cycling conditions including denaturation, annealing, extension (94°C, 5 min; then 30 cycles of 94°C for 30 s, 50°C for 60s, 72°C for 1 min 30 s), termination (72°C, 10 min). Gel electrophoresis was performed to confirm amplification. Purification of the amplified product involved ethanol precipitation, centrifugation, washing, air-drying, and resuspension in distilled water [29]. The PCR products were run on agarose gel to confirm integrity of the amplified gene fragments. Electrophoresis was done (120 v, 45 min), visualized by ultraviolet (UV) transillumination and photographed. The PCR product sizes were estimated by comparison with the mobility of a molecular weight ladder that was ran alongside experimental samples. The amplified fragments were ethanol purified in order to remove the PCR reagents. Products were resuspended in 20 μl sterile distilled water and kept at 20°C prior to sequencing. The purified fragments were checked on a 1.5% agarose gel at 110 volts for 1 h to confirm presence of the purified product, and quantified using a nanodrop (Thermo Fischer Scientific, Model 2000). Sequencing was done using a Genetic Analyzer 3130xl sequencer (Applied Biosystems, USA) and the BigDye terminator v3.1 cycle sequencing kit (Applied Biosystems, USA), while genetic analysis was performed using BioEdit software and MEGA 6.

3. Results and Discussion

3.1. Food Products Microbial Load, Isolates Biochemical Features, and Distribution

The total viable bacteria count (TVBC) and total viable colony count (TVCC) of the food products ranged from 19×10^{5}cfu/ml (turkey samples) to 26×10^{5}cfu/ml (shrimp samples), while the TVCC varied from 3×10^{5}cfu/ml in shrimp samples to 55×10^{5}cfu/ml in turkey samples. The highest TVCC and TVBC were recorded for turkey and shrimp samples (Figure 1). The biochemical characteristics of bacteria isolated from the food produces were shown in Table 1 with the probable microorganisms identified as *Enterobacter asburiae, Klebsiella planticola, Paenibacillus pectinilyticus, Bacillus subtilis, Klebsiella pneumoniae, Bacillus niacin,* and *Serratia grimesii. Table 2* showed the percentage occurrence of bacteria that were isolated from various food samples. The analysis indicated that *Paenibacillus pectinilyticus* had the highest percentage of occurrences at 38.5%, followed by *Klebsiella planticola, Klebsiella pneumonia*, and *Bacillus niacin*, each with a distribution of 15.4%. However, *Enterobacter asburiae, Bacillus subtilis*, and *Serratia grimesii* each had a percentage distribution of 7.7%.



Total viable colony count of bac (TVBC); Total viable coliform count (TVCC)

Figure 1. Total viable colony count of bacteria and coliforms

The findings presented in this study shed light on the bacterial counts observed in various foods sampled, and provide valuable insights into food safety and quality assessment. The observed range of total viable bacteria count (TVBC) and total viable colony count (TVCC) among the food products underscores the variability in bacterial contamination levels across different food types.

Notably, the highest TVBC and TVCC were found in shrimp and turkey samples, respectively, indicating the presence of potentially harmful bacteria populations in these food products. A similar observation was reported by Nkere *et al*. [30] where harmful presence of *Klebsiella pneumoniae, Enterobacter*, and *Escherichia coli* were detected in vended foods. However, our total coliform counts across samples were higher which may be linked to our conducting TVCC, and not only *E. coli* counts. The authors also reported a bacterial percentage distribution of 45% for *Klebsiella pneumoniae*, 51% for *E. coli*, and 4% for *Enterobacter*. Variations in bacterial distribution trends may be linked to differences in food nature, as their study sampled ready-to-eat processed foods, while our study sampled fresh proteinaceous food products. Overall, in terms of products quality, coliforms are considered as integral indicators of faecal contamination, and the counts obtained in this study are above the tolerable limit of 10^4 cfu/g used in developed world regions and nations [31]. This points to the unhygienic and unsafe nature of these foods. The ingestion of foods with excessive levels and presence of coliforms is linked with diarrhoea, cramps in the abdomen, vomiting, and nausea, among other symptoms [32,33]. As such, their presence in high concentrations even in the absence of common enteric pathogens could result in high incidence of morbidity among persons consuming these foods.

Table 1. Biochemical characteristics of bacteria isolated from different food sources

S/N	Gram	Shape	Spore	Gly	H_2S	Lac	Gluc	MR	VP	Starch	Casein	Lip	Nit	Mot	Ind	Cit	Probable identity
1	-	Rod	-	+	-	+	+	+	-	-	-	-	+	-	-	+	*Enterobacter asburiae*
2	-	Rod	-	+	+	+	+	+	-	-	-	+	+	+	-	+	*Klebsiella planticola*
3	+	Rod	+	+	-	-	+	-	-	-	-	-	+	-	-	+	*Paenibacillus pectinilyticus*
4	+	Rod	+	+	-	-	-	-	+	-	-	-	+	-	-	+	*Paenibacillus pectinilyticus*
5	+	Rod	+	+	-	-	-	-	+	-	-	-	+	-	-	+	*Paenibacillus pectinilyticus*
6	+	Rod	+	+	+	-	+	-	-	+	+	+	+	-	-	+	*Bacillus subtilis*
7	+	Rod	+	+	-	-	+	-	+	-	-	-	+	-	-	+	*Paenibacillus pectinilyticus*
8	+	Rod	+	+	-	-	-	-	+	-	+	-	+	-	-	+	*Bacillus niacin*
9	-	Rod	-	+	-	-	+	+	-	-	-	-	+	-	-	+	*Serratia grinesii*
10	+	Rod	+	+	-	-	+	-	-	-	-	-	+	-	-	+	*Paenibacillus pectinilyticus*
11	+	Rod	+	+	-	-	+	+	-	-	-	-	+	-	-	+	*Bacillus niacin*
12	-	Rod	-	+	-	+	+	+	-	+	+	+	+	-	-	+	*Klebsiella planticola*
13	-	Rod	-	+	-	-	-	-	+	+	-	-	+	-	-	+	*Klebsiella pneumonia*

Gly, Glycerol; H_2S, Hydrogen sulphide; Lac, Lactose; Glu, Glucose; MR, Methyl red; VP, Voges-Proskauer; Lip, Lipase; Nit, Nitrate; Mot, Motility; Ind, Indole; and Cit, Citrate.

Table 2. Percentage distribution of bacteria isolated from different food samples

Isolates	Beef	Shrimp	Turkey	Frequency (N)	Percentage (%)
Enterobacter asburiae			1	1	7.7
Klebsiella planticola		1	1	2	15.4
Paenibacillus pectinilyticus	2	1	2	5	38.5
Bacillus subtilis			1	1	7.7
Klebsiella pneumonia		1		1	7.7
Bacillus niacin	1		1	2	15.4
Serratia grinesii		1		1	7.7
Total	3	4	6	13	100.0

3.2. Identification of Bacteriophage Plaques

Table 3 showed the identification of bacteriophage from bacteria isolated from food sources. The results revealed that across all samples 2 major bacteria of 13 isolates were good phage hosts based on the formation of plaques. The bacteria include *Klebsiella planticola*, and *Paenibacillus pectinilyticus* (Table 3). Bacteriophages are being explored as potential alternatives to antibiotics for combating bacterial infections. Their specificity to bacterial hosts and benign effect on beneficial bacteria make them promising therapeutic agents [34], hence the current surge in interests in characterizing bacteriophages and phage hosts from diverse sources, including food [35]. Food presents an attractive reservoir due to its diverse bacterial strains, including pathogens [36]. This study identified the presence of potential bacteriophages hosts (*Klebsiella planticola*, *Paenibacillus pectinilyticus*) from fresh food products. Yang *et al*. [37] found *Escherichia coli* and *Salmonella* associated phages from wastewater samples. El-Shibiny *et al*. [38] also isolated bacteriophages from raw milk, cheese, and meat products. These studies collectively demonstrate the diverse sources from which bacteriophages could be detected and isolated,

highlighting their ubiquitous presence in various food environments.

Table 3. Overview of detected phage plaques using isolated bacteria as hosts

Isolate No.	Microorganisms	Shrimp	Turkey	Beef
1	*Enterobacter asburiae*	No Plaque Formed	No Plaque Formed	No Plaque Formed
2, 12	*Klebsiella planticola*	Plaque Formed	Plaque Formed	Plaque Formed
4, 7	*Paenibacillus pectinilyticus*	Plaque Formed	Plaque Formed	Plaque Formed
6	*Bacillus subtilis*	No Plaque Formed	No Plaque Formed	No Plaque Formed
8, 11	*Bacillus niacin*	No Plaque Formed	No Plaque Formed	No Plaque Formed
9	*Serratia grinesii*	No Plaque Formed	No Plaque Formed	No Plaque Formed
13	*Klebsiella pneumonia*	No Plaque Formed	No Plaque Formed	No Plaque Formed

3.3. Molecular Identification of Bacteria

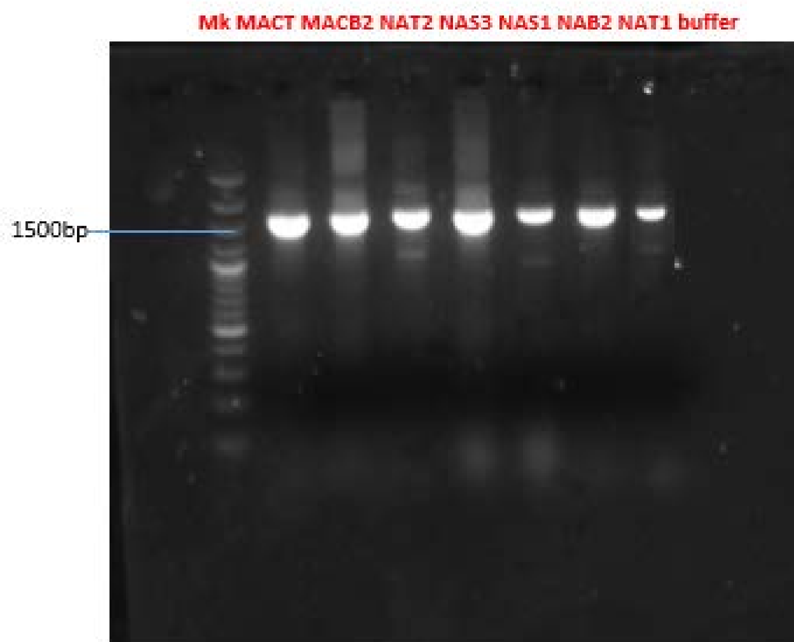
The study utilized agarose gel electrophoresis to analyse PCR products which revealed sharp bands around 1500 bp (Figure 2). Subsequent sequencing and comparison with GenBank entries identified seven potential bacterial phage host. Phylogenetic analysis depicted the relationships among these bacterial strains as shown in Figure 3. Notably, the bacterial isolate 1 showed 99.93% similarity to *Enterobacter asburiae*, isolates 2 and 6 to *Raoultella planticola* (99.64% and 99.71%, respectively), isolates 4 and 7 to *Paenibacillus pectinilyticus* (99.7% and 100%, respectively), isolate 12 to *Serratia grimesii* (99.28%), and isolate 3 to *Neobacillus niacini* (99.93%) (Table 4). In addition, about 57% of the time, probable bacterial identities from conventional biochemical tests did not align with 16S rRNA molecular characteristics/identities (Table 5). These findings underscore the diversity and genetic relatedness of the studied bacterial isolates.

Table 4. Molecular profile of characterized bacterial isolates

Sample ID	Scientific Name	Max score	Total score	Query cover	Per. (%) Identity	Accession number
1	*Enterobacter asburiae*	2534	2534	100%	99.93%	ON366413
2	*Raoultella planticola*	2547	20359	99%	99.64%	ON366414
4	*Paenibacillus pectinilyticus*	2518	2518	99%	100.00%	ON366415
7	*Paenibacillus pectinilyticus*	2551	2551	100%	99.71%	ON366416
12	*Serratia grimesii*	2531	2531	100%	99.28%	ON366417
3	*Neobacillus niacin*	2579	2579	100%	99.93%	ON366418
6	*Raoultella planticola*	2553	20386	99%	99.71%	ON366419

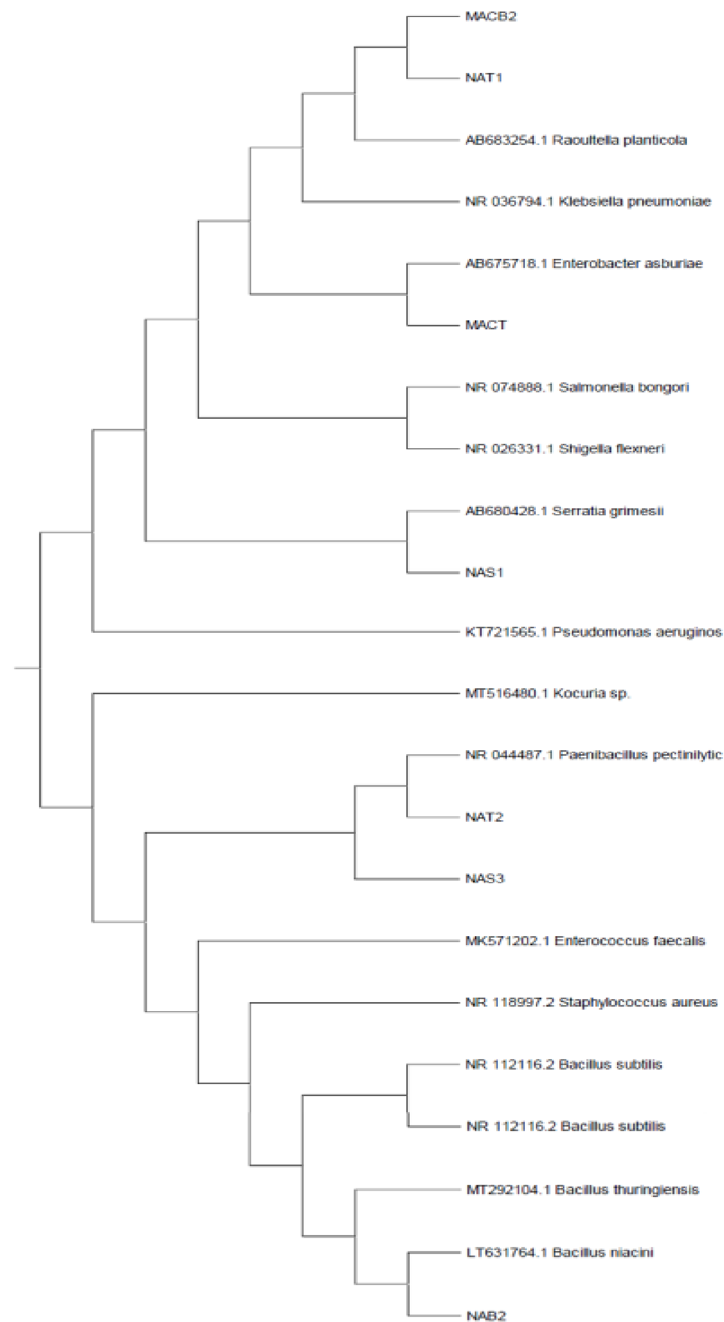
Table 5. Comparison of bacterial identities from conventional and molecular techniques

Sample ID	Identities from molecular characterization	Identities from conventional characterization
1	*Enterobacter asburiae* (>99%)	*Enterobacter asburiae*
2	*Raoultella planticola* (>99%)	*Klebsiella planticola*
4	*Paenibacillus pectinilyticus* (100%)	*Paenibacillus pectinilyticus*
7	*Paenibacillus pectinilyticus* (>99%)	*Paenibacillus pectinilyticus*
12	*Serratia grimesii* (>99%)	*Klebsiella planticola*
3	*Neobacillus niacin* (>99%)	*Paenibacillus pectinilyticus*
6	*Raoultella planticola* (>99%)	*Bacillus subtilis*



MACB2 and NAT1 = *Raoultella planticola*, MACT = *Enterobacter asburiae*, NAT2 and NAS3 = *Paenibacillus pectinilyticus*, NAS1 = *Serratia grimesii*, NAB2 = *Bacillus niacin*.

Figure 2. Agarose gel depiction of amplifications of the seven bacterial isolates 16S genes analysed with universal primers



MACB2 and NAT1 = *Raoultella planticola*, MACT = *Enterobacter asburiae*, NAT2 and NAS3 = *Paenibacillus pectinilyticus*, NAS1 = *Serratia grimesii*, NAB2 = *Bacillus niacin*.

Figure 3. Phylogenetic tree depicting closely related clades of the seven bacterial isolates

The identification of phage host bacteria in food samples is crucial to understanding the dynamics of microbial communities and their impact on food safety [39]. *Klebsiella planticola* and *Paenibacillus pectinilyticus* are known to have relevance in food spoilage and foodborne illness, making their association with phages particularly significant. While members of the *Paenibacillus* genus are resplendent in nature and do not usually cause infections in humans, some species like *P. macerans* and *P. silvae* are increasingly being detected in immunocompromised individuals [40,41] and others implicated in the spoilage of dairy products [42]. *Raoultella planticola* is an emerging, rare pathogen, and member of the family *Enterobacteriaceae* that is associated with urinary tract infections (UTI). It is also closely related to *K. pneumoniae* and can be isolated from sewage, soil, sewage, water environs [43]. For the first time, we report its presence from fresh food products which may have been contaminated from any of the earlier highlighted primary sources. On the other hand, the presence of *E. asburiae* has been associated with septicaemia, UTIs, pneumonia, neurodegenerative disease, and is an emerging clinically relevant antibiotic resistant pathogen [44]. In addition, it has been reported that food products derived from animals serve as vehicles for the transfer of bacteria from foods to humans, and are a leading cause for the increased prevalence of foodborne zoonotic infections [45]. On the other hand, *S. grimesii*, is a highly evolved pathogen with a wide host range and ability to express the grimelysin virulence factor embedded within formed outer membrane versicles (OMVs) for invading host cells and establishing infection

especially in immunocompromised individuals [46].

Shousha *et al*. [45] further showed that *E. coli*-associated phages were isolated from meat samples, and identified using metagenomic techniques. In this study, all the potential bacterial phage hosts analysed on a molecular level showed similar bands at 1,500 bp. However, while the genome size of the microbes seems to be similar it does not automatically translate into similar genotypic or phenotypic traits. In the same vein, a similar host range does not equate with genetic relatedness of phages [47]. Also, the phylogenetic tree showed five clear clade clusters for clear identification of bacterial isolates relative to closely related gene bank species sequences and neighbours for easy identification of *R. planticola*, *E. asburiae*, *P. pectinilyticus*, *S. grimesii*, and *B. niacin* (Figure 3). The phylogenetic tree is an informative depiction of the origin, evolutionary relatedness, and similarities and differences among identified bacterial species based on 16S rRNA molecular fingerprints [48]. This study has once more shown that the application of more than one characterization workflow ensures improved precision and accurate determination of bacterial identities and detection of false positives and false negatives outcomes. Molecular methods have proved to be more effective at detecting and identifying new or emerging pathogens, since they are highly automated and standardized, for derivation of highly reproducible microbial identifications [49]. Molecular methods are also able to identify microorganisms that are difficult to culture, detect multiple pathogens in a single assay, and provide information on the relatedness of isolates for epidemiological studies and outbreak investigations [49].

4. Conclusions

The use of comparative methods, that is, traditional and molecular (16S rRNA sequencing) for the identification potential bacterial phage hosts from fresh food products (beef, shrimp, turkey) showed the presence of two potential bacteriophages, as well as spoilage, and emerging bacterial pathogens. The molecular characterization technique was again shown to supersede conventional and laborious biochemical test methods for the accurate identification of microbial species. Our findings of high coliform and viable bacterial counts indicated contamination of products and their unsafe and unhygienic nature at the points of purchase. Our study provided valuable insights into the dynamic biochemical and molecular microbial interactions that take place in the studied food products. Nevertheless, further studies targeting the isolation of phages, and their lysis efficacy are encouraged to better determine potential food quality or safety applications. As we navigate the challenges surrounding food safety and protection, the impact of in-depth research into phage-based biocontrol interventions cannot be overemphasized as it may hold considerable promise for the food industry.

ACKNOWLEDGMENTS

Authors acknowledge the support received in part from the National Research Foundation (NRF)/Department of Science and Innovation (DSI) of South Africa (Reference number PSTD2204133389) to C. E. Aruwa; and the Directorate of Research and Postgraduate Support, Durban University of Technology (DUT), South Africa.

Statement of Competing Interests

The authors have no competing interests.

References

[1] Todd, E, "Food-borne disease prevention and risk assessment". *International Journal of Environmental Research and Public Health*, 17 (14). 5129. 2020.

[2] Hoffmann, S. and Ahn, J.W, "Economic cost of major foodborne illnesses increased $2 billion from 2013 to 2018", *Amber waves: The Economics of Food, Farming, Natural Resources, and Rural America*, 2021 (04). 2021.

[3] Lin, P., Tsai, H. and Ho, T, "Food safety gaps between consumers' expectations and perceptions: development and verification of a gap-assessment tool", *International Journal of Environmental Research and Public Health*, 17 (17), 6328. 2020.

[4] Lianou, A., Panagou, E.Z. and Nychas, G.J.E, Meat safety—I foodborne pathogens and other biological issues. In Lawrie's meat science, Woodhead Publishing, 2023, 549-590.

[5] Sadiku, M.N., Ashaolu, T.J. and Musa, S.M, "Food contamination: A primer", *International Journal of Advance in Scientific Research and Engineering*, 6 (03). 01-07. 2020.

[6] Gourama, H, Foodborne pathogens. Food safety engineering, Springer International Publishing, Cham, 2020, 25-49.

[7] Schirone, M., Visciano, P., Tofalo, R. and Suzzi, G, "Foodborne pathogens: Hygiene and safety. *Frontiers in Microbiology*, 10. 481870. 2019.

[8] Kamboj, S., Gupta, N., Bandral, J.D., Gandotra, G. and Anjum, N, "Food safety and hygiene: A review", *International Journal of Chemical Studies*, 8 (2), 358-368. 2020.

[9] Farooq, U., Shafi, A., Shahbaz, M., Khan, M.Z., Hayat, K., Baqir, M. and Iqbal, M, Food Quality and Food Safety: An Introduction, In Sequencing Technologies in Microbial Food Safety and Quality, CRC Press, 2021, 3-24.

[10] Sehrawat, A. and Sindhu, S.S, "Potential of biocontrol agents in plant disease control for improving food safety", *Defence Life Science Journal*, 4 (4). 220-225. 2019.

[11] Jongman, M., Carmichael, P., Loeto, D. and Gomba, A, "Advances in the use of biocontrol applications in preharvest and postharvest environments: A food safety milestone", *Journal of Food Safety*, 42 (2). e12957. 2022.

[12] Zdolec, N., Lorenzo, J.M., and Ray, R.C, "Use of microbes for improving food safety and quality", *BioMed Research International*, 2018. 3902698. 2018

[13] Acuff, J.C., Dickson, J.S., Farber, J.M., Grasso-Kelley, E.M., Hedberg, C., Lee, A. and Zhu, M.J, "Practice and Progress: updates on outbreaks, advances in research, and processing technologies for low-moisture food safety", *Journal of Food Protection*, 86 (1). 100018. 2023.

[14] Sibisi, A.S, Food hygiene, safety, handling practices and knowledge of food handlers in a food retail company in Durban, KwaZulu-Natal, South Africa (Doctoral dissertation), 2019.

[15] Suárez, V.B., Quiberoni, A., Binetti, A.G. and Reinheimer, J.A., 2002. Thermophilic lactic acid bacteria phages isolated from Argentinian dairy industries. *Journal of Food Protection*, *65*(10), 1597-1604.

[16] Moye, Z.D., Woolston, J. and Sulakvelidze, A, "Bacteriophage applications for food production and processing", *Viruses*, 10 (4). 205. 2018.

[17] Islam, M.S., Zhou, Y., Liang, L., Nime, I., Liu, K., Yan, T., Wang, X. and Li, J, "Application of a phage cocktail for control of *Salmonella* in foods and reducing biofilms", *Viruses*, 11 (9). 841. 2019.

[18] Garvey, M, "Bacteriophages and food production: Biocontrol and

bio-preservation options for food safety", *Antibiotics*, 11 (10), 1324, 2022.

[19] Lavilla, M., Domingo-Calap, P., Sevilla-Navarro, S. and Lasagabaster, A, "Natural Killers: Opportunities and challenges for the use of bacteriophages in microbial food safety from the one health perspective", *Foods*, 12 (3). 552. 2023.

[20] Aruwa, C.E., and Ogunlade, S.T, "Classical identification, 16S rDNA sequencing, and molecular characterization of Bacillus species from convenience foods", *British Journal of Applied Science & Technology*, 15 (5). 1-11. 2016.

[21] Aruwa, C.E., Akindusoye, A.J. and Awala, S.I, "Socio-demographic characteristics and food hygiene level assessment of food handlers in cafeterias around a Federal University in Nigeria", *Journal of Scientific Research and Reports*, 14 (4). 1-9. 2017.

[22] Martínez-Laorden, A., Arraiz-Fernández, C. and González-Fandos, E, "Microbiological quality and safety of fresh turkey meat at retail level, including the presence of ESBL-producing Enterobacteriaceae and methicillin-resistant *S. aureus*", *Foods*, 12 (6), 1274. 2023.

[23] Anie, C.O., Jemikalajah, D.J. and Eke, C.N, "Determination of microbial load of selected smoked fish sold in Abraka markets delta state" *Nigerian Journal of Pure and Applied Science,* 32 (1). 3281-3288. 2019.

[24] Fawole, M.O. and Oso, B.A, Characterization of bacteria: Laboratory manual of microbiology, Spectrum Publishers, Ibadan, 2004, 24.

[25] Van Twest, R. and Kropinski, A.M, Bacteriophage enrichment from water and soil. Bacteriophages: Methods and Protocols - Isolation, Characterization, and Interactions, 1. 15-21. 2009.

[26] Cappuccino, J. G. and Sherman, N, Microbiology. A laboratory manual (5th ed.). Benjamin/Cummings Science Publishing, 1999.

[27] Glonti, T. and Pirnay, J.P, "*In vitro* techniques and measurements of phage characteristics that are important for phage therapy success", *Viruses*, 14 (7). 1490. 2022.

[28] Rijpens, N.P. and Herman, L.M, "Molecular methods for identification and detection of bacterial food pathogens", *Journal of AOAC International*, 85 (4). 984-995. 2002.

[29] Shin, J.H, "Nucleic acid extraction techniques", *Advanced Techniques in Diagnostic Microbiology*, 209-225. 2013.

[30] Nkere, C.K., Ibe, N.I. and Iroegbu, C.U, "Bacteriological quality of foods and water sold by vendors and in restaurants in Nsukka, Enugu State, Nigeria: A comparative study of three microbiological methods", *Journal of Health, Population, and Nutrition*, 29 (6). 560. 2011.

[31] Cooke. E.M., and Gibson, G.L, Essential clinical microbiology. John Wiley and Sons, New York, 1990, 16-21.

[32] Merlin, S.B, "Food contamination and gastroenteritis", *Annual Reviews in Public Health*, 6. 12-14. 1969.

[33] World Health Organization Food Safety Unit. Contaminated food: a major cause of diarrhoea and associated malnutrition among infants and young children. Facts Infant Feed, 1-4, 1993.

[34] Ling, H., Lou, X., Luo, Q., He, Z., Sun, M. and Sun, J, "Recent advances in bacteriophage-based therapeutics: Insight into the post-antibiotic era", *Acta Pharmaceutica Sinica B*, 12 (12), 4348-4364. 2022.

[35] Huang, C., Shi, J., Ma, W., Li, Z., Wang, J., Li, J. and Wang, X, "Isolation, characterization, and application of a novel specific *Salmonella* bacteriophage in different food matrices", *Food Research International*, 111. 631-641. 2018.

[36] Abebe, E., Gugsa, G. and Ahmed, M, "Review on major food-borne zoonotic bacterial pathogens", *Journal of Tropical Medicine*, 2020 (4674235). 1-19. June 2020.

[37] Yang, Q., Ding, Y., Nie, R., Yao, L., Wang, X., Zhou, M., Wang, J. and Wang, X, "Characterization of a novel T7-like *Salmonella typhimurium* (ATCC13311) bacteriophage LPST144 and its endolysin", *LWT*, 123. 109034. 2020.

[38] El-Shibiny, A. and El-Sahhar, S, "Bacteriophages: the possible solution to treat infections caused by pathogenic bacteria", *Canadian Journal of Microbiology*, 63 (11). 865-879. 2017.

[39] Suja, E. and Gummadi, S.N., 2023. Advances in the applications of bacteriophages and phage products against food-contaminating bacteria. *Critical Reviews in Microbiology*, 1-26.

[40] Vecchia, I.D., Fasan, D., Pegoraro, M. and Benedetti, P, "Febrile sepsis: First report of human disease due to *Paenibacillus sylvae*", *Access Microbiology*, 5 (6). 000580-v3. 2023.

[41] Szaniawski, M.A. and Spivak, A.M, "Recurrent *Paenibacillus* infection", Oxford Medical Case Reports, 2019 (5). p.omz034. 2019.

[42] Moreno, S.A.I., Andrus, A.D., Ranieri, M.L., Orsi, R.H., Ivy, R, "Genomic comparison of spore forming bacilli isolated from milk", *BMC Genomics*, 15. 26. 2014.

[43] Mihu, A.G., Susan, M.M., Strauti, C.N., Mot, M.D., Muresanu, H.D., Balta, C. and Nesiu, A, "First Case of *Raoultella planticola* urinary tract infection reported in western Romania", *Medicina*, 59 (3). 506. 2023.

[44] Vittoria, M.M., Angela, K., Aurora, P., Stefano, G., Cristina, M., Mariangela, S., Patrizia, C., Roberta, M. and Fausto, B, "*Enterobacter asburiae* ST229: an emerging carbapenemases producer", *Scientific Reports*, 14 (1). p.6220. 2024.

[45] Shousha, A., Awaiwanont, N., Sofka, D., Smulders, F.J., Paulsen, P., Szostak, M.P., Humphrey, T. and Hilbert, F, "Bacteriophages isolated from chicken meat and the horizontal transfer of antimicrobial resistance genes", *Applied and Environmental Microbiology*, 81 (14). 4600-4606. 2015.

[46] Bozhokina, E., Kever, L. and Khaitlina, S., "The *Serratia grimesii* outer membrane vesicles - associated grimelysin triggers bacterial invasion of eukaryotic cells", *Cell Biology International*, 44 (11). 2275-2283. 2020.

[47] Stenholm, A.R., Dalsgaard, I. and Middelboe, M, "Isolation and characterization of bacteriophages infecting the fish pathogen *Flavobacterium psychrophilum*", *Applied and Environmental Microbiology*, 74 (13). 4070-4078. 2008.

[48] Pavlopoulos, G.A., Soldatos, T.G., Barbosa-Silva, A., and Schneider, R, "A reference guide for tree analysis and visualization", *BioData mining*, 3 (1). 1. 2010.

[49] Mirzaei, M.K. and Pourmand, M.R, "Applications of molecular methods for identification and characterization of microbial communities in contaminated sites", *Journal of Applied Microbiology*, 121 (3). 655-670. 2023.

© The Author(s) 2024. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).