Conventional and Molecular Characterization Based Microbial Assessment of Street Vended *(Vada pav)* Samples from Anand City, Gujarat, India

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HIGHLIGHTS

- The total viable count in *Vada pav* samples ranged from 3.94 to 6.09 CFU/g, while the Yeast and Mold Count ranged from 1.15 to 2.85 log CFU/g tested in seven different locations.
- The dominant microbes identified in *Vada pav* sample, using both methods, were *Escherichia coli* (42.85%) followed by *Bacillus cereus* (28.5%).
- *Staphylococcus aureus* (28.5%) was isolated by the conventional culture technique but not confirmed using molecular characterization.
- *Salmonella* spp. was not detected in any of the samples.

ABSTRACT

Background: Street foods offer convenient meal options for the consumer, but pose safety concerns if not handled or served with proper hygiene. The purpose of the present study was the microbial evaluation of street vended *Vada pav* samples sold at popular locations in Anand city using the conventional culture technique and molecular characterization via Polymerase Chain Reaction.

Methods: Duplicate samples were collected from seven different locations (n=14) across five zones: East (2), West (1), North (1), South (1), and Central (2) during June 2023. *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *Salmonella* spp. were isolated and identified. For the microbial screening, bacterial enumeration, colony morphology, Gram's reaction, and biochemical characterization were performed. Amplification of *nuc* (*S. aureus*), *nheA* (*B. cereus*), *phoA* (*E. coli*), and 16S rRNA (*Salmonella* spp.) genes were carried out via Polymerase Chain Reaction assay.

Results: Total Viable Count (TVC) ranged from 3.93 to 6.08 log Colony Forming Units (CFU)/g while the Yeast and Mold Counts ranged from 2.30 to 4.28 log CFU/g. Using the conventional culture technique, the prevalence of *S. aureus*, *B. cereus*, and *E. coli* were found to be 3/14, 2/14, and 3/14, respectively; whereas based on molecular characterization, the prevalence was 0/14, 2/14, and 3/14, respectively. *Salmonella* spp. was not detected in any of the samples.

Conclusion: The study indicates a potential health hazard for consumers due to microbial contamination of street vended *Vada pav* samples. Consequently, it is crucial to regulate and improve hygienic practices in street food vendors.

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Introduction

Street Vended Foods (SVFs) are readily available meals for the consumer. India is famous for its unique street foods which contribute up to 40% of the daily diet of the urban population (Hirani et al., 2019). Street foods are popular among city dwellers because they are inexpensive, easily accessible, and convenient on the sidewalk, especially for low to middle income populations (Alelign et al., 2023). *Vada pav* is a popular Indian street food that consists of a spicy mashed potato patty called Vada, which is deep-fried after being coated in chickpea batter. This *vada* is placed inside a soft bread roll called *pav*, usually topped with various *chutneys* or sauces for added flavour (Solomon et al., 2015). The safety of these foods is always doubtful. These foods are perceived as potential risks for food-borne illnesses if not prepared or served hygienically. According to WHO (2022) estimates, unsafe food is responsible for 420,000 deaths and 600 million incidents of food-borne illnesses globally each year. Children under the age of five account for 30% of food-borne deaths. Eating unsafe food results in the loss of 33 million years of healthy life annually worldwide; however, this figure is probably underestimated. *Staphylococcus aureus, Bacillus cereus, Salmonella, Campylobacter jejuni, Listeria monocytogenes, Escherichia coli, Clostridium botulinum, C. perfringens, Shigella,* and *Vibrio* are food-borne pathogens that cause several food-borne illnesses (FSSAI, 2021).

The second or third most common cause of food-borne illnesses is *S. aureus*. Food products are considered one of the important sources from which this bacterium can infect humans and cause illness in both animals and humans. *S. aureus* demonstrates various pathogenic factors that contribute to its pathogenicity and ability to colonize (Rajabi et al., 2023). Food contamination with enterotoxigenic *S. aureus* leads to staphylococcal enterotoxin intoxication, resulting in acute symptoms such as nausea, vomiting, abdominal cramps, diarrhoea, and chills, with or without fever (Fisher et al., 2018). *B. cereus* is an opportunistic pathogen, ranked as the fifth most common bacterium responsible for foodborne infections and food poisoning. It is widely distributed in the environment and is undoubtedly the most significant among aerobic spore forming species. *B. cereus* has the capacity to produce an emetic toxin and diarrheal enterotoxins (Hefny et al., 2020). *E. coli* typically inhabits the intestinal tract of vertebrate animals. Therefore, the identification of *E. coli* in food suggests the potential for faecal contamination and the presence of additional faecal bacteria, including pathogens. *E. coli* is one of the most effective faecal contamination indicator bacteria among commonly used faecal indicator bacteria (Braz et al., 2020). *Salmonella* spp. are found in both domestic and wild animals as well as in the environment. *Salmonella* spp. cause salmonellosis, a common food-borne infection in humans. *Salmonella* exposure can cause mild symptoms, severe disease, and even death (Food Standards Australia New Zealand, 2023).

Traditional microbiological methods, such as International Organisation for Standardisation (ISO) standards provide reliable and standardised procedures for detecting food-borne pathogens. However, enrichment, isolation, and identification steps need to be followed, which can lead to time consuming analysis that may not be compatible with the requirement for rapid results (Foddai and Grant., 2020). Rapid methods for identifying food-borne pathogens, including immunological, biosensor, and nucleic acid-based techniques, have been developed to address the limitations of traditional methods (Kim et al., 2020). Polymerase Chain Reaction (PCR) is the most widely used nucleic acid amplification technique to detect food-borne pathogens. PCR assay detects pathogens by amplifying target genes using primers which correspond to a certain base sequences present in a microorganism however, electrophoresis is required to confirm both the presence and size of the desired final amplified product (Zhao et al., 2014).

The *nuc* gene encodes the thermonuclease enzyme and can be used as a valuable genetic marker for the identification of *S. aureus* using PCR (Al-Ashmawy et al., 2016). *B. cereus* produces enterotoxins such as cytotoxin K (*CytK*), non hemolytic enterotoxin (*Nhe*), and hemolysis BL (*Hbl*), which cause food-borne poisoning. The NHE gene complex consists of *Nhe*A, *Nhe*B, and *Nhe*C. The non-haemolytic enterotoxin (*Nhe*) is responsible for the diarrhoeal food-poisoning syndrome (Liu et al., 2020). The alkaline phosphatase encoding gene *phoA* is a housekeeping gene present in all *E. coli* and can be utilised for specific identification (Luo et al., 2023). *Salmonella* spp. are amplified for the 16S rRNA gene because it is the most prevalent housekeeping genetic marker due to its presence across bacterial species (Ibal et al., 2019).

A few studies have been conducted in Gujarat state on the microbiological hazards of street vended foods. These studies were focused on pizza (Solanki and Dave, 2012) *Pani puri* (Mehta et al., 2020), *Bhel* (Sheth et al., 2005), and hotdogs (Jotangiya, 2018). Further, based on the literature reviewed, no studies have been reported from Anand city on street vended foods. *Vada pav* is a popular ready-to-eat food; however, its microbial contamination has not been reported from any part of India except for a study by Chumber et al. (2007) from Pune. Thus, the purpose of this study was to carry out a microbial evaluation of street vended *Vada pav* samples sold in popular locations in Anand city of central Gujarat with

reference to *S. aureus*, *B. cereus*, *E. coli*, and *Salmonella* spp.

Materials and methods

Sample collection

Samples of *Vada pav* were purposively collected in June 2023 in two trials (a and b) from seven popular locations out of a total of nine in Anand city, as follows: two from the east (E1 and E2), one from the west (W1), one from the north (N1), one from the south (S1), and two from the central zone (C1 and C2). Samples were brought to the laboratory in plastic bags as sold by the vendor, along with tomato sauce, with or without added *chutney* in a separate smaller plastic bag and then transported in an ice box and processed within 2 h.

Sample preparation

In the laboratory, under aseptic conditions, *Vada pav* was carefully opened, the *pav* (bread) was separated and a small quantity (2 g) of tomato sauce or a mixture of tomato sauce and *chutney* was spread aseptically on the *Vada,* as *Vada pav* is generally consumed with tomato sauce and *chutney*. The *Vada pav* was reassembled according to the vendor's presentation. For sampling, four small pieces of *Vada pav* were collected from four different sides, along with a portion from the centre. All pieces were combined and weighed to obtain a total weight of 25 g. This sample was placed inside a sterile filtered sampling bag (PW391, HiMedia) with the addition of 225 ml of Buffered Peptone Water (BPW; M1275, HiMedia). A homogenized mixture was prepared by crushing it with a pestle (for up to one min). Since the sampling bag contains a filter, the peptone water passes through to the opposite side while the homogenized solid material remains inside. The BPW obtained was further serially diluted up to 10^{-3} decimal dilutions. For plating, 100 µl of the sample was pipetted out and spread onto basal as well as selective and differential media.

Conventional culture technique

-Identification and enumeration of bacteria

Samples were inoculated on total plate count agar (M091A, HiMedia, Mumbai, India) and potato dextrose agar (M096, HiMedia,Mumbai, India) for Total Viable Count (TVC) and Yeast and Mold Count (YMC), respectively. Baird Parker Agar (BPA; M043,HiMedia,Mumbai, India) containing 5% egg yolk tellurite emulsion (FD046), Polymyxin Pyruvate Egg Yolk Mannitol Bromothymol Blue Agar (PEMBA; M1484, HiMedia,Mumbai, India) with the addition of egg yolk emulsion (5%) (FD045, HiMedia,Mumbai, India) and

PEMBA supplement (5%) (FD200, HiMedia,Mumbai, India)*,* Hicrome *E. coli* Agar (HEA; M1295I HiMedia,Mumbai, India), and *Salmonella Shigella* Agar modified (SSA; M1032, HiMedia,Mumbai, India) were used for the identification of *S. aureus, B. cereus, E. coli,* and *Salmonella* species, respectively. Plates were incubated at 37 °C for 24 to 48 h and colonies were counted and replated on respective media for isolation. Results were expressed in log Colony Forming Units (CFU)/g. Isolated bacteria were characterized based on colony morphology, Gram's reaction, motility test, and biochemical tests. For biochemical tests, carbohydrate utilization (glucose, lactose, sucrose, mannose, arabinose, dulcitol, and mannitol), Indole, Methyl Red, Voges–Proskauer, and Citrate (IMViC), hemolysin production; Triple Sugar Iron agar (TSI) test; and enzyme production test such as catalase, oxidase, coagulase, nitratase, urease; as well as the hydrolysis of starch, gelatin, and casein were performed (Patel and Patel, 2016). Media and reagents for phenotypic characterization were purchased from HiMedia (Mumbai, India) and prepared in the laboratory.

-Reference strains

All bacterial strains used as positive controls, *S. aureus -* 7443*, B. cereus* (6840)*, E. coli* – (1692), and *Salmonella* spp. (734) were purchased from the Microbial Type Culture Collection and Gene Bank (MTCC) (Chandigarh, India).

Molecular characterisation using PCR assay

-DNA extraction

DNA was extracted using the thermal lysis method. Pure cultures were suspended in 100 µl of nuclease free water in a sterile microcentrifuge tube. The bacterial suspension was vortexed and heated in a thermal cycler (Applied biosystems, California- 2720) at 95 °C for 10 min. The samples were centrifuged at 10,000 rpm for 10 min to settle the cell debris. The 50 ul upper aqueous phase containing bacterial DNA was transferred to another microcentrifuge tube and stored at -20 °C until further use (Likhitha et al., 2022).

-PCR protocol

Presumptive isolates were subjected to PCR assay for species specific thermonuclease gene *nuc* for *S. aureus,* the diarrheal enterotoxin gene *nheA* for *B. cereus*, the housekeeping gene *phoA* for *E. coli,* and 16S rRNA for *Salmonella* spp., respectively. Table 1 provides details on the oligonucleotide primers used for all four genes. The reaction mixture was prepared according to the method outlined by Thakur et al. (2020)

Table 1: Details of primers used for Polymerase Chain Reaction (PCR) assay

Bacteria	Target Gene	Primer Sequence (5′–3′)	Amplicon size (bp)	GenBank accession number	Reference
Staphylococcus aureus	*nuc*	F: GCGATTGATGGTGATACGGTT R: GCCAAGCCTTGACGAACTAAAGC	270	NC_007795.1	Budiarso et al. (2019)
Bacillus cereus	*nheA*	F: AAGGCGAATGTACGAGAGTGG R: CTTCTCTCGTTTGACTATCTGCAG	553	NZ_CP017060.1	Kumar et al. (2009)
Escherichia coli	*phoA*	F: CGATTCTGGAAATGGCAAAAG R: CGTGATCAGCGGTGACTATGAC	720	NC_002695.2	Hu et al. (2011)
Salmonella spp.	16S rRNA	F: TGTTGTGGTTAATAACCGCA R: CACAAATCCATCTCTGGA	572	NC_003197.2	Nyabundi et al. (2015)

-PCR conditions for the detection of the various genes

For *S. aureus*, the *nuc* gene was targeted using an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 95 °C for one min, annealing at 58 °C for 30 s, and extension at 72 °C for 45 s. A final extension was carried out at 72 °C for seven min. For *B. cereus*, the *nheA* gene was amplified with an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for one min, annealing at 61°C for 30 s, and extension at 72 °C for one min. For *E. coli*, the *phoA* gene was targeted with an initial denaturation at 94 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 45 s, annealing at 59 °C for 45 s, and extension at 72 °C for 45 s. For *Salmonella* spp., the 16S rRNA gene was amplified with an initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 49 °C foronemin, and extension at 72 °C foronemin. A final extension step was performed at 72 °C for 7 min. The amplified product was subjected to gel electrophoresis on a 2% agarose gel and visualized using the Syngene Gbox gel documentation system (United kingdom).

Statistical analysis

One-way ANOVA was performed using IBM SPSS version 26.0 to determine significant differences ($p \leq 0.05$), with results presented as mean±Standard Deviation (SD) for TVC and YMC.

Results and discussion

Conventional culture technique

-Enumeration of TVC and YMC

Table 2 shows the TVC and YMC of *Vada pav* samples. TVC ranged from 3.94 to 6.09 log CFU/g, with the highest count found in a sample procured from one of the eastern zones (E1) and the lowest observed in the western zone (W3). YMC ranged from 1.15 to 2.85 log CFU/g with the highest YMC observed in the central zone (C1) and the lowest in one of the eastern zones (E2). Three locations (E1, N1, and S1) showed no yeast and mold contamination in the *Vada pav* samples. L4 and L5 showed low TVC and no contamination detected for YMC. TVC was significantly different ($p \leq 0.05$); however, YMC did not show significant differences among the analyzed *Vada pav* samples.

Table 2: Results of microbial counts in *Vada pav* samples (log Colony Forming Units (CFU)/g)

Location	*TVC	*YMC
E1	6.09±0.32 [b]	*ND
E2	5.11±0.04 [b]	1.15±1.63 [a]
W1	3.94±0.64 [a]	2.14±3.03 [a]
N1	4.12±0.37 [a]	ND
S1	4.36±0.32 [ab]	ND
C1	4.36±0.14 [ab]	2.85±0.52 [a]
C2	4.17±0.42 [a]	1.35±1.91 [a]
F-value	8.410 [*]	1.162

ND=Not Detected; TV=Total Viable Count; YMC=Yeast and Mold Count
*=Significant difference ($p \leq 0.05$); Values are mean±Standard Deviation (SD) of 2 trials; means carrying similar superscripts within a column are not significantly different.
E1 and E2=East zone; W1=West zone; N1=North zone; S1=South zone; C1 and C2=Central zone; a and b=trial

Sharma et al. (2020) studied the total viable count of selected street foods, i.e., *tikki*, veggie burger, *samosa*, *pakoda*, momo, and spring rolls in Palampur city, Himachal Pradesh, India. They reported a TVC of 32×10^2 CFU/g for burgers, which is similar to the TVC obtained for all locations in the present study except E1 and E2. Asiegbu et al. (2020) studied the microbial quality of ready-to-eat street vended foods namely starch-based foods (n=75), beef-based foods (n=45), poultry-based foods (n=30), fish-based foods (n=10), vegetable-based foods (n=20), and sandwich-based foods (n=25). A count of 3.95±0.45 log CFU/g TVC was noted for sandwich-based foods; within this category, the highest count were observed for cheese burgers (n=5) and cheese/egg burgers (n=5) which was 4.12±0.28 and 3.77±0.30 CFU/g, respectively, which is close to the value obtained in the present investigation.

-Enumeration of S. aureus, B. cereus, *E. coli* and *Salmonella* spp.

Colonies observed on BPA exhibited a grey-black shiny appearance with or without an opaque zone surrounding the colony, indicative of *S. aureus*. PEMBA revealed colonies displaying a blue colour with precipitation, characteristic of *B. cereus*. On HEA agar, colonies displayed a bluish-green hue, indicating the presence of *E. coli*. Colonies of *Salmonella* spp. were not observed in

any of the samples. The count for *S. aureus* ranged from 3.93 to 4.25 log CFU/g, while *B. cereus* count ranged from 2.00 to 2.30 log CFU/g. The count for *E. coli* was consistent at 2.00 log CFU/g, with the highest count of *S. aureus* found in samples obtained from the central zone. Samples acquired from the East and South zones were contaminated with *B. cereus*, while *E. coli* was isolated from the samples collected from both eastern and central zones (Figure 1).
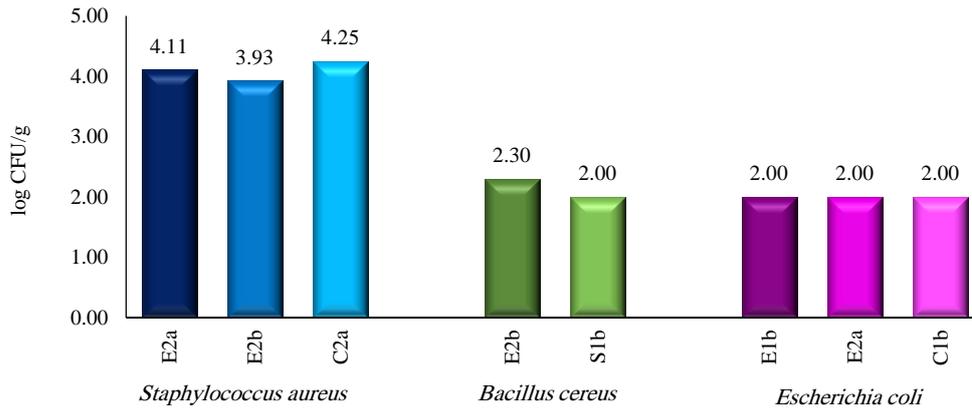

Figure 1: Bacterial counts of different *Vada pav* samples
CFU=Colony Forming Units, first two letters=location, a and b=trial

Yogesh et al. (2019) examined 30 burger samples from moderate fast food centres as well as reputed brands and street vendors and found significant contamination by *Staphylococcus* spp., *E. coli, Salmonella* spp., *Vibrio* spp., and *Listeria* spp. Adhikari et al. (2023) analyzed 150 *chutney* samples served at street foods sold in Bharatpur city, Nepal, reportingaverage CFU counts of 1.33×10^6, 1.83×10^5, and 1.24×10^5 for TVC, coliform, and *Salmonella-Shigella* counts, respectively. This indicates that *chutneys* used in *Vada pav* samples could be a source of microbial contamination. In the present investigation, a higher *E. coli* prevalence (42.85%) was observed compared to previous studies. Bezerra et al. (2010) evaluated hamburgers from Brazil and found contamination by *Staphylococcus*, *B. cereus,* and *Salmonella* spp. These authors noted high counts of *Staphylococcus* and *B. cereus,* but *Salmonella* spp. was not detected. The present study aligns with findings by Bezerra et al. (2010) as they also reported an absence of *Salmonella* spp.

Based on the FSSAI (2023) guidelines for food grain products such as bread, cakes, and doughnuts, the presence of *Salmonella* spp. is prohibited in these foods, which align with the findings of the present study. The microbial guidelines for ready-to-eat foods peovided by

the Centre for Food Safety (2014) indicate that counts of *S. aureus* and *E. coli* should be <20, while *B. cereus* should be $<10^3$/g. However, the UKHSA (2024) has established a higher threshold for the presence of *B. Cereus,* set at $<10^5$. In the present study, the counts for *S. aureus* and *E. coli* exceeded the limit, while the count for *B. cereus* was lower.

Isolating *Salmonella* spp. from food is sometimes susceptible to failuredue to the loss of bacteria during enrichment, despite obtaining a contaminated portion. *Salmonella* spp. have the capability to enter a Viable but Non-Culturable (VBNC) state under unfavourable conditions, which adds to the challenges of culturing using various conventional culture media and enrichment protocols proposed for isolating *Salmonella* spp. from food and environmental samples (Pui et al., 2011).

-Morphological and biochemical characteristics

Subcultured isolates were examined for Gram's reaction and motility. Isolates of *S. aureus* were identified as Gram-positive cocci occurring singly or in diplo or staphylo arrangements. *B. cereus* isolates were confirmed as positive, rod-shaped bacteria occurring in short and long chains. Meanwhile, *E. coli* isolates exhibited Gram-negative, rod-shaped characteristics occurring singly or in

diplo as observed under the microscope. In the motility test, *S. aureus* was found to be negative. All strains of *B. cereus* and *E. coli* demonstrated a diffuse cloud of growth away from the line of inoculation, indicating that all the strains were motile.

-IMViC test

Table 3 demonstrates the biochemical characteristics of the isolated bacteria. IMViC test showed that all *S. aureus* and *B. cereus* isolates tested negative for indole production and citrate utilisation. *S. aureus* isolates were positive for Methyl Red (MR) while *B. cereus* showed variable results. Variable results were also observed among *S. aureus* and *B. cereus* for Voges Proskauer (VP). Indole production and MR were positive for *E. coli* isolates, while VP and citrate utilisation were negative.

Table 3: Biochemical characterization of the isolates obtained from *Vada pav* samples

Isolate	IMViC* Indole	MR	VP	Citrate	Enzymatic test Catalase	Oxidase	Urease	Nitratase	Gelatinase	Coagulase	Caseinase	Amylase	Hemolysis	Sugar fermentation Glucose	Sucrose	Lactose	Mannose	Arabinose	Mannitol	Dulcitol	TSI** agar Slant	Butt	H$_2$S	Gas	Probable bacteria
C2a	-	+	+	-	+	-	+	+	-	-	NA	NA	-	+	+	+	+	+	+	+	A	A	-	-	*Staphylococcus aureus*
E2a	-	+	-	-	+	-	+	-	+	-	NA	NA	-	+	+	+	+	+	+	+	A	A	-	-	
E2b	-	+	-	-	+	-	+	-	+	-	NA	NA	-	+	+	+	+	+	+	+	A	O	-	-	
S1b	-	-	+	-	+	-	+	+	+	NA	+	+	+	+	+	+	+	+	+	+	K	A	-	-	*Bacillus cereus*
E2b	-	+	-	-	+	-	+	+	-	NA	+	+	+	+	+	+	+	+	+	+	A	A	-	-	
C1b	+	+	-	-	+	-	-	+	-	NA	NA	NA	-	+G	+G	+G	+G	+G	+G	+G	A	A	-	+	*Escherichia coli*
E1b	+	+	-	-	+	-	-	+	-	NA	NA	NA	-	+G	+G	+G	+G	+G	+G	+G	A	A	-	+	
E2a	+	+	-	-	+	-	-	+	-	NA	NA	NA	-	+G	+G	+G	+G	+G	+G	+G	A	A	-	+	

IMViC*=Indole, Methyl Red, Voges-Proskauer and Citrate test; TSI**=Triple Sugar Iron agar test; MR=Methyl Red; VP=Voges Proskauer; G=Gas; K=Alkaline; A=Acid; O=no colour change; NA=Not Applicable
E1 and E2=East zone; W1=West zone; N1=North zone; S1=South zone; C1 and C2=Central zone; a and b=trial

-Hemolysin production test

All *B. cereus* isolates were positive for hemolysin production; however, all *S. aureus* and *E. coli* isolates were found to be negative.

-Carbohydrate utilization test

S. aureus and *B. cereus* showed moderate to high carbohydrate utilization with no gas formation, while high utilization of carbohydrate with gas formation was observed in *E. coli* isolates.

-Triple sugar iron agar test

S. aureus isolates in general showed yellow-coloured slant and butts (indicating acid production) without gas or H$_2$S production. Variable results were found in *B. cereus* isolates regarding slant colour showing either pink (alkaline) or yellow (acid) hue with acidic butt, no gas formation, and no H$_2$S production. In contrast, *E. coli* isolates depicted an acidic slant and butt with gas formation and no H$_2$S production.

-Enzyme production test

Most *S. aureus* isolates showed positive results for catalase, urea hydrolysis and gelatinase production, while they showed negative results for oxidase, nitratase, and coagulase tests. The majority of the *B. cereus* isolates were positive for all the enzyme tests except for the oxidase test, while *E. coli* isolates were negative for nearly all tests other than the catalase and nitratase tests.

Mandal and Mandal (2018) reported that *S. aureus* isolates were positive for MR, VP, citrate, catalase, gelatinase, and glucose utilization but negative for indole, oxidase, and urease. They noted variable results for nitratase production as well as sucrose, mannitol, and mannose utilization. The present study is consistent with these findings except for VP, urease, and gelatinase; furthermore, the isolates in this study utilized sucrose, mannitol, and mannose. Tewari et al. (2015) found that *B. cereus* isolates were motile and produced hemolysis while showing variable results for nitratase and VP tests; this study agrees with their findings. Bhutia et al. (2021)

found that *E. coli* isolates were gram negative, motile, and gave positive results for arabinose utilization as well as catalase, nitratase, and MR tests while being negative for gelatinase, urease, and VP tests. Other tests such as indole, citrate, and carbohydrate utilization (glucose, lactose, sucrose, mannitol, and mannose) showed variable results. The present study showed similar results but *E. coli* strains were able to utilise almost all sugars with high acid and gas production.

Based on morphological and biochemical characterization, from a total of 14 samples tested: *S. aureus* (n=3), *B. cereus* (n=2), and *E. coli* (n=3) were identified.

Chumber et al. (2007) conducted the microbial assessment of street vended *Vada pav* samples and identified contamination with *E. coli* and *Pseudomonas aeruginosa*. Among the eight *Vada pav* samples tested, these bacterium was present in six samples. Similarly, in the present study, *E. coli* contamination was noted in three *Vada pav* samples from seven locations.

Severe contamination with *S. aureus* occurs due to improper handling, while the presence of *E. coli* and other coliform bacteria may result from insufficient hand washing and a lack of good manufacturing practices by food vendors (Mehta et al., 2020). *B. cereus* is extensively dispersed in natural environments and can be obtained from soil, water, and vegetation. The occurrence of *Bacillus* spp. isolates could result from the ability of *Bacillus* species to resist desiccation, allowing them to persist in dry products like grain and flour (Muhammad and Galadima, 2022).

Vendor location, raw material, utensils and equipment, storage and reheating, as well as the personal hygiene of the vendor are significant hazards and sources of microbial risks associated with contamination (Mohammed and Shehasen, 2020). According to Abdulkareem et al. (2014), vendors prepared food in unhygienic conditions, with flies present and stalls located near trash sites to prevent obstructions; these practices increase microbial hazards. Vendors often reuse water for hand washing, food preparation, and utensil cleaning, which can pose a risk to microbiological food safety (Prevolsek et al., 2021). The time and temperature of cooking are important as they may inactivate infectious bacteria that can develop during prolonged storage. Reheating temperatures must be sufficiently high or prolonged to effectively inactivate microbes. Some food vendors prepare food ahead of time and store it, then reheat the food at the time of sale. However, reheating the food is not always adequate for the destruction of microbes because the bacteria present in the food may have germinated from spores that survived cooking or multiplied after cooking (Rane, 2011).

Molecular characterization using PCR assay

The isolates were later subjected to PCR assay. Out of the isolates, two *B. cereus* isolatesand three *E. coli* isolates produced amplicons of the expected size as shown in Figure 2. Abdulrahman (2020) evaluated 200 whole chicken carcasses from Duhok, Kurdistan region, Iraq. Results showed that 28 out of 100 local chicken carcasses and 80 out of 100 imported frozen chicken carcasses were found to be contaminated with *S. aureus* using the conventional method. From the local chicken carcasses only 18 out of 22 coagulase-positive isolates were confirmed as *S. aureus* by amplification of the nuc gene; similarly, from imported chicken carcasses 57 out of 68 coagulase-positive isolates were confirmed. The author concluded that PCR assay is more accurate and specific for the detection of *S. aureus* in food samples and that it seems to be more reliable than conventional methods for evaluating bacteriological safety of foods. In the present study out of three *S. aureus* isolates obtained using the conventional culture technique, none showed positive *nuc* gene amplification by PCR.
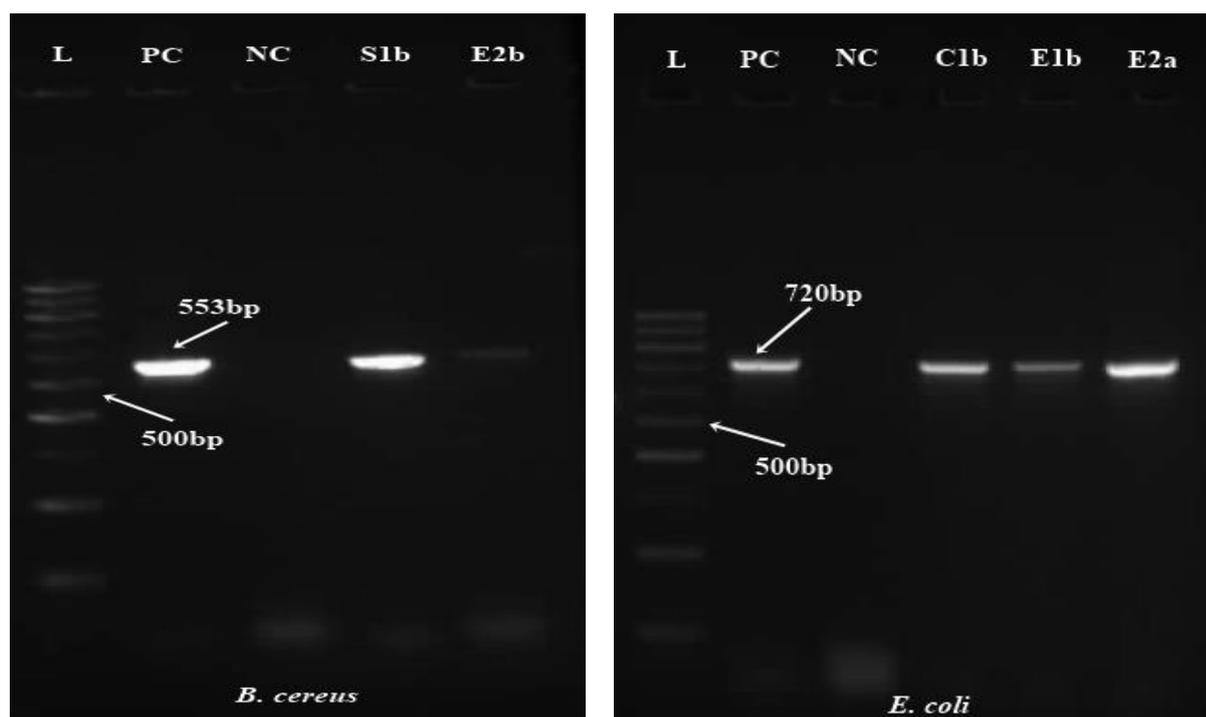
Figure 2: Polymerase Chain Reaction (PCR) amplification of *nheA* gene for *Bacillus cereus* and *phoA* gene for *Escherichia coli* isolates obtained from *Vada pav* samples from different locations

L=DNA Ladder (100bp), PC=Positive Control, NC=Negative Control, E 1 and E2=East 1 and East 2, S1=South 1, C1=Central 1, a and b=trial

In a study conducted by Tewari et al. (2015) on *B. cereus* contamination in raw meat and meat products, it was found that all *B. cereus* contained at least one of the four enterotoxin genes that encodingvirulence factors, 26 isolates out of a total 29 (89.7%) showed one gene from the NHE complex. The present study is comparable to this studies as all the isolates obtained (n = 2) were amplified for the *nheA* gene except for one.

In the study by Hegab et al. (2020), *E. coli* isolated using the conventional methods from Ras cheese (4/50), Domiati cheese (2/50) and Mish cheese (2/50) from Egypt also tested positive for the *phoA* gene in similar proportions. Eid et al. (2019) observed the presence of *E. coli* in minced meat, raw meat, sausage, burger, pastirma, luncheon, salami, and frankfurter using the conventional culture method; PCR results indicated that all *E. coli* isolates showed the presence of the *phoA* gene. The present study also showed similar findings where all *E. coli* isolates identified by the conventional culture method also amplified the *phoA* gene.

Table 4 shows the microbial contamination by location using both methods. Location E2 showed the presence of *S. aureus, B. cereus*, and *E. coli,* although *S. aureus* was not confirmed by the molecular method. Location E2 was an extremely congested spot close to heavy traffic and a railway line, which could explain the higher microbial contamination there. Overall, location E2 can be considered the lowest in hygienic practices while locations N1 and S1 were superior based on lower TVC values, absence of YMC, and all the other bacteria.

Table 5 shows a comparison between conventional and molecular methods for analysing microbial contamination in *Vada pav* samples.

Table 4: Microbial contamination of *Vada pav* samples by location

Location	Trial	TVC log CFU/g	YMC log CFU/g	*Staphylococcus aureus*	*Bacillus cereus*	*Escherichia coli*
E1	a	5.86	-	-	-	-
	b	6.31	-	-	-	P
E2	a	5.13	2.30	P/N [*]	-	P
	b	5.08	-	P/N [*]	P	-
W1	a	3.48	4.28	-	-	-
	b	4.39	-	-	-	-
N1	a	4.38	-	-	-	-
	b	3.86	-	-	-	-
S1	a	4.13	-	-	-	-
	b	4.58	-	-	P	-
C1	a	4.26	2.48	-	-	-
	b	4.46	3.22	-	-	P
C2	a	3.87	2.70	P/N [*]	-	-
	b	4.47	-	-	-	-

CFU=Colony Forming Units; TVC=Total viable count; YMC=Yeast and Mold Count; P=Positive; *N=Negative result by PCR assay; E1 and E2=East zone; W1=West zone; N1=North zone; S1=South zone; C1 and C2=Central zone; a and b=trial

Table 5: Comparison of microbial conventional and molecular methods in *Vada pav* samples (n=7)

Bacteria	Conventional culture technique		Molecular characterization	
	Positive examples (%)	Negative samples (%)	Positive examples (%)	Negative samples (%)
Staphylococcus aureus	2 (28.5%)	5 (71.42%)	0	7 (100%)
Bacillus cereus	2 (28.5%)	5 (71.42%)	2 (28.5%)	5 (71.42%)
Escherichia coli	3 (42.85%)	4 (57.14%)	3 (42.85%)	4 (57.14%)
Salmonella spp.	0	7 (100%)	0	7 (100%)

Conclusion

Different locations significantly affected total viable count in *Vada pav* samples. The study also revealed contamination with *S. aureus, B. cereus,* and *E. coli*, with a higher incidence of *E. coli* (42.85%) compared to both *B. cereus* (28.57%) and *S. aureus* (28.57%). *Salmonella* spp. was not detected in any *Vada pav* samples. This study indicates a potential microbial health risk to humans through consumption of street vended *Vada pav* samples. Therefore, it is important to monitor and enhance hygienic practices among street food vendors.

Author contributions

M.D.P., P.V.H., and S.R. designed the study; M.D.P conducted the experimental work, analyzed the data, and wrote the manuscript. All authors reviewed and approved the final manuscript.

Conflicts of interest

The authors declare that there is no conflict of interest.

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Ethical consideration

Not applicable.

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