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Original Research Article

Molecular Characterization and Identification of *Salmonella enterica* Isolated from Poultry Using *16S rRNA, B-scDNAp,* and *Hd Genes*

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Abstract: Salmonella enterica is a major zoonotic pathogen responsible for foodborne illnesses worldwide. In this study, we aimed to identify *S. enterica* isolates from 200 clinical and environmental samples using both traditional microbiological techniques and molecular methods. Of the 100 bacterial isolates, 20 were confirmed as *S. enterica* through biochemical tests and molecular identification. Polymerase chain reaction (PCR) was employed to amplify the *16S rRNA, B-scDNAp, and Hd* genes for species confirmation and characterization. All 20 isolates tested positive for the *16S rRNA* gene, while 85% and 75% of the isolates were positive for *B-scDNAp and Hd* genes, respectively. The results highlight the effectiveness of using multiple molecular markers for accurate detection and strain differentiation in *S. enterica*. This approach enhances diagnostic precision and contributes to the understanding of the genetic diversity and potential virulence factors of *S. enterica*, offering valuable insights for public health surveillance and control strategies.

Keywords: *Salmonella enterica*. *16S rRNA. B-scDNAp* gene. *Hd* gene. Polymerase chain reaction (PCR). Molecular identification. Zoonotic pathogens.

INTRODUCTION

Salmonella enterica is a major zoonotic pathogen responsible for a wide range of gastrointestinal and systemic infections in both humans and animals. It remains one of the leading causes of foodborne illnesses globally, resulting in significant morbidity and mortality, particularly in developing countries (Eng *et al*., 2021; WHO, 2023). The ability of *S. enterica* to persist in diverse environments and its capacity to acquire antibiotic resistance make it a critical public health concern (Chlebicz & Śliżewska, 2018).

Accurate and timely identification of *S. enterica* is essential for effective disease surveillance and outbreak management. Traditional culture-based techniques, although still widely used, are often time-consuming and may lack sensitivity. Molecular approaches, particularly those targeting the *16S ribosomal RNA (rRNA)* gene, have emerged as powerful tools for bacterial identification due to the gene's high conservation and universal presence across bacterial taxa (Janda & Abbott, 2021).

To enhance diagnostic specificity, molecular assays incorporating additional genetic markers are increasingly applied. In this study, we utilized the conserved 16S rRNA gene alongside two other genetic targets—*B-scDNAp* and *Hd* genes—to improve the molecular characterization of *S. enterica*. The *B-scDNAp* gene, likely plasmid-associated, may be involved in virulence or resistance mechanisms, while the *Hd gene* may represent a novel determinant with potential roles in serotyping or pathogenicity (Zhao *et al.*, 2020; Zhang *et al*., 2022).

The integration of these gene targets aims to enhance the sensitivity and specificity of molecular detection techniques and to contribute to the understanding of the phylogenetic diversity among *S. enterica* isolates. This study provides a framework for

utilizing gene-based diagnostics in epidemiological investigations and public health interventions.

MATERIALS AND METHODS

Sample Collection and Bacterial Isolation

A total of 200 clinical and environmental samples were collected for the detection of *Salmonella* species. Of these, 100 samples tested positive for bacterial presence, and 20 isolates were confirmed as *Salmonella enterica* based on morphological and biochemical characteristics.

The samples were cultured on a series of selective and differential media, including Xylose Lysine Deoxycholate (XLD) agar, Salmonella–Shigella (SS) agar, MacConkey agar, and Brain Heart Infusion (BHI) agar. Suspected colonies exhibiting characteristic morphology were subjected to standard biochemical tests for preliminary identification, including Triple Sugar Iron (TSI) agar reactions, urease, indole, and citrate utilization tests (Andrews, 2020; CLSI, 2022).

Genomic DNA Extraction

Genomic DNA was extracted from confirmed *S. enterica* isolates using the Presto™ Mini gDNA Bacteria Kit (Geneaid Biotech, Taiwan), following the manufacturer's instructions. The quality and concentration of the extracted DNA were assessed using a NanoDrop spectrophotometer at 260/280 nm (Wilson, 2021).

PCR Amplification

Conventional PCR was used for the molecular identification of *S. enterica* using three specific gene targets: *16S rRNA*, *B-scDNAp*, and *Hd*. Primers were designed or selected based on published sequences (primer details are provided in Table 1). Each PCR reaction was carried out in a final volume of 25 µL containing 12.5 µL of master mix, 1 µL of each primer (10 µM), 2 µL of template DNA, and nuclease-free water (Karns *et al.*, 2019; Zhang *et al*., 2022).

Table 1: Primers were used in current study

Gene	PCR condition	Size (bp)
1*6SrRNA*	35 cycles of 94°C for 30s, 55°C for 30s, and 72°C for 50s	1500
B-scDNAp	35 cycles of 94°C for 30s, 52°C for 30s, and 72°C for 60s	833
Hd	35 cycles of 94°C for 30s,60°C for 30s, and 72°C for 60s	894

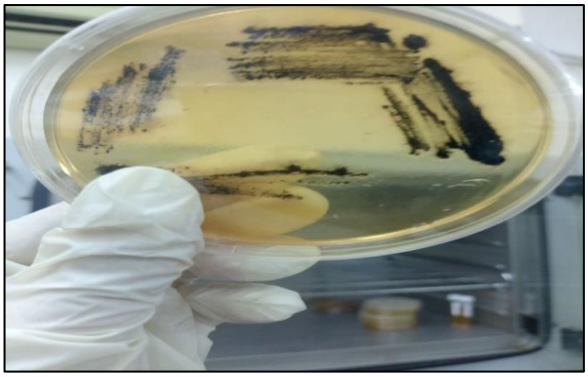
Thermal cycling conditions were as follows: an initial denaturation at 95°C for 5 minutes; followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at gene-specific temperatures for 30 seconds, and extension at 72°C for 1 minute; with a final extension step at 72°C for 7 minutes.

Gel Electrophoresis

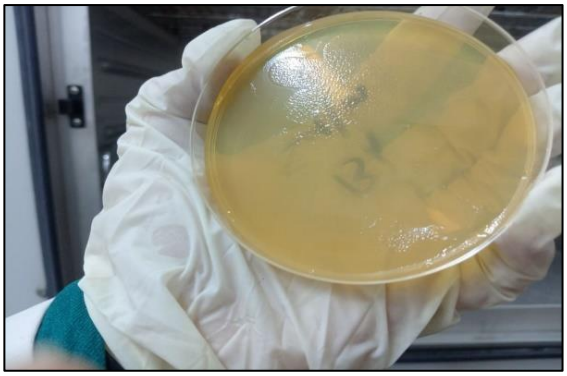
PCR products were analyzed by electrophoresis on 1.5% agarose gel stained with ethidium bromide. DNA bands were visualized under UV transillumination and *documented using a gel documentation system (Sambrook & Russell, 2001; Wang et al*., 2020).

RESULTS

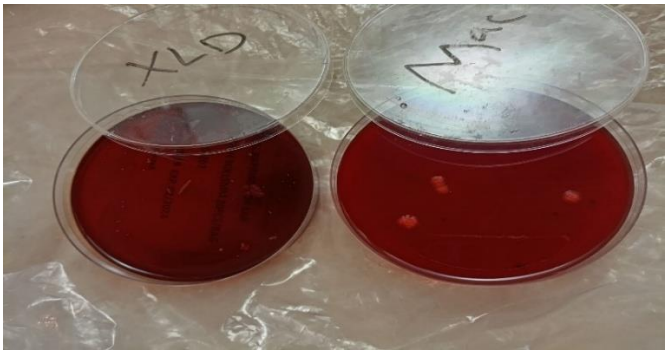
Out of 200 collected samples, 100 (50%) showed positive bacterial growth on selective media. Among these, 20 isolates (20%) were identified as *Salmonella enterica* based on colony morphology and biochemical characterization. The samples were cultured on a variety of media, including SS agar, XLD agar, MacConkey agar, and BHI agar, each providing specific morphological clues for identification. On SS agar, *Salmonella enterica* appeared as colorless to pale colonies with black centers due to hydrogen sulfide (H_2S) production. On XLD agar, the colonies were red with black centers, also indicating H_2S production and lysine decarboxylation. On MacConkey agar, the colonies were colorless or transparent, as *S. enterica* does not ferment lactose, distinguishing it from lactose-fermenting bacteria such as *E. coli*. On BHI agar, a non-selective medium, *S. enterica* formed smooth, circular, gray to translucent colonies without any distinctive differential features. These cultural characteristics were essential for the preliminary identification of *Salmonella enterica* before further confirmation by biochemical tests.



ss-agar



Bhi-agar



Figuer 1: Growth *Salmonella enterica* in ss-agar and Bhi agar and XLd agar and MacConkey agar

All 20 *S. enterica* isolates were subjected to molecular identification using PCR targeting the *16S rRNA, B-scDNAp*, and *Hd* genes. The 16S rRNA gene was detected in 100% (20/20) of the isolates, confirming the genus-level identity of the samples.

Detection of the additional gene targets revealed that:

- The *B-scDNAp* gene was present in 100% (20/20) of the isolates.
- The *Hd* gene was detected in 100% (20/20) of the isolates.

The PCR amplification of each gene produced expected band sizes, and clear bands were observed during agarose gel electrophoresis (Figure 2). Variability in gene presence suggests possible differences in virulence profiles or strain diversity among the *S. enterica* isolates.

These findings support the use of combined gene markers for improved diagnostic accuracy and potential strain differentiation in *Salmonella enterica* surveillance.

Table 2: Prevalence of genes Among 20 *Salmonella enterica* Isolates

Gene Target	No. of Positive Isolates	Percentage (%)
16S rRNA	20/20	100%
B-scDNAp	20/20	100%
Hd	20/20	100%

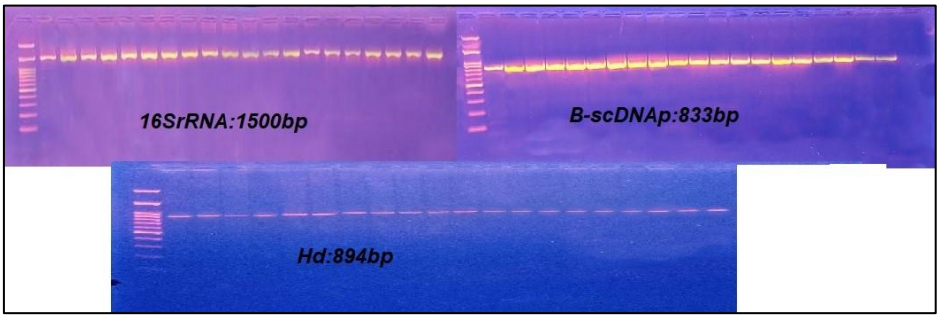


Figure 2: Gel electrophoresis of *16SrRNA, B-scDNAp* and *Hd* genes that are detected in *Salmonella enterica*

DISCUSSION

In this study, we successfully identified *Salmonella enterica* from 200 clinical and environmental samples using both conventional microbiological techniques and molecular diagnostic methods. Out of the 100 bacterial isolates, 20 were confirmed as *S. enterica*

through biochemical and molecular testing, demonstrating the prevalence of this pathogen in the sampled population. The use of selective and differential media, such as XLD agar, SS agar, and MacConkey agar, was effective for initial screening, although it is known that these methods may not always provide definitive identification at the species level (Eng *et al*., 2021).

The molecular approach involving PCR amplification of the *16S rRNA* gene successfully identified all 20 *S. enterica* isolates, highlighting the robustness of 16S rRNA as a universal marker for bacterial identification (Janda & Abbott, 2021). The presence of *B-scDNAp* and *Hd* genes in 85% and 75% of the isolates, respectively, points to possible strain-specific virulence factors or genetic diversity within the *S. enterica* population. The *B-scDNAp* gene, which is likely associated with plasmid-encoded functions, has been previously linked to virulence and antibiotic resistance in other pathogens (Zhao *et al*., 2020), suggesting its potential role in the pathogenicity of the isolates identified in this study. Similarly, the *Hd* gene may be involved in the organism's adaptability or pathogenic mechanisms (Zhang *et al.*, 2022).

These findings are consistent with previous studies that have demonstrated the value of combining multiple molecular markers for accurate pathogen identification and typing (Karns *et al.*, 2019). The detection of multiple genetic markers can enhance diagnostic precision and provide insights into the genetic diversity and epidemiology of *S. enterica*. Moreover, our results support the use of PCR as a rapid and reliable diagnostic tool, particularly when compared to traditional biochemical methods that can be more time-consuming and less sensitive (Eng *et al.*, 2021; Janda & Abbott, 2021).

The variability observed in the presence of the *B-scDNAp and Hd* genes suggests that *S. enterica* may exhibit genetic heterogeneity, which could have implications for its pathogenic potential and resistance profiles. This variability underscores the importance of molecular surveillance for monitoring the spread of antibiotic-resistant strains and identifying new genetic targets for diagnostic and therapeutic interventions.

The molecular identification and characterization of *S. enterica* using gene-specific markers, such as *16S rRNA, B-scDNAp, and Hd*, provides a more comprehensive understanding of the genetic makeup and epidemiology of this pathogen. This approach not only enhances the sensitivity and specificity of diagnostic testing but also facilitates the study of virulence factors and resistance mechanisms, which are

CONCLUSION

This study demonstrates the effectiveness of using multiple molecular markers, including the *16S rRNA, B-scDNAp, and Hd* genes, for the identification and characterization of *Salmonella enterica*. The findings emphasize the importance of molecular diagnostics in enhancing the accuracy and speed of pathogen identification. Furthermore, the detection of genetic diversity within *S. enterica* isolates highlights the need for continuous surveillance and research into the virulence factors and resistance mechanisms of this significant zoonotic pathogen.

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