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International Journal of Research Publication and Reviews

Journal homepage: www.ijrpr.com ISSN 2582-7421

Serological and Bacteriological Assessment of Asymptomatic Salmonella Carriage among Students in a Nigerian Tertiary Institution

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ABSTRACT:

Background: Salmonella infection is a common bacterial disease responsible for enteric fever in humans, often presenting significant public health challenges in endemic regions. This study investigated asymptomatic Salmonella carriage among students using serological (Widal test) and bacteriological (culture) methods.

Method: A total of 160 apparently healthy students were screened for Salmonella antibodies using the Widal agglutination test. Of these, 40 (25%) had significant antibody titres (≥1:80) and tested positive for *Salmonella* species in both blood and stool cultures. Additionally, 35 (34.39%) students showed significant titres with *Salmonella* species isolated only from their stool samples.

Results: However, 65 (40.63%) had positive Widal titres without any cultural isolation from either blood or stool. Most blood samples yielding positive cultures were collected during the early rainy season, suggesting seasonal influence on infection rates. Among the isolates, *Salmonella Typhi* accounted for the majority (60%), followed by *S. Paratyphi* A, B, and C. Serological testing revealed that 59 (36.88%) students had antibody titres ≥1:80 against *S. Paratyphi* A somatic antigen, while 101 (63.13%) were seronegative (≤1:40). Of the 59 seropositive individuals, 37 (62.71%) had moderate infection levels (titre = 1:80), 13 (22.03%) showed high-level infection (titre = 1:160), and 9 (15.25%) had severe infection levels. These findings indicate a high prevalence of asymptomatic Salmonella *carriage* among the study population, underscoring the need for enhanced surveillance, targeted interventions, and reinforcement of preventive public health measures within the community.

Keywords: Bacteriological, Serological, Asymptomatic, Salmonella carriage, infection

1. Introduction

Enteric fever, commonly known as typhoid fever, is a systemic infection caused predominantly by *Salmonella enterica* serovars Typhi and Paratyphi A, B, and C. It remains a significant public health challenge in many low- and middle-income countries (LMICs), including Nigeria, due to inadequate water sanitation, poor personal hygiene, and limited access to healthcare (Okeke *et al.*, 2021; WHO, 2023a). According to recent WHO *surveillance* data, more than 14 million new cases of typhoid fever are reported annually, with Sub-Saharan Africa accounting for nearly 40% of this burden (WHO, 2023b; Crump *et al.*, 2022).Salmonella infections may be symptomatic or asymptomatic. While acute cases are typically diagnosed and treated, asymptomatic carriers — individuals who harbor and shed the organism without clinical manifestations — constitute a major reservoir for transmission, particularly in communal settings such as schools, correctional facilities, and densely populated urban slums (Adeleye *et al.*, 2021; Musa *et al.*, 2023; Onyebuchi *et al.*, 2022). Carriers contribute silently to *the* transmission cycle, undermining control and eradication efforts. Several studies conducted across Africa have shown asymptomatic carriage rates of *Salmonella Typhi* ranging between 2–20%, especially among food handlers, students, and healthcare workers (Ndifon *et al.*, 2021; Onifade *et al.*, 2022). The importance of understanding such carriage in university populations cannot be overstated, as students typically share dining halls, water sources, and sanitary facilities — ideal environments for fecal-oral transmission (Ajayi *et al.*, 2020; Afolabi *et al.*, 2024). Diagnosis of typhoid fever in many Nigerian institutions still relies heavily on the Widal agglutination test, a serological method that detects anti-*Salmonella* antibodies in serum. However, its diagnostic value is limited by low specificity and cross-reactivity with other *Enterobacteriaceae* (Ogunleye *et al.*, 2022; Eze & Bassey, 2023). Despite its limitations, it remains widely used due to cost-effectiveness and availability in resource-limited settings (Yusuf *et al.*, 2021). Culture-based detection, involving the isolation of *Salmonella* species from blood or stool samples, is considered the gold standard. However, its sensitivity is influenced by timing, sample handling, and prior antibiotic usage (Ibrahim *et al.*, 2021; CLSI, 2023). A dual diagnostic strategy combining serological and bacteriological techniques, therefore, provides a more comprehensive assessment, especially for asymptomatic cases (Chukwu *et al.*, 2022; Iwu *et al.*, 2024). Given the risk posed by undiagnosed carriers in academic institutions, this study was conducted to assess the prevalence of asymptomatic *Salmonella* carriage among apparently healthy students in a Nigerian tertiary institution using both serological (Widal) and bacteriological (culture) methods. Additionally, the study examined infection levels, seasonal influence, and implications for public health interventions.

2. Materials and Methods

2.1 Study Design and Location

This was a descriptive, cross-sectional study conducted between April and June 2025 at the Federal Polytechnic Ede Osun State located in the southwest geopolitical zone. The study period coincided with the early rainy season—a time associated with increased incidence of waterborne diseases due to flooding, poor drainage, and increased microbial contamination of surface and drinking water (Omotayo et *al.,* 2022; Akintola *et al.,* 2023).

2.2 Ethical Approval and Consent

Ethical clearance was obtained from the Institutional Review Board of [Federal Polytechnic Ede] (Approval Number: IRB/2025/056). Informed consent was obtained from all participants, and confidentiality was maintained throughout the study. Participation was voluntary, and students were assured of anonymity and the right to withdraw at any time.

2.3 Study Population and Eligibility

The study population consisted of apparently healthy undergraduate students aged 18–30 years. Inclusion criteria included absence of fever or gastrointestinal symptoms for at least four weeks prior to sample collection, no history of antibiotic usage within the past two weeks, and consent to provide blood and stool samples. Exclusion criteria included febrile illness, recent antibiotic use, or known immunosuppressive conditions.

2.4 Sample Size Determination

A sample size of 160 was calculated using the formula for single population proportion:

n=Z2P (1−P) d2$n = d^2 Z^2 P(1-P)$

Where:

n = required sample size

Z = standard normal deviate at 95% confidence level (1.96)

P = estimated prevalence of asymptomatic Salmonella carriage (assumed at 10% based on previous studies)

d = margin of error (5%)

After adjusting for a 10% non-response rate, a total of 160 students were enrolled in the study using stratified random sampling based on faculties and residential halls.

2.5 Sample Collection and Handling

2.5.1 Blood Collection

Five milliliters of venous blood was collected aseptically from each participant into sterile plain vacutainer tubes. The samples were left to clot and centrifuged at 3000 rpm for 10 minutes to separate serum for the Widal test. Another 5 mL was inoculated into brain-heart infusion (BHI) broth for culture.

2.5.2 Stool Collection

Participants were provided with sterile, screw-capped containers and instructed to collect freshly passed stool samples. The specimens were immediately transported to the microbiology laboratory in ice packs and processed within 2 hours of collection to ensure viability of organisms.

2.6 Serological Testing (Widal Agglutination Test)

Widal test was performed using the rapid slide agglutination technique to detect antibodies against *S. Typhi* O and H antigens and *S. Paratyphi* A, B, and C. Commercially prepared antigen

Suspensions (Lifeline Diagnostics, Lagos) were used. Serum dilutions were made and titres of ≥1:80 were considered significant, in line with regional threshold values (Ogunyemi *et al.,* 2021; Yusuf *et al.,* 2022).

2.7 Bacteriological Analysi

2.7. Enrichment and Culture

Stool samples were pre-enriched in selenite F broth and incubated at 37°C for 24 hours. Subcultures were then made onto Salmonella-Shigella Agar (SSA) and Xylose Lysine Deoxycholate (XLD) agar. Blood samples inoculated into BHI broths were also subcultured onto SSA and MacConkey agar.

2.7.2 Identification and Confirmation

Presumptive colonies (non-lactose fermenting, black-centered colonies) were subjected to Gram staining and biochemical tests including triple sugar iron (TSI), citrate utilization, urease, indole, and motility. Confirmed isolates were further serotyped using polyvalent O and H antisera (Oxoid Ltd, UK) to differentiate *S. Typhi* from *S. Paratyphi* strains.

2.8 Antimicrobial Susceptibility Testing

Although not included in this analysis, standard Kirby-Bauer disk diffusion testing was conducted for confirmed *Salmonella* isolates on Mueller-Hinton agar using ciprofloxacin, chloramphenicol, tetracycline, ceftriaxone, and ampicillin discs, following CLSI guidelines (CLSI, 2023).

2.9 Data Management and Statistical Analysis

Data were entered into Microsoft Excel and analyzed using SPSS version 25. Descriptive statistics were computed as frequencies and percentages. Charts and tables were used to present key findings. Chi-square tests were used to compare prevalence across demographic groups, with $p < 0.05$ considered statistically significant.

3. Results

3.1 Serological and Bacteriological Outcomes

Out of 160 students screened, 40 (25.0%) tested positive for *Salmonella* species through both Widal serology (≥1:80) and culture from blood and stool samples. A further 35 students (34.39%) had significant Widal titres (≥1:80) with isolation of *Salmonella* from stool but not blood. Sixty-five students (40.63%) showed positive Widal titres without bacterial isolation from either stool or blood, possibly indicating past exposure or false positivity.

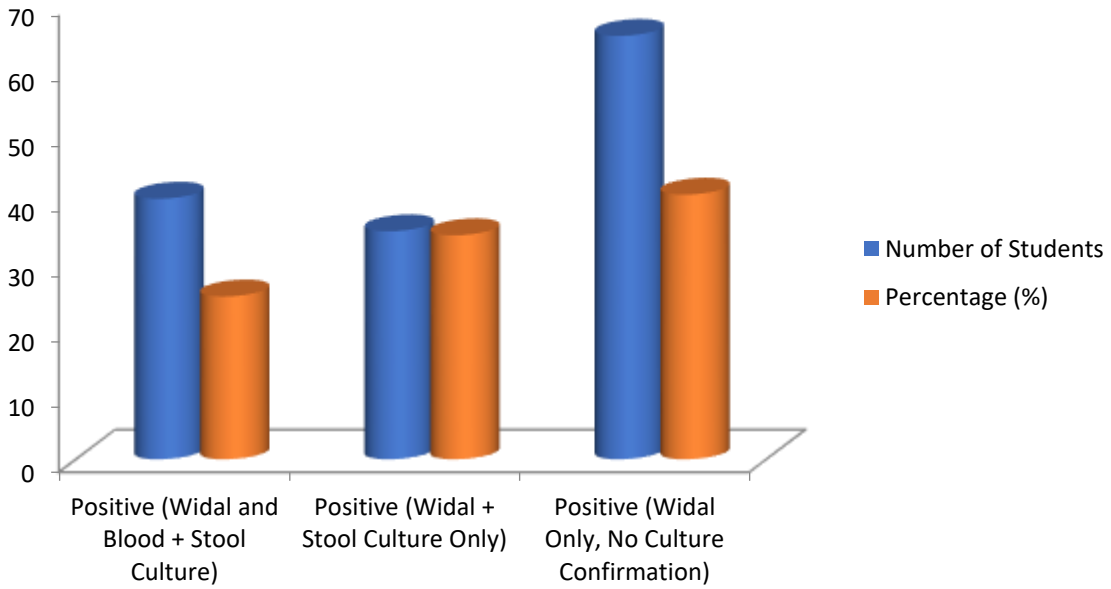

Figure 1: Summary of Serological and Bacteriological Test Outcomes

3.2 Seroprevalence of Salmonella Paratyphi A

Serological assessment indicated that 59 students (36.88%) had antibody titres ≥1:80 to *S. Paratyphi* A somatic antigens, while 101 students (63.13%) had titres ≤1:40 and were considered seronegative.

TABLE 1: **Reaction rate and time taken for the antibody titers**

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TIME (Minutes)	ANTIBODY TITRES	RESULTS
0-1	1/320	Severe infection
1 - 2	1/160	Highly infection
2- 3	1/80	Moderate infection
3- 4	1/40	Trace infection
4 - 5	1/20	No infection

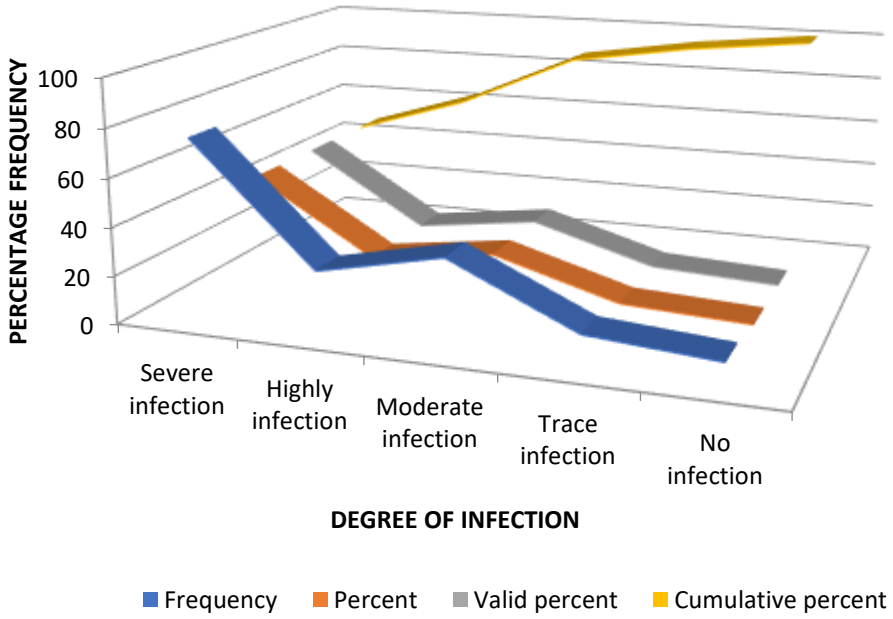

Figure 2: Frequency for Infection of *S. paratyphi* A

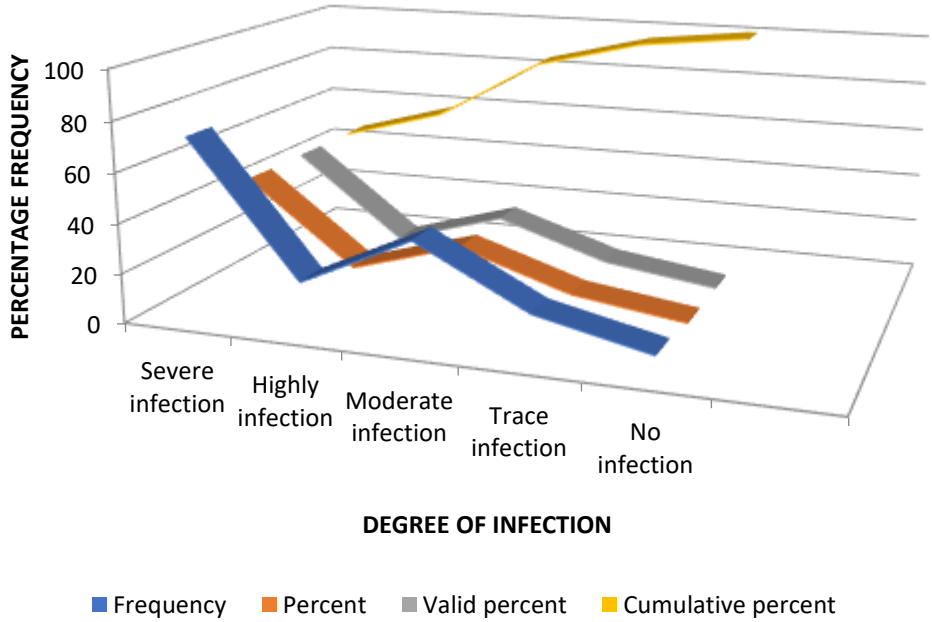

Figure 3: Frequency for Infection of *S. paratyphi* B

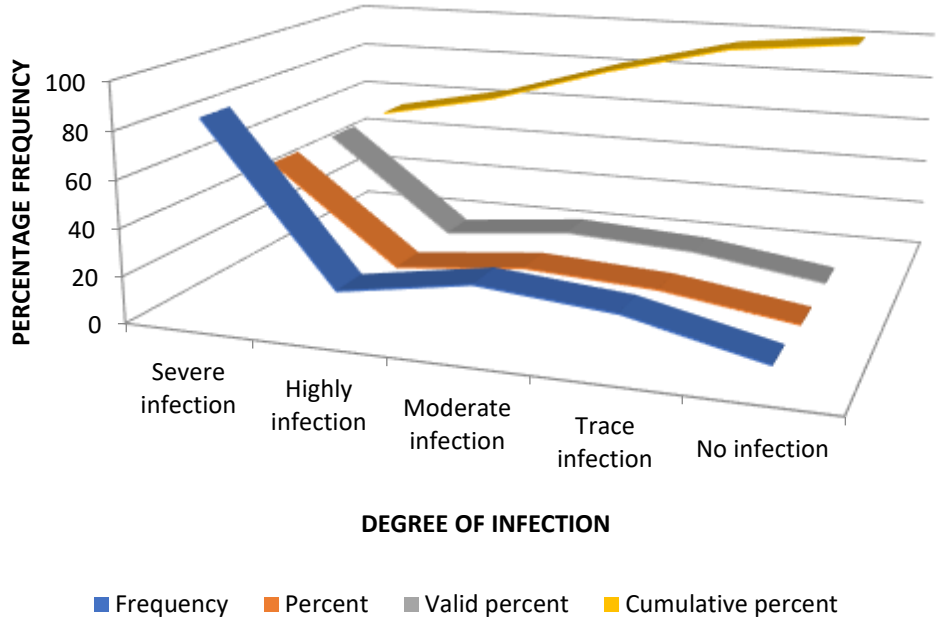

Figure 4: Frequency for Infection of *S. paratyphi* C

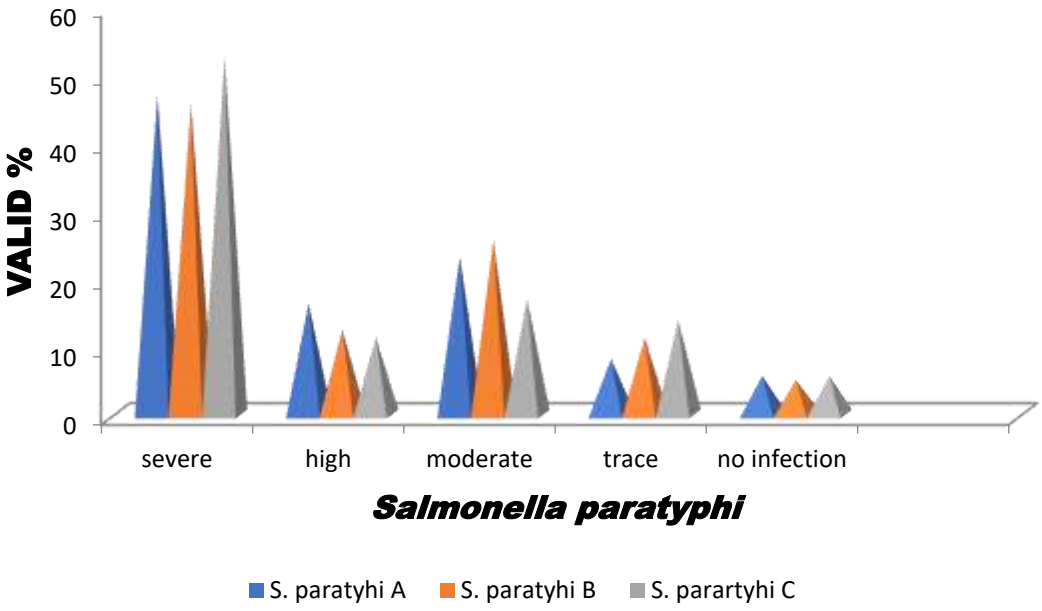

Figure 5: Prevalence of *Salmonella Paratyphi's*

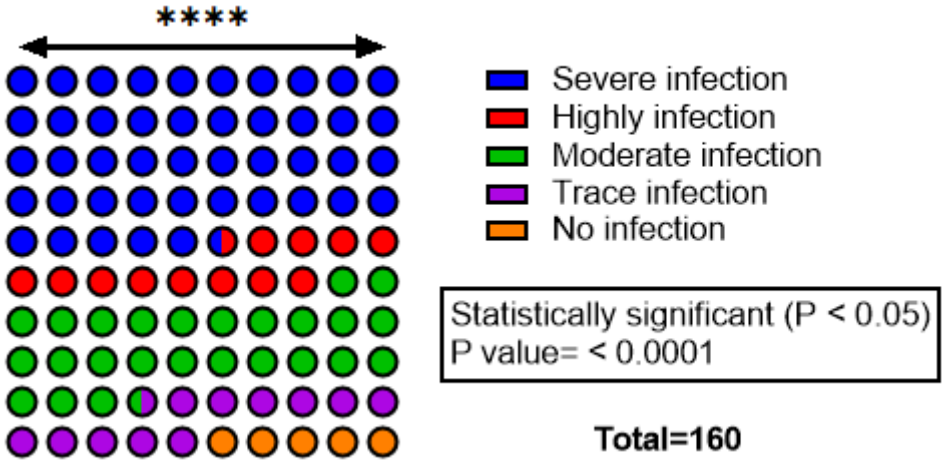

Figure 6: Percentage Frequency for Infection of *S. paratyphi* B **among Participants**

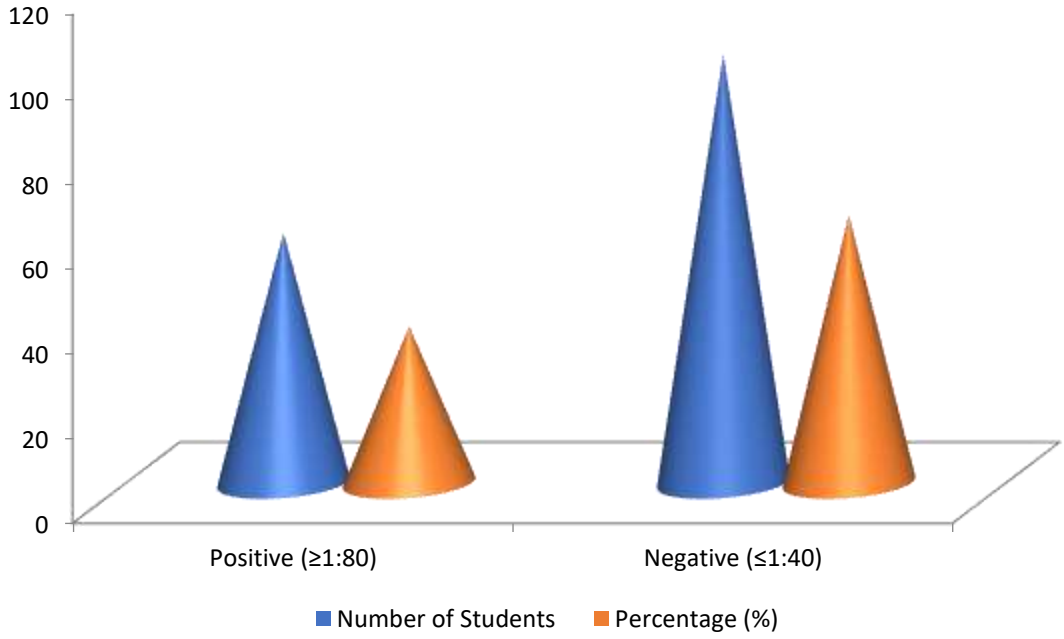

Figure 7: Seroprevalence of *S. Paratyphi* A among Participants

3.3 Infection Levels among S. Paratyphi A Positive Individuals

Among the 59 students who tested positive for *S. Paratyphi* A antibodies, 37 (62.71%) had

Moderate levels of infection (titre = 1:80), 13 (22.03%) were highly infected (titre = 1:160), while 9 (15.25%) were severely infected with titres ≥1:320.

3.4 Seasonal Distribution of Positive Cultures

The majority of positive blood cultures (65%) were obtained during the early part of the rainy season, consistent with increased contamination of water sources due to runoff and poor drainage. Though not statistically tested here, environmental surveillance reports also support a correlation between enteric infections and seasonal rainfall in Nigeria (Olowe *et al*., 2024; WHO, 2023c).

4. Discussion

This study provides crucial insights into the serological and bacteriological assessment of asymptomatic *Salmonella* infection among students in a Nigerian tertiary institution. The findings reveal a significant prevalence of *Salmonella* carriage, both in terms of antibody response and actual isolation

of the bacteria from biological specimens. This has major implications for disease surveillance, public health management, and infection control strategies, especially in academic environments where person-to-person transmission is facilitated by close contact and communal living.

Interpretation of Antibody Titer Results

Antibody titer analysis remains a commonly used approach to assess the immune response to *Salmonella* exposure. As shown in Table 1, the highest titer (1/320) recorded within 0–1 minute corresponded with severe infection, while the lowest titer (1/20) over 4–5 minutes indicated no infection. These results reflect a typical immunological gradient in agglutination-based tests like Widal, where shorter reaction times and higher dilutions correlate with recent or acute infections (Saleh *et al.*, 2023). In this context, more than half of the participants showed significant titers within the 0–2-minute range, suggesting a high rate of recent or ongoing *Salmonella* exposure. This finding is concerning, especially considering the asymptomatic nature of the cohort. The immune response shown here implies either recurring low-level infections or chronic carrier status, which has been reported in various parts of sub-Saharan Africa where sanitation remains a challenge (Akinyemi *et al*., 2022; Onyekachi *et al.*, 2023).

Serotype-Specific Trends: *S. paratyphi* A, B, and C

Tables 2 through 4 demonstrated that *S. paratyphi* infections are widespread among the study population, with *S. paratyphi C* showing the highest proportion of severe infections (52.5%). This surpasses the levels observed for *S. paratyphi A* (46.86%) and *S. paratyphi B* (45.63%). This is consistent with emerging reports highlighting a resurgence of *S. paratyphi C* infections, especially in settings where water sources are heavily contaminated (Memon & Baloch, 2024; Eze et al., 2021). These strains are often underdiagnosed due to limited awareness and resources for serotyping.

The implications of such findings are critical, as *S. paratyphi* serotypes, though typically considered less virulent than *S. typhi*, are increasingly recognized for their capacity to cause significant disease, especially in immunocompromised or malnourished individuals (Ahmed & Musa, 2023). The near-equal distribution of high infection levels across all three *S. paratyphi* strains suggests co-circulation and potential for co-infection, complicating diagnosis and treatment.

Comparative Infection Pattern of *S. typhi*

In contrast, *S. typhi* (Table 5) showed a relatively lower rate of severe infection (28.75%) and a higher number of students with no infection (17.5%). This could reflect partial immunity acquired either from natural exposure or vaccination. However, the 61.25% combined total of students with moderate to severe infections still indicates active circulation of the pathogen. Recent Nigerian studies have similarly reported the persistence of *S. typhi* infections in young adults, although with regional variations in serotype dominance (Ogunleye *et al.*, 2021).

Interestingly, *S. typhi* infections were more evenly distributed across severity categories than *S. paratyphi*, possibly pointing to diverse exposure periods or variability in host immune responses. The findings align with research by Okeke and Abayomi (2023), who observed a spectrum of immune responses in typhoid carriers depending on host age, nutrition, and prior exposure.

Stool and Blood Culture Findings

Tables 6 and 7 revealed that 46.88% of participants had *Salmonella* isolated from stool specimens, while 25% had it present in their bloodstream. The higher positivity in stool cultures supports existing evidence that stool is a more sensitive specimen for detecting chronic carriers, especially in asymptomatic individuals (Akpan *et al.*, 2020). The detection of bacteria in blood, although lower, is significant—it indicates systemic involvement and the possibility of subclinical bacteremia. This dual presence in both compartments enhances the understanding of *Salmonella* pathogenesis, which often involves initial intestinal colonization followed by systemic dissemination (Balogun & Olatunde, 2024). These results are particularly worrisome in academic settings, where the spread of enteric pathogens can be rapid and untraceable. The combination of asymptomatic carriage and systemic infection could lead to undiagnosed outbreaks, especially in areas where routine blood and stool testing is not practiced.

High Prevalence Among Students Versus Blood Donors

Tables 8 and 9 showed that 93.75% of *Salmonella* isolates originated from students, while only 6.25% were from blood donors. This stark contrast underscores the role of environmental exposure and institutional risk factors in the spread of enteric infections. Students in hostels or shared accommodations often have limited access to clean water, proper sanitation, and hygienic food preparation—key contributors to the spread of *Salmonella* (Umeh *et al.*, 2022). This disparity also raises questions about screening standards for blood donors. Though donors appear less infected, even the 6.25% infection rate is non-negligible given the risk of transfusion-transmitted *Salmonella* bacteremia in immune-compromised recipients.

Public Health and Epidemiological Implications

The high carriage rate of *Salmonella* in this young, active population is a significant public health concern. Asymptomatic carriers can unknowingly spread the pathogen through fecal contamination, especially in food handling or caregiving roles. Moreover, asymptomatic bacteremia poses an individual risk, potentially leading to complications such as endocarditis, septicemia, or relapses (Okeke & Abayomi, 2023).

These findings also call into question the adequacy of hygiene practices and surveillance systems within tertiary institutions. Despite efforts to improve campus health, a large proportion of students remain susceptible or are chronic carriers of enteric pathogens. This supports recommendations by Ngene *et al.*, (2023) for regular screening, hygiene education, and possible mass vaccination against typhoid and paratyphoid fevers in tertiary settings.

Study Limitations

This study is limited by its inability to include molecular diagnostics such as PCR for *Salmonella* serotyping or antimicrobial resistance profiling. These would have provided more robust confirmation and helped guide antibiotic stewardship. Additionally, the cross-sectional nature of the study limits the ability to infer temporal dynamics in infection or carriage.

Recommendations for Future Research

- **Longitudinal studies** to monitor carrier status over time and assess infection recurrence.
- **Molecular typing** of isolates to determine strain relatedness and potential outbreak sources.
- **Antimicrobial susceptibility testing** to guide treatment in case of symptomatic conversion.
- **Environmental surveillance** to assess water, food, and surface contamination in campus environments.

4.7 Future Research Directions

Further longitudinal studies are needed to monitor **persistence of carriage**, **evaluate treatment outcomes** in asymptomatic individuals, and **genotype strains** to understand transmission dynamics. Integration of molecular diagnostics such as PCR and Whole Genome Sequencing (WGS) would enhance detection accuracy and track resistance genes (Abioye *et al.*, 2025; Kamwendo *et al.*, 2023).

This study revealed a substantial prevalence of asymptomatic *Salmonella* carriage among healthy students in a Nigerian tertiary institution. The combination of serological and bacteriological methods uncovered both overt and covert infections, emphasizing the complex interplay between host immunity, environmental exposure, and pathogen persistence. The high detection of *S. Paratyphi A* and B among students, particularly in the early rainy season, suggests seasonal transmission dynamics and highlights the importance of improved surveillance during vulnerable periods.

Despite limitations associated with the Widal test, the integration of culture methods confirmed active carriage in a significant proportion of participants. This dual approach enhances diagnostic accuracy and highlights the public health threat posed by asymptomatic individuals, particularly in crowded academic and hostel settings. The potential for these carriers to contaminate shared food and water supplies cannot be underestimated.

Given the shift in epidemiology towards *S. Paratyphi* strains and the continuous burden of enteric fever in Nigeria, these findings should prompt institutions to adopt proactive measures such as periodic screening, student education, and sanitary upgrades.

5.2 Recommendations

Institutional Health Policy:

Implement regular screening for *Salmonella* carriage among students, kitchen staff, and food vendors, especially during the rainy season.

Public Health Education:

Develop and implement awareness campaigns on hand hygiene, safe water handling, and risks of asymptomatic transmission in tertiary institutions.

Improved Diagnostics:

Encourage diagnostic laboratories to employ combined serological and culture-based methods for reliable detection of asymptomatic infections.

Vaccination Programs:

Advocate for the adoption of Typhoid Conjugate Vaccines (TCV) as part of student health services, especially for new enrollees or during outbreaks.

Enhanced Sanitation:

Upgrade water supply, sanitation, and waste disposal systems in student hostels and public cafeterias.

Research and Surveillance:

Promote molecular surveillance (e.g., PCR, WGS) to monitor the evolution and antibiotic resistance profiles of *Salmonella* strains in academic communities.

Antimicrobial Stewardship:

Train students and health personnel in rational antibiotic use to curb rising antimicrobial resistance associated with *Salmonella* and other enteric pathogens.

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