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IDENTIFICATION OF SALMONELLA TYPHI IN BLOOD SAMPLES FROM VARIOUS ETHNIC ADULT POPULATIONS IN QUETTA THROUGH SELECTIVE CULTURE AND MOLECULAR APPROACHES

Syed Rafiq Shah[1], Shabir Ahmad Khan[1]*, Muhammad Shafee[1], Muhammad Ali Khan[1]

[1]**Department Center for Advanced Studies in Vaccinology and Biotechnology (CASVAB),** University of Balochistan, Quetta, Pakistan

***Corresponding Author:** Syed Rafiq Shah. E. mail: shabirahmadkhan@gmail.com

Abstract

Objective: *Salmonella typhi, the etiological agent of typhoid fever, remains a significant public health concern in developing countries. This study aimed to identify and confirm S. typhi in blood samples obtained from various ethnic adult populations in Quetta, Pakistan, using selective culture techniques and molecular approaches.*

Methodology: *A total of 234 blood samples were collected from adult patients belonging to different ethnic communities over a period of ten months. Samples were processed using selective media (XLD agar and MacConkey agar), biochemical tests, Gram staining, and polymerase chain reaction (PCR) for confirmation of S. typhi. Antimicrobial susceptibility testing was performed using standard disc diffusion method.*

Results: *Among the 234 samples, 26% (n=61) tested positive for typhoid fever, while 74% (n=173) tested negative. Gender-based analysis revealed higher prevalence in males (18.0%) compared to females (8.0%). Age-wise distribution showed prevalence rates of 10.25% in the 18-20 years age group, 10.40% in the 18-20 years group, and 4.25% in the 18-25 years group. Ethnic distribution demonstrated the highest prevalence among Pashtoon (9.40%), followed by Baloch (7.30%), Punjabi (5.10%), and Hazara (4.20%) populations. PCR amplification confirmed S. typhi with specific bands at 230 bp. Antimicrobial susceptibility testing revealed resistance to multiple antibiotics including chloramphenicol, amoxicillin, penicillin G, polymyxin B, ciprofloxacin, metronidazole, lincomycin, and trimethoprim, while susceptibility was observed only to vancomycin.*

Conclusion: *The findings highlight the significant burden of typhoid fever in Quetta, particularly among specific ethnic groups and male populations. The study emphasizes the urgent need for improved water supplies, enhanced vaccination coverage, better sanitation infrastructure, and community awareness programs to control typhoid transmission in high-risk areas.*

Keywords: *Blood tests, ELISA, Ethnic groups, PCR, Salmonella typhi*

INTRODUCTION

Salmonella typhi, a motile, flagellated, Gram-negative, facultative anaerobic bacterium, measures approximately 0.7-1.5 µm in width and 2.0-5.0 µm in length (1). This pathogen exclusively causes typhoid fever in humans and is primarily transmitted through contaminated water, food, vegetables, and raw meat products (1). The bacterium frequently causes gastrointestinal infections, particularly in regions with poor sanitation, inadequate hygiene practices, and unsafe water sources (2). Both humans and animals can contract salmonellosis, with over one-third of cases occurring in individuals under ten years of age, indicating higher susceptibility among younger populations (3).

Typhoid fever exhibits geographical variation in incidence rates. Southeast and South-Central Asian regions demonstrate particularly high susceptibility, while incidence rates are generally lower in Africa, North America, Latin America, the Caribbean, and Europe (4). Among South and Southeast Asian countries, the disease is more frequently reported among pre-schoolers and young adults (5). Several nations, including China, Vietnam, India, Indonesia, and Pakistan, have assessed typhoid fever incidence (6). Pakistan reported substantially higher rates of 451.7 cases per 100,000 populations annually in individuals aged 2-15 years, compared to China's reported rate of 15.3 cases per 100,000 populations annually (7).

Furthermore, India reported 430.1 cases, Indonesia reported 148.7 cases, and Pakistan reported 573.2 cases per 100,000 populations annually among children (6). These data indicate that South Asian countries, particularly Pakistan and India, bear a significantly higher disease burden compared to Northeast and Southeast Asian nations.

In Pakistan, specifically in Karachi, blood culture-based methods indicate an incidence rate of 170 cases per 100,000 populations, while serology-based studies suggest a much higher rate of 710 cases per 100,000 populations (8). The prevalence of typhoid fever in Pakistan is attributed to multiple factors including contaminated water sources, inadequate drainage systems, lack of education, and insufficient treatment leading to increased mortality (9). Effective control measures include improving water supplies, enhancing vaccination coverage, and implementing better sanitation practices (9). Rapid and appropriate laboratory diagnostic methods are crucial for addressing this public health challenge, with blood tests, PCR, and ELISA playing important roles in typhoid detection (10).

Biochemically, *S. typhi* typically demonstrates negative results for indole, urease, and tryptophan deamination tests. It ferments various carbohydrates, reduces nitrates, and produces acid, while showing negative Voges-Proskauer (VP) reactions (11). Enteric fever remains prevalent in Quetta city due to overcrowding, poor sanitation, and limited access to safe drinking water. Local healthcare facilities often rely on less accurate serological tests such as the Widal test, leading to misdiagnosis and inappropriate antibiotic use, which contributes to the emergence of multi-drug resistant *S. typhi* strains (12). *Salmonella typhi* can survive in both aerobic and anaerobic environments, particularly during human infection, though the mechanisms underlying its host specificity remain incompletely understood (13). Chronic carriers of *S. typhi* are at increased risk of developing malignancies in the gallbladder, pancreas, and colon, causing significant morbidity (13). The bacterium exhibits distinctive biochemical properties, including the inability to produce indole, hydrolyze urea, or deaminate phenylalanine or tryptophan. Most salmonella serotypes reduce nitrates to nitrites and produce acid through carbohydrate fermentation. Notably, *S. enterica* serovar Typhi and *S. paratyphi* A do not ferment lactose and cannot utilize citrate, distinguishing them from other salmonella serotypes. Objectives of the present study were to isolate *S. typhi* from blood specimens collected from the adult population in Quetta city, to identify local strains of *S. typhi* using a combination of PCR and biochemical tests and to evaluate drug sensitivity patterns in relation to *S. typhi*.

MATERIALS AND METHODS

A total of 234 blood samples were collected from adult patients over a period of ten months. Fresh blood samples were collected and immediately stored in Cary Blair transport medium (Oxoid Ltd, Basingstoke, UK). All samples were transported to the laboratory within six hours of collection. Random sampling was employed to ensure a representative dataset for the study.

SAMPLE PROCESSING

Samples were inoculated into selenite cysteine broth medium and incubated at 37°C for 18-24 hours. Following incubation, aliquots were streaked onto xylose lysine deoxycholate (XLD) agar and incubated at 37°C for 18-24 hours. Bacterial colonies obtained from XLD agar were subsequently subcultured onto MacConkey agar for confirmation of lactose fermentation. Pure cultures of non-lactose fermenting organisms were subjected to Gram staining and standard biochemical tests for confirmation.

SELECTIVE MEDIA

Selective media were utilized for *Salmonella* isolation, involving preliminary enrichment steps followed by cultivation on selective and differential agar media. Identification was achieved through biochemical and serological tests.

Stained slides were air-dried and examined under an oil-immersion objective lens (100x) using a microscope, with results recorded accordingly.

BIOCHEMICAL TESTS

Isolated colonies were subjected to a series of biochemical tests for identification of *S. typhi*. The catalase test was performed to differentiate catalase-producing bacterial colonies from non-catalase-producing colonies. A drop of 3% hydrogen peroxide was placed on a clean glass slide, and a small portion of the test colony was mixed into the reagent. Positive catalase reaction was indicated by the immediate appearance of bubbles (14).

Tetramethyl-p-phenylenediamine dihydrochloride was added to the colony, and results were recorded (15).

Straight platinum loops were used for culturing in SIM media tubes, which were incubated for 24 hours at 37°C before reading results. For the indole test, two or three drops of Kovacs reagent were added to the same tube and the control tube (15).

The ability to produce acid through glucose fermentation was assessed using the methyl red (MR) test. Pure cultures of *S. typhi* were inoculated into MR-VP broth and incubated for 48 hours at 37°C. Following incubation, a few drops of methyl red indicator were added. A red color appearance, indicating mixed acid fermentation, confirmed a positive MR reaction (14).

POLYMERASE CHAIN REACTION (PCR)

PCR was employed for molecular identification of *S. typhi*. Forward primer sequences (5'-GTT ATT TCA GCA TAA GGA G-3') and reverse primer sequences (5'-ACT TGT CCG TGT TTT ACT C-3') were used for amplification. PCR products were visualized using gel electrophoresis, with clear bands measuring 230 bp in length.

RESULTS

A total of 234 blood samples from adult patients across different ethnic communities were collected and processed for identification and confirmation of *Salmonella typhi*. The overall positivity rate was 26.0% (n=61), while 74.0% (n=173) tested negative (Fig. 1a).

RACE-WISE DISTRIBUTION OF TYPHOID PATIENTS

Race-wise distribution of typhoid fever cases demonstrated distinct trends, with Pashtoon ethnicity showing the highest prevalence (9.40%), followed by Baloch (7.30%), Punjabi (5.10%), and Hazara (4.20%) (Fig. 1b). Gender-based analysis revealed higher prevalence in males (18.0%) compared to females (8.0%). Age-wise distribution showed prevalence rates of 10.25% in the 18-20 years age group, 10.40% in the 18-20 years group, and 4.25% in the 18-25 years group.

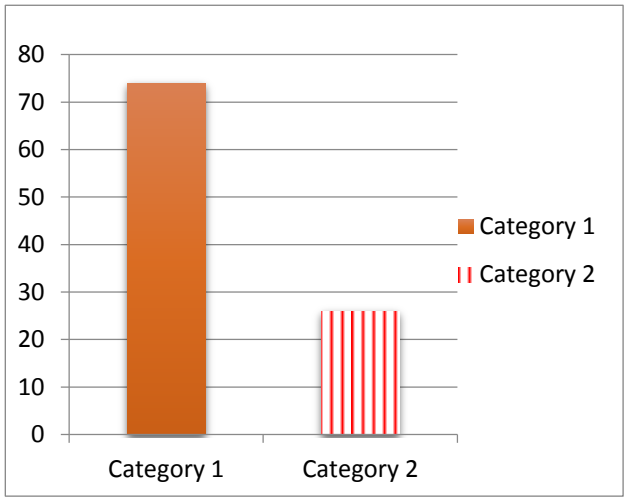
SALMONELLA TYPHI MORPHOLOGICAL FEATURES ON XLD MEDIUM

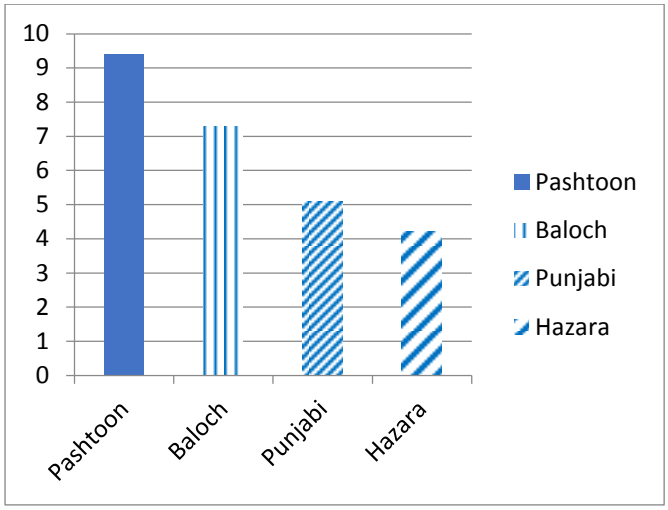
Following growth on XLD agar and incubation at 37°C for 24 hours, *S. typhi* colonies exhibited characteristic features. Colonies were approximately 2-3 mm in diameter, appearing as red colonies with black centers, with a clear surface and flattened appearance (Fig. 1c).

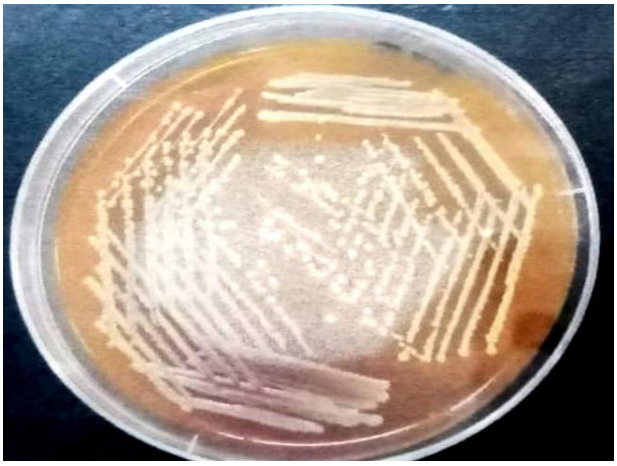
Gram staining and examination under 100x oil immersion microscopy revealed the presence of single and paired Gram-negative rods measuring 0.7-1.5 μm in size (Fig. 1d).

ANTIBIOTIC SENSITIVITY TESTING

Antimicrobial susceptibility testing revealed varying resistance patterns. The isolates demonstrated resistance to multiple antibiotics, including chloramphenicol (30 μg), amoxicillin (10 μg), penicillin G (10 μg), polymyxin B (10 μg), ciprofloxacin (5 μg), metronidazole (25 μg), lincomycin (30 μg), and trimethoprim (5 μg). Notably, susceptibility was observed only to vancomycin (30 μg), with a zone of inhibition of 23 mm (Table I).







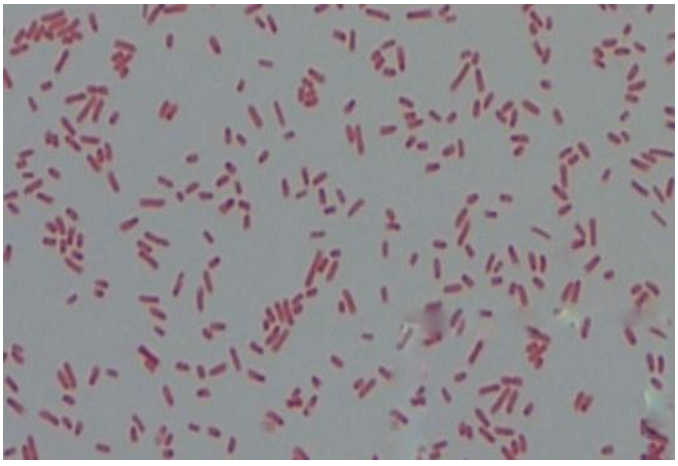


Fig. 1(a). Overall occurrence of Typhoid fever in Quetta City; **(b).** Race-wise Distribution of Typhoid Fever in Population of Quetta City**; (c).** *Salmonella typhi* growth on XLD media after 24 hours incubation at 37°C; **(d).** Gram staining of specimen showing single and paired Gram-negative rods (0.7-1.5 µm) observed under 100X oil immersion objective

Table I. Antimicrobial Susceptibility Pattern of ***S. typhi*** Isolates

Class	Antibiotic	Abbreviation	Concentration (µg)	Zone of Inhibition
Chloramphenicol	Chloramphenicol	C	30	0 mm
	Amoxicillin	AML	10	0 mm
Penicillin	Penicillin G	P	10	0 mm
	Polymyxin B	POL	10	0 mm
Glycopeptides	Vancomycin	VA	30	23 mm
Quinolones	Ciprofloxacin	CIP	5	0 mm
Flagyl	Metronidazole	MTZ	25	0 mm
Lincosamides	Lincomycin	L	30	0 mm
Sulfonamides	Trimethoprim	W	5	0 mm

PCR AMPLIFICATION

PCR successfully amplified DNA extracted from *S. typhi* isolates using specific primers. The forward primer sequence (5'-GTT ATT TCA GCA TAA GGA G-3') and reverse primer sequence (5'-ACT TGT CCG TGT TTT ACT C-3') yielded clear bands measuring 230 bp in length, confirming the presence of *S. typhi* (Fig. 2).

DISCUSSION

The present study identified *Salmonella typhi* in 26% of blood samples collected from adult patients in Quetta, demonstrating a substantial burden of typhoid fever in the region. This finding is consistent with previous reports from Pakistan, which have documented high incidence rates of typhoid fever, particularly in urban and peri-urban settings with inadequate sanitation infrastructure (6,8,9). The overall positivity rate

of 26% is comparable to studies conducted in other South Asian countries, reflecting the endemic nature of typhoid fever in the region (6).

Fig. 2. DNA extraction and PCR amplification from ***S. typhi***

Gender-based analysis revealed a higher prevalence among males (18.0%) compared to females (8.0%). This disparity may be attributed to differences in occupational exposure, healthcare-seeking behavior, and social practices that increase male exposure to contaminated food and water sources (9). Similar gender differences have been reported in other studies from Pakistan and neighboring countries (6, 9). The higher prevalence among males warrants targeted public health interventions addressing occupational and behavioral risk factors specific to male populations.

The age distribution of typhoid cases showed higher prevalence in younger adults (18-20 years), consistent with global epidemiological patterns indicating that typhoid fever predominantly affects children and young adults in endemic regions (4,5). The relatively high prevalence in this age group may be related to increased social mobility, dietary habits, and exposure to contaminated sources outside the home environment (5). Public health strategies should prioritize vaccination campaigns targeting this vulnerable age group.

Ethnic distribution of typhoid cases demonstrated the highest prevalence among Pashtoon (9.40%), followed by Baloch (7.30%), Punjabi (5.10%), and Hazara (4.20%) populations. These differences may reflect variations in socioeconomic status, living conditions, access to clean water, sanitation facilities, and healthcare services among different ethnic communities in Quetta (9). The higher prevalence among Pashtoon and Baloch populations warrants further investigation into specific risk factors and culturally appropriate interventions for these communities.

The morphological characteristics of *S. typhi* colonies on XLD agar (red colonies with black centers, 2-3 mm in diameter) are consistent with standard descriptions and confirm the identity of the organism (16). Similarly, Gram staining results revealing single and paired Gram-negative rods measuring 0.7-1.5 μm align with established morphological criteria for *S. typhi* (1). These findings underscore the utility of selective culture methods in the routine diagnosis of typhoid fever.

PCR amplification successfully identified *S. typhi* with specific bands at 230 bp, confirming the molecular detection of the pathogen. The use of PCR has been advocated as a rapid and highly sensitive diagnostic tool for typhoid fever, particularly in resource-limited settings where conventional culture methods may be less sensitive due to prior antibiotic use (10, 17). The molecular confirmation of *S. typhi* in this study validates the culture-based findings and provides robust evidence for the presence of the pathogen in the study population.

Antimicrobial susceptibility testing revealed a concerning pattern of multidrug resistance among *S. typhi* isolates. Resistance to chloramphenicol, amoxicillin, penicillin G, polymyxin B, ciprofloxacin,

metronidazole, lincomycin, and trimethoprim was observed, with susceptibility only to vancomycin. This pattern is consistent with reports of increasing antimicrobial resistance among *S. typhi* isolates in Pakistan and other South Asian countries (8, 12). The emergence of multidrug-resistant *S. typhi* strains poses significant challenges for clinical management and public health control (12). The susceptibility to vancomycin observed in this study requires further investigation, as vancomycin is not typically used for *Salmonella* infections and may not be clinically relevant.

The findings of this study have important implications for public health policy and practice in Quetta and similar settings. The high prevalence of typhoid fever, particularly among specific ethnic groups and male populations, underscores the need for targeted interventions. Improving water supplies, enhancing vaccination coverage, and implementing better sanitation practices are critical measures for reducing typhoid transmission (9). Additionally, raising community awareness about the importance of hand hygiene, food safety, and proper waste disposal is essential for sustainable disease control.

The study also highlights the limitations of relying solely on serological tests such as the Widal test for diagnosis, which may lead to misdiagnosis and inappropriate antibiotic use (11). The integration of culture-based methods and molecular techniques such as PCR can improve diagnostic accuracy and guide appropriate antimicrobial therapy, potentially reducing the selection pressure that drives antimicrobial resistance (17).

CONCLUSION

This study identified a substantial burden of typhoid fever in Quetta, with 26% of blood samples testing positive for *S. typhi*. The disease demonstrated higher prevalence among males and specific ethnic groups, particularly Pashtoon and Baloch populations. The high proportion of multidrug-resistant isolates emphasizes the urgency of implementing effective control measures and surveillance programs.

Conflict of interest:

Authors declared no conflict of interest.

Authors' contribution:

SRS Sample collection, laboratory experiments, data analysis, manuscript drafting; SAK Research supervision & design, technical guidance, manuscript review and revision; MS Sample processing, biochemical identification, antimicrobial susceptibility testing, data interpretation; MAK Sample collection and transport, PCR optimization, gel electrophoresis, statistical analysis.

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