International Journal of Science and Research Archive
eISSN: 2582-8185
Cross Ref DOI: 10.30574/ijsra
Journal homepage: https://ijsra.net/

(RESEARCH ARTICLE)

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# INTEGRATED ANALYSIS OF ANTIMICROBIAL RESISTANCE AND CYTOKINE PROFILES (IL-12A, IFN-Γ) IN TYPHOID FEVER PATIENTS FROM NAJAF PROVINCE, IRAQ

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International Journal of Science and Research Archive, 2026, 20(02), 425–431

Publication history: Received on 03 July 2026; revised on 15 August 2026; accepted on 17 August 2026

Article DOI: https://doi.org/10.30574/ijsra.2026.20.2.1632



## ABSTRACT

**Background:** Typhoid fever, caused by *Salmonella Typhi*. The rise of extensively drug-resistant (XDR) strains has reduced treatment options. Control depends on the Th1 immune response, especially the IL-12/IFN-γ pathway.

**Objective:** To assess antimicrobial susceptibility of *S. Typhi* isolates and compare serum IL-12α and IFN-γ levels between typhoid patients and healthy controls in Najaf, Iraq.

**Methods:** The cross-sectional study included 30 blood culture-confirmed *S. Typhi* patients from Najaf hospitals. Isolates were tested for antimicrobial susceptibility using VITEK 2 (AST-GN card). Serum IL-12α and IFN-γ were measured by ELISA in patients and 30 matched controls. Data were analyzed in SPSS. $p < 0.05$ was considered significant.

**Results:** Isolates retained the highest susceptibility to imipenem (83.3%), meropenem (80.0%), and trimethoprim-sulfamethoxazole (60.0%), with low susceptibility to third-generation cephalosporins and azithromycin (≤20%). Patients and controls were well matched for age (17.6 ± 7.2 vs 18.7 ± 8.4 years, $p = 0.58$) and sex (56.7% male in both groups, $p = 1.00$). Both cytokines were significantly higher in patients than controls: IFN-γ (111.99 ± 26.55 vs 62.97 ± 11.94 ng/mL; $p < 0.001$) and IL-12α (382.29 ± 29.45 vs 253.56 ± 16.01 ng/L; $p < 0.001$). Within patients, IFN-γ and IL-12α were positively correlated (Spearman $r = 0.41$, $p = 0.023$). ROC curve analysis showed excellent discrimination between patients and controls for both markers (IL-12α AUC = 0.992; IFN-γ AUC = 0.982).

**Conclusions:** *S. Typhi* isolates remained most susceptible to carbapenems, with high resistance to cephalosporins and azithromycin. Larger studies linking resistance phenotypes to cytokine responses and validating cutoffs across diverse fevers are required.

**Keywords:** *Salmonella typhi*; Antimicrobial Resistance; Interferon-Gamma; Interleukin-12; Typhoid Fever; Najaf; Iraq

## 1 INTRODUCTION

Typhoid fever, caused by *Salmonella enterica* serovar Typhi, continues to impose a substantial burden in areas with inadequate sanitation and unsafe drinking water. A recent Global Burden of Disease analysis estimated millions of enteric fever illnesses and well over 100,000 deaths in 2021 alone, with the highest incidence concentrated in South-Central and Southeast Asia and a persistent burden across the Middle East and North Africa [1,2]. Antimicrobial resistance has progressively eroded the therapeutic options for this disease: extensively drug-resistant (XDR) *S. Typhi,* first described at scale in Hyderabad, Pakistan, has since spread internationally and is now resistant to first-line agents, fluoroquinolones, and third-generation cephalosporins, leaving carbapenems and, in some regions, azithromycin as the remaining reliable options [3,4].

Beyond the microbiological dimension, control of *S. Typhi*, an intracellular pathogen, depends heavily on a coordinated cell-mediated immune response. Interleukin-12 (IL-12), produced by antigen-presenting cells, drives differentiation of naïve T cells toward a T-helper type 1 (Th1) phenotype and induces interferon-gamma (IFN-γ) production, which in turn activates macrophages to kill intracellular bacteria; recent work using human gallbladder organoid and epithelial models has reaffirmed that *S. Typhi* actively engages host cytokine signaling during both acute and chronic infection [5]. Iraqi case-control data have likewise shown that immune marker profiles are significantly altered in patients with confirmed *S. Typhi* infection compared with healthy controls, supporting an active, detectable host response in peripheral blood [6]. Defects in the IL-12/IFN-γ axis have also been implicated in cases of recurrent typhoid fever [7], underscoring the clinical relevance of this axis to disease outcome, independent of the antimicrobial susceptibility of the infecting strain.

Despite the parallel importance of resistance phenotype and host immune response, few studies examine both in the same population, and population-level data on *S. Typhi* antimicrobial susceptibility from Iraq remain limited, though recent local reports describe substantial resistance among clinical, food-associated, and pediatric isolates [8-11]. A recent hospital-based study of pediatric patients in Najaf reported high resistance to ceftazidime, ceftriaxone, ciprofloxacin, and the aminoglycosides, with the highest retained susceptibility to meropenem, imipenem, and trimethoprim-sulfamethoxazole [8], but did not include any assessment of host cytokine responses. The present study was therefore designed to jointly characterize, in the same setting, (1) the antimicrobial susceptibility profile of blood culture-confirmed *S. Typhi* isolates and (2) serum IL-12α and IFN-γ concentrations in typhoid fever patients compared with matched healthy controls, from hospitals and clinics across Najaf Province, Iraq.

## 2 MATERIALS AND METHODS

### 2.1 Study design and setting

This was a cross-sectional, observational study conducted at hospitals and clinics across Najaf Province, Iraq, between 15 June and September 2025.

### 2.2 Sample collection and microbiological processing

Venous blood was collected from each patient and inoculated into blood culture bottles processed on the BacT/Alert 3D system. Positive cultures were subcultured onto blood agar, MacConkey agar, and xylose-lysine-deoxycholate (XLD) agar. Colonies producing hydrogen sulfide were further identified using the VITEK 2 automated system (Gram-negative identification card), confirming 30 isolates as S. Typhi.

### 2.3 Antimicrobial susceptibility testing

Confirmed isolates underwent antimicrobial susceptibility testing using the VITEK 2 Gram-negative AST card (AST-GN) in accordance with the manufacturer's instructions, with results interpreted according to Clinical and Laboratory Standards Institute (CLSI) breakpoints [18].

### 2.4 Cytokine measurement

In parallel, venous blood was collected into gel (serum-separator) tubes from the same 30 patients and from 30 age- and sex-matched controls. Serum interferon-gamma and interleukin-12 subunit alpha (IL-12α) concentrations were measured by sandwich enzyme-linked immunosorbent assay (ELISA) using commercial kits (Bioassay Technology Laboratory [BT LAB]; Human IFN-γ ELISA kit, Cat. No. E0105Hu, standard curve range 1–400 ng/mL, sensitivity 0.49 ng/mL; Human IL-12A ELISA kit, Cat. No. E5508Hu, standard curve range 10–2000 ng/L, sensitivity 4.67 ng/L), performed according to the manufacturers' protocols. Optical density was read at 450 nm, and concentrations were derived from the standard curve generated for each assay run.

### 2.5 Statistical analysis

Data were analyzed using IBM SPSS Statistics version 29.0. Continuous variables were expressed as mean ± standard deviation (SD). Data normality was assessed using the Shapiro–Wilk test. Depending on data distribution, comparisons between groups were performed using the independent-samples t-test or Mann–Whitney U test. Spearman's rank correlation was used to assess the relationship between variables. Receiver operating characteristic (ROC) curve analysis was performed to evaluate diagnostic performance. Categorical variables were presented as frequencies and percentages. A p-value < 0.05 was considered statistically significant.

## 3 RESULTS

### 3.1 Demographic characteristics

Thirty typhoid fever patients (17 male, 13 female; mean age 17.6 ± 7.2 years) and 30 healthy controls (17 male, 13 female; mean age 18.7 ± 8.4 years) were included. Patients and controls did not differ significantly in age (independent-samples t-test, p = 0.58) or sex distribution (chi-square, p = 1.00), confirming adequate matching Table 1.

**Table 1: Demographic characteristics of typhoid fever patients and matched controls.**

| Variable | Patients (n=30) | Controls (n=30) | p-value |
|---|---|---|---|
| Age, years (mean ± SD) | 17.6 ± 7.2 | 18.7 ± 8.4 | 0.58 |
| Male, n (%) | 17 (56.7) | 17 (56.7) | 1.00 |
| Female, n (%) | 13 (43.3) | 13 (43.3) | 1.00 |

### 3.2 Antimicrobial susceptibility profile

Among the 30 confirmed *S. Typhi* isolates, the highest susceptibility rates were observed for imipenem (83.3%), meropenem (80.0%), and trimethoprim-sulfamethoxazole (60.0%). Susceptibility was markedly lower for (ceftazidime 10.0%, ceftriaxone 30.0%, cefixime 16.7%, cefotaxime 20.0%) and for azithromycin (20.0%), consistent with the circulation of extensively resistant strains in this setting Table 2.

**Table 2: Antibiotic susceptibility profile of S. Typhi isolates (n = 30) using the VITEK 2 AST-GN card. Percentages are calculated from the total number of isolates tested.**

| Antibiotic | n Susceptible | % Susceptible | Antibiotic | n Susceptible | % Susceptible |
|---|---|---|---|---|---|
| Imipenem | 25 | 83.3 | Azithromycin | 6 | 20.0 |
| Meropenem | 24 | 80.0 | Ertapenem | 5 | 16.7 |
| Trimethoprim-sulfamethoxazole | 18 | 60.0 | Tetracycline | 4 | 13.3 |
| Levofloxacin | 12 | 40.0 | Ceftazidime | 3 | 10.0 |
| Ciprofloxacin | 10 | 33.3 | Vancomycin | 2 | 6.7 |
| Gentamicin | 9 | 30.0 | Latamoxef | 2 | 6.7 |
| Ceftriaxone | 9 | 30.0 | Tigecycline | 2 | 6.7 |
| Amikacin | 8 | 26.7 | Piperacillin | 2 | 6.7 |
| Ofloxacin | 7 | 23.3 | Aztreonam | 1 | 3.3 |
| Cefotaxime | 6 | 20.0 | Minocycline | 1 | 3.3 |
| Cefixime | 5 | 16.7 | Ticarcillin | 1 | 3.3 |
| Piperacillin-tazobactam | 4 | 13.3 | – | – | – |

### 3.3 Cytokine profile

IFN-γ values indicated in Shapiro-Wilk testing that were not normally distributed in either group (patients: $p < 0.001$; controls: $p = 0.004$), whereas IL-12α values were normally distributed in both groups (patients: $p = 0.52$; controls: $p = 0.84$). Accordingly, the Mann-Whitney U test was used for IFN-γ and the independent-samples t-test for IL-12α.

Serum IFN-γ was significantly higher in patients (111.99 ± 26.55 ng/mL; median 112.37) than in controls (62.97 ± 11.94 ng/mL; median 64.99) (Mann-Whitney $U = 896.0$, $p < 0.001$). Serum IL-12α was likewise significantly higher in patients (382.29 ± 29.45 ng/L) than in controls (253.56 ± 16.01 ng/L) ($t = 21.03$, $p < 0.001$) Table 3.

**Table 3: Serum IFN-γ and IL-12α concentrations in typhoid fever patients versus healthy controls.**

| Cytokine | Patients (mean ± SD) | Controls (mean ± SD) | Test statistic | p-value |
|---|---|---|---|---|
| IFN-γ (ng/mL) | 111.99 ± 26.55 | 62.97 ± 11.94 | U = 896.0 | < 0.001 |
| IL-12α (ng/L) | 382.29 ± 29.45 | 253.56 ± 16.01 | t = 21.03 | < 0.001 |

### 3.4 Correlation between IFN-γ and IL-12α

Within the patient group, IFN-γ and IL-12α concentrations were positively and significantly correlated (Spearman $r = 0.41$, $p = 0.023$), consistent with coordinated activation of the IL-12/IFN-γ Th1 axis during acute infection Fig1.

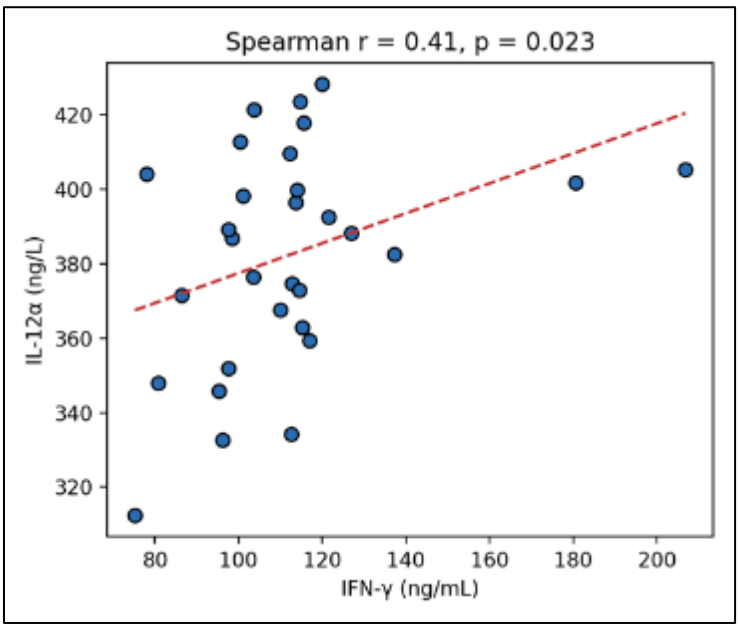


**Figure 1: Correlation between serum IFN-γ and IL-12α concentrations in typhoid fever patients.**

### 3.5 Diagnostic performance (ROC curve analysis)

Receiver operating characteristic (ROC) curve analysis was performed to evaluate the ability of serum IFN-γ and IL-12α to discriminate typhoid fever patients from healthy controls. Both cytokines showed excellent discriminative performance. IL-12α achieved an area under the curve (AUC) of 0.992 (0.969–1.000), with an optimal cutoff of 312.45 ng/L (Youden index), yielding 93.3% sensitivity and 96.7% specificity. IFN-γ achieved an AUC of 0.982 (0.947–1.000), with an optimal cutoff of 77.96 ng/mL, yielding 93.3% sensitivity and 90.0% specificity (Table 4, Fig 2).

**Table 4: ROC curve analysis of serum IFN-γ and IL-12α for discriminating typhoid fever patients from healthy controls.**

| Marker | AUC (95% CI) | Optimal cutoff | Sensitivity (%) | Specificity (%) |
|---|---|---|---|---|
| IFN-γ (ng/mL) | 0.982 (0.947–1.000) | 77.96 | 93.3% | 90.0% |
| IL-12α (ng/L) | 0.992 (0.969–1.000) | 312.45 | 93.3% | 96.7% |

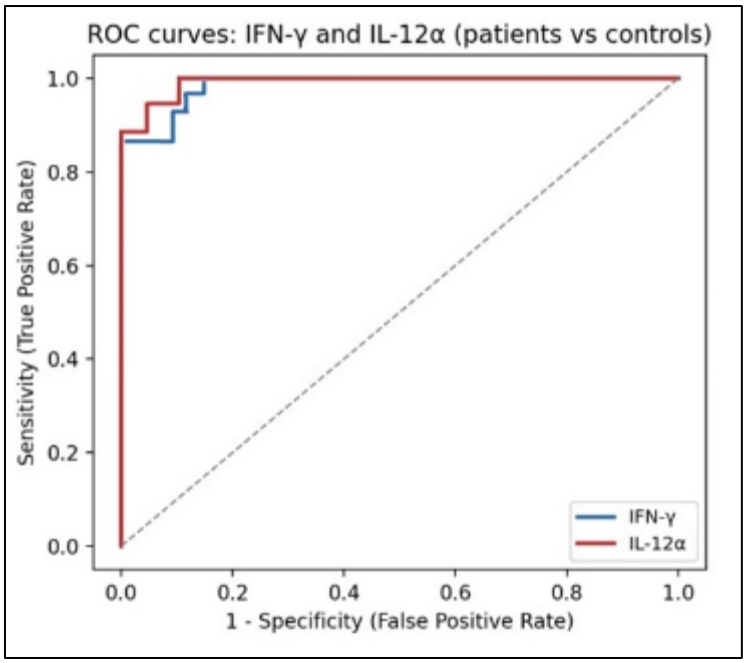


**Figure 2: ROC curves for serum IFN-γ and IL-12α in distinguishing typhoid fever patients from healthy controls.**

## 4 DISCUSSION

This study characterized, in the same geographic setting, both the antimicrobial susceptibility profile of *S. Typhi* and the host IL-12α/IFN-γ cytokine response during acute typhoid fever. Carbapenems retained the highest in vitro activity, and trimethoprim-sulfamethoxazole showed partial recovery of susceptibility, while resistance to third-generation cephalosporins and azithromycin was substantial. This pattern closely mirrors a recently published pediatric series from the same province, which similarly found meropenem, imipenem, and trimethoprim-sulfamethoxazole as the most reliably active agents and reported extensive resistance to ceftazidime, ceftriaxone, and ciprofloxacin [8], and is broadly consistent with other recent Iraqi surveillance from Baghdad, Wasit, and Erbil describing extensive drug resistance among circulating *Salmonella* isolates from clinical and food sources [9-11]. Taken together, these independent samples suggest that XDR *S. Typhi*, first described at scale in South Asia and now recognized as a growing international threat [3,4], is an established feature of the local circulating strain population in Najaf across different age groups and specimen types, reinforcing the case for local antimicrobial stewardship and empirical regimens built around carbapenems rather than third-generation cephalosporins or fluoroquinolones. The low azithromycin susceptibility observed in this series is also consistent with the global emergence of azithromycin-resistant *S. Typhi* lineages, first documented via *acrB* efflux-pump mutations in South and Southeast Asia and increasingly tracked as a marker of extensively resistant clones [12-14], further narrowing oral treatment options and strengthening the case for expanded typhoid conjugate vaccination alongside stewardship as a preventive strategy in resistance-endemic settings [15, 16].

The cytokine findings extend this picture to the host side of the host–pathogen interaction. Both IFN-γ and IL-12α were markedly elevated in patients relative to matched healthy controls, and the two cytokines correlated with one another within the patient group. This is consistent with recent evidence that *S. Typhi* actively engages host cytokine signaling pathways during infection [5], and with Iraqi case-control data showing significantly altered immune marker profiles in confirmed typhoid patients relative to healthy controls [6]. The magnitude of Th1 activation observed here supports the view that acute typhoid fever provokes a robust, coordinated cell-mediated response irrespective of the resistance phenotype of the infecting strain a distinction worth emphasizing, since in vitro antimicrobial susceptibility describes the pathogen, while the cytokine profile describes the host, and the two do not necessarily move together. Case reports of recurrent typhoid fever linked to inborn defects of the IL-12/IFN-γ axis further illustrate that when this axis fails, even antimicrobial-susceptible strains can cause relapsing disease [7], reinforcing that host immune competence is an independent determinant of outcome alongside strain resistance. The excellent ROC discrimination achieved by both markers in this study parallels diagnostic-accuracy work using interferon-gamma-based assays in other infectious contexts, such as tuberculous meningitis, where comparably high accuracy has similarly been reported primarily against non-infected or low-suspicion comparator groups rather than close clinical mimics [17] — a parallel that reinforces the limitation noted below regarding the choice of comparator group.

A genuinely integrated interpretation — for example, testing whether patients infected with more resistant isolates mount a different cytokine response than those infected with susceptible isolates — would require pairing individual-level resistance phenotypes with individual-level cytokine measurements in a larger cohort; the present study's aggregate susceptibility reporting does not support that isolate-by-isolate linkage. This is an important direction for future work in this population.

## 5 CONCLUSION

Circulating *S. Typhi* isolates among typhoid fever patients in Najaf Province, Iraq, retained the highest susceptibility to carbapenems and trimethoprim-sulfamethoxazole, with substantial resistance to third-generation cephalosporins and azithromycin, corroborating recent local pediatric surveillance data. In parallel, patients mounted a significantly elevated and internally correlated IL-12α/IFN-γ response compared with matched healthy controls, consistent with active Th1-mediated immunity during acute infection. Continued antimicrobial surveillance alongside immunological characterization may help refine both empirical treatment strategies and understanding of host determinants of disease outcome in this setting.

## STATEMENTS ON COMPLIANCE WITH ETHICAL STANDARDS

### *Disclosure of conflict of interest*

No conflict of interest to be disclosed.

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