RESEARCH ARTICLE

Prevalence of malaria, dengue, and typhoid fever among children presenting with acute undifferentiated febrile illness (AUFI) landing at the emergency department of the National Institute of Child Health, Karachi

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Abstract

Objective: To determine the prevalence of malaria, dengue and typhoid fever among children presenting with acute undifferentiated febrile illness.

Method: The descriptive, cross-sectional study was conducted from September 2022 to February 2023 at the emergency department of the National Institute of Child Health, Karachi, and comprised children aged ≤14 years with fever of unknown cause over the preceding 3-15 days. Whole blood samples were used for malaria slides and malarial parasite immunochromatographic tests. The serum was subjected to dengue non-structural antigen 1, immunoglobulin M and immunoglobulin G rapid diagnostic tests. Typhoid was diagnosed via blood culture. Antimicrobial susceptibility testing was also performed. Data was analysed using SPSS 22.

Results: There were 290 patients with mean age 6.08±3.32 years. Out of 290,156(54%) were male while 134(46%) were female. Typhoid fever was identified in 25(8.8%) cases, followed by malaria 11(3.8%) and dengue 4(1.2%). The majority of patients 260(86.2%) had no confirmed diagnosis for these infections. No co-infections were identified. Nineteen out of 25 (75%) Salmonella typhi isolates demonstrated resistance to quinolones whereas 18 out of 25 (73%) were resistant to third-generation cephalosporins, while all the isolates 25(100%) were sensitive to macrolides and carbapenems. No statistically significant associations were observed between demographic variables and infection status (p>0.05).

Conclusion: Typhoid fever was the most frequently identified infection among the three causes of acute undifferentiated febrile illness. However, most cases remained undiagnosed, indicating the need for broader diagnostic evaluation.

Keywords: Acute undifferentiated febrile illness, Children, Dengue, Malaria, Typhoid, Antimicrobial resistance.

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Introduction

Fever is one of the major reasons for children to visit the emergency department (ED). Fever is associated with numerous diseases, ranging from mild to severe infections. While some of these infections may subside on their own, others require hospital admission. Similarly, the outcome of these diseases varies from recovery in a few hours to death after a lengthy stay in intensive care units (ICUs).[1]

Acute undifferentiated fever is a type of fever that causes a rise in body temperature, ranging from 37.5° Celsius to 38.5° Celsius, and it does not have a definite diagnosis, or a diagnosis is yet to be made.[2]

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Initial investigations do not suggest a particular underlying cause of such fever, and, therefore, certain advanced diagnostic techniques are required.[3]

In this regard, developing countries are struggling to meet the challenges of sufficient provision of resources and technology to cope with diseases, especially the endemic diseases, including dengue, malaria, chikungunya and typhoid fever. Most of the basic health units (BHUs), especially those in rural communities, are lacking proper and sufficient diagnostic facilities. Therefore, cases of undifferentiated fever are mismanaged most of the time and are referred in critical condition to EDs of tertiary care hospitals. Hence, EDs of hospitals are overcrowded.[4-6]

Although the majority of fever cases are mild and easily treated, in some cases, fever can be brought on by serious and potentially fatal illnesses, like brain or blood infections and cancer, that may go undetected despite multiple pathological tests.[5]

In this scenario, the decision to administer antimicrobial drugs gains critical importance. Until the arrival of laboratory confirmation, clinicians have to rely on information obtained from physical examinations, presenting complaints and histories of antibiotic use to

identify the source of infection. However, a final diagnosis is dependent upon certain laboratory and radiographic imaging for further differentiation of the fever.[7]

The causes of fever also vary from patient to patient. Therefore, it is imperative to adopt a systematic approach to deal with every patient. Increased self-medication and administration of antibiotics at the mere onset of fever, even in most emergency settings, is the biggest cause of antimicrobial resistance (AMR).[8,9]

Unfortunately, Pakistan is one of the endemic areas for malaria, dengue and enteric fever, and during endemic seasons, EDs are overburdened with undifferentiated fever cases.[10] Therefore, epidemiological data along with physical examination, presenting complaints and history of antibiotic use are necessary to guide the health resources in the right direction. This helps clinicians in early differentiation and treatment of the cases, along with a reduction in inappropriate use of antibiotics and hospitalisation.

Malaria, dengue and enteric fever mimic each other in signs and symptoms. Therefore, it is imperative to differentiate them from each other on the basis of laboratory evidence. The current study was planned to determine the prevalence of malaria, dengue and typhoid fever among children presenting with acute undifferentiated febrile illness (AUIF).

Patients and Methods

The descriptive, cross-sectional study was conducted from September 2022 to February 2023 at the ED of the National Institute of Child Health (NICH), Karachi, after approval from the institutional ethics review board. The sample size was calculated using the single population proportion formula

$$n = Z^2 \times P \times (1-P) / d^2,$$

where Z with 95% confidence level was 1.96, expected prevalence (P) of malaria based on previous study.[2] was 25.2%, and margin of error (d) was 5%. by putting all these values in the formula we got the final sample size that was 290.[11] All patients were enrolled by using non-probability convenience sampling technique.

Those included were patients aged ≥2 months and ≤14 years, presenting ED with a body temperature of ≥38°C with duration of fever ≤15 days without any identifiable source of infection. The patients were enrolled after initial clinical evaluation and written informed consent from the parents/guardians. Patients who were on antibiotics for >24 hours were excluded.

Demographic data, clinical history and presenting symptoms were noted using a structured questionnaire. Whole blood was used to produce malaria slides and to carry out malarial parasite immunochromatographic tests (MP-ICTs). The malaria parasite was detected by Giemsa staining of blood smears. Thick and thin blood films were prepared using clean, grease-free, non-silicate glass slides. These films were dried and stained with 10% freshly prepared Giemsa stain maintained at a potential of hydrogen (pH) of 7.2. Thin blood films were fixed with 100% methanol before staining. The stained blood films were viewed under a light microscope at x1000 magnification (X100 oil immersion lens).[12] In MP-ICT, each rapid test device had about two drops of whole blood poured on the sample pad, and they were left undisturbed on a clean surface for 15-20 minutes. The chromogenic indicator in the test device produced two lines in case of positive results: one line for the control, and the other for either of the two serotypes; plasmodium falciparum or vivax. One line was observed in negative strips, which referred to the control line. Samples were repeated in case no line was produced. Antigen detection for Plasmodium falciparum and other species of Malaria was performed using the Bioline Malaria Ag P.f/pan test (Abbott Diagnostics Korea Inc., Yongin, Gyeonggi-do, Republic of Korea) according to the manufacturer's instructions. The serum was used to carry out dengue non-structural antigen-1 (NS1Ag), immunoglobulin M (IgM) and IgG rapid diagnostic tests (RDTs). In each rapid test device, about 100µl of serum was added to the sample pad, and it was left undisturbed on a clean surface for 15-20 minutes. The chromogenic indicator in the test device produced two lines in case of positive results: one line for the control, and the other for a positive reaction with IgG or IgM. Only one line was produced in negative strips, which referred to the control line. Samples were repeated in case no line was produced. Serum samples were tested using the SD Bioline™ Dengue IgG/IgM rapid diagnostic test (Standard Diagnostics, Inc., Yongin-si, Republic of Korea), following the manufacturer's protocol.

Blood 2ml was collected for culture, and it was inoculated directly into a single, paediatric sample-sized resin-containing blood culture bottle (Peds Plus; Becton Dickinson, Sparks, MD, USA, 2022). It was then loaded onto the BACTEC 9240 automated blood culture instrument (Becton Dickinson, Sparks, MD, USA) within 1 hour from the time of receipt in the laboratory. A few drops of the sample were taken out by a sterile syringe from the positively detected bottle, loaded onto the BACTEC instrument, and inoculated on blood and MacConkey agar culture plates. Purified colonies were gram-stained and identified by different biochemical reactions. All laboratory procedures were performed as per the institutional standard operating

protocols (SOPs). Blood culture samples were processed within 1 hour of collection. Antimicrobial susceptibility testing was conducted using the Kirby-Bauer disc diffusion method in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines.[13] Repeat testing was performed in cases of invalid or inconclusive RDT results.

Data was analysed using SPSS 22. Mean±standard deviation were calculated for quantitative variables, while frequencies and percentages were calculated for qualitative variables. Associations of categorical variables with the infection status were assessed using the chi-square test. P≤0.05 was considered statistically significant.

Results

There were 290 patients with mean age 6.08±3.32 years (Table 1). Out of 290,156(54%) were male while 134(46%) were female (Table-3). Typhoid fever was identified in 25(8.8%) cases, followed by malaria 11(3.8%) and dengue 4(1.2%). The majority of patients 260(86.2%) had no confirmed diagnosis for these infections. No co-infections were identified (Table 2). Nineteen out of 25 (75%) Salmonella typhi isolates demonstrated resistance to quinolones while18 out of 25 (73%) were resistant to third-generation cephalosporins, while all the isolates 25(100%) were sensitive to macrolides and carbapenems (Figure). Inferential analysis did not show statistically significant association of the infection status with demographic variables (p>0.05) (Table 3).

Table-1: Demographic characteristics of the study subjects.

Variable	n	Range	Minimum	Maximum	Mean±SD
Age (years)	290	13.50	.50	14.00	6.08±3.33
Size of family	290	20	2	22	7.95±3.52
Number of rooms	290	6	1	7	1.94±.911
Age (years) of attendant	290	29	21	50	31.73±6.361

Table-2: Frequency of dengue, malaria and typhoid.

Variable	n (%)
Dengue ICT	
Positive	4 (1.2)
Negative	286 (98.8)
MP ICT	
Positive	11 (3.8)
Negative	279 (96.2)
Growth on blood culture	
Yes	25 (8.8)
No	265 (91.2)

MP: Malarial parasite, ICT: Immunochromatographic test.

Table-1: Association of infection with demographic features.

Variable	Infection Present n (%)	Infection Absent n (%)	p-value
Age group			0.62
≤5 years	14 (10.9)	115 (89.1)	
>5 years	26 (16.1)	135 (83.9)	
Gender			0.74
Male	21 (13.5)	135 (86.5)	
Female	19 (14.1)	115 (85.9)	
Socioeconomic status*			0.58
≤2 rooms	28 (14.9)	160 (85.1)	
>2 rooms	12 (11.8)	90 (88.2)	

*Socioeconomic status assessed indirectly using number of rooms in the household.

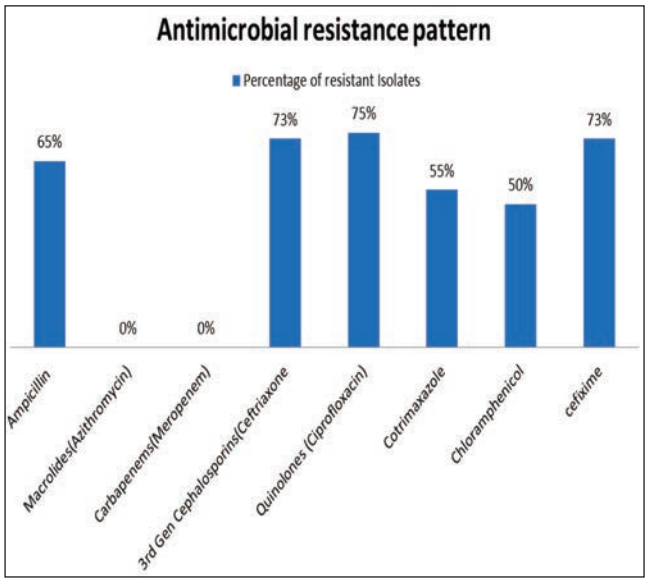

Figure: Antimicrobial resistance (AMR) pattern of salmonella typhi isolated from blood cultures (n=25).

Discussion

The current study evaluated the prevalence of three major endemic infections —typhoid, malaria and dengue — among children presenting with AUFI in a tertiary care ED setting. Among these, typhoid fever was the most frequently identified infection (8.8%), followed by malaria (3.8%) and dengue (1.2%). The findings are consistent with a study in India which estimated that 7.5% of cases were typhoid-positive among the subjects, but their dengue positivity rate was comparatively high (12.2%) and malaria was low (0.9%).[14] A study in Cameroon reported malaria on top (44.79%), followed by typhoid fever 37.15% while co-infection of dengue and malaria was the least prevalent (19.79%).[15] Interestingly, another study in India reported dengue as the most common (38%) cause of acute febrile illness, followed by typhoid fever (10%) and malaria (7%).[16] The variability in prevalence observed across different studies may be attributed to geographical, seasonal and methodological differences.[17-19] These findings underscore the importance of localised epidemiological data to guide clinical decision-making.

Notably, the majority of children (86.2%) presenting with AUFI in the current study remained undiagnosed for these

three infections. This suggests that a substantial proportion of febrile illnesses may be attributable to other aetiologies, including viral infections, alternative bacterial pathogens, or non-infectious causes, which were not evaluated in the current study. This highlights the need for broader diagnostic approaches in the evaluation of AUFI.

The current findings should be interpreted in the context of the study's duration, as the data was collected over a six-month period from September to February. This time frame may reflect seasonal patterns of infectious diseases, particularly for malaria and dengue, and may not represent the year-round prevalence of these infections.

The AMR pattern observed in this study is concerning, with high resistance rates noted for quinolones and third-generation cephalosporins. However, all the isolates demonstrated sensitivity to macrolides and carbapenems. This finding should be interpreted cautiously due to the relatively small number of isolates, and further large-scale studies are required to validate these results.

Even though a study in Bangladesh reported similar results regarding ampicillin, it reported a very high rate of sensitivity against ceftriaxone, cefixime, gentamicin, ciprofloxacin, cotrimoxazole and azithromycin.[20]

These findings suggest that drug-resistant strains of S. typhi are prevalent in South Asia, and azithromycin and meropenem are the last hope against these deadly bugs.[20]

A few causes of rapidly increasing drug resistance, especially extensive drug resistance, may be irrational empirical prescriptions, over-the-counter sales, the re-use of leftover antibiotics, the tendency to suggest drugs to relatives on the basis of similarity of symptoms, and withdrawing antibiotics intake on improvement of symptoms, etc.[21,22]

The current study has several limitations. First, it was conducted over a limited time period, which may not reflect variations in disease prevalence over time. Second, only three infectious aetiologies were investigated, and a large proportion of AUFI cases remained undiagnosed. Third, the use of non-probability convenience sampling may limit generalisability of the findings. Future studies incorporating broader diagnostic panels and year-round data-collection are recommended.

Conclusion

Typhoid fever was the most frequently identified infection among the three studied causes of AUFI, but most of the cases remained undiagnosed, indicating the need for broader diagnostic evaluation.

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References

1. Tan CD, Vermont CL, Zachariasse JM, von Both U, Eleftheriou I, Emonts M, et al. Emergency medical services utilisation among febrile children attending emergency departments across Europe: an observational multicentre study. Eur J Pediatr. 2023;182:3939-47. doi:10.1007/s00431-023-05056-3.
2. Ali N, Khan NU, Waheed S, Mustahsan S. Aetiology of acute undifferentiated fever in patients presenting to the emergency department of a tertiary care centre in Karachi, Pakistan. Pak J Med Sci. 2020;36:1285-90. doi:10.12669/pjms.36.6.2334.
3. Goel A, Bansal R, Bansal P. Triple infection with dengue, chikungunya and malaria. Trop Parasitol. 2022;12:131-2. doi:10.4103/tp.tp_9_21.
4. Shahid U, Farooqi JQ, Barr KL, Mahmood SF, Jamil B, Imitaz K, et al. Comparison of clinical presentation and outcomes of Chikungunya and Dengue virus infections in patients with acute undifferentiated febrile illness from the Sindh region of Pakistan. PLoS Negl Trop Dis. 2020;14:e0008086. doi:10.1371/journal.pntd.0008086.
5. Kavirayani V, Madiyal M, Aroor S, Chhabra S. Clinical profile and role of serology in paediatric acute febrile illness: experience from a tertiary care hospital in South India. Clin Epidemiol Glob Health. 2021;12:100898. doi:10.1016/j.cegh.2021.100898.
6. Saint S, Chopra V, Shuman E. Approach to fever. In: The Saint-Chopra Guide to Inpatient Medicine. 4th ed. Oxford: Oxford University Press; 2018. p. 275-80. doi:10.1093/med/9780190862800.003.0048.
7. Wright WF, Auwaerter PG. Fever and fever of unknown origin: review, recent advances, and lingering dogma. Open Forum Infect Dis. 2020;7:ofaa132. doi:10.1093/ofid/ofaa132.
8. Lewin M, Mary T, Sushma K. Etiology of prolonged fever in children using a protocol-based approach from a tertiary-level hospital in India: a descriptive study. Pediatr Infect Dis. 2025;7:54-8. doi:10.5005/jp-journals-10081-1465.
9. Amin S, Noor M, Rahim F, Khan MY, Ullah R. Co-infection with dengue virus and extensively drug-resistant Salmonella typhi. Cureus. 2021;13:e19653. doi:10.7759/cureus.19653.
10. Mishra V, Shukla D, Bano F, Sharma S, Nigam S, Kumar S, et al. To study the prevalence of aetiologies of acute undifferentiated febrile illnesses of the patients at a tertiary care centre in Uttar Pradesh, India. J Popul Ther Clin Pharmacol. 2023;30:1719-26.
11. Ahmed SK. How to choose a sampling technique and determine sample size for research: a simplified guide for researchers. Oral Oncol Rep. 2024;12:100662. doi:10.1016/j.oor.2024.100662.
12. Siagian FE. The use of immersion oil in parasitology light microscopic examination. Int J Pathogen Res. 2024;13:1-8. doi:10.9734/ijpr/2024/v13i2274.
13. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 32nd ed. CLSI supplement M100. Wayne (PA): Clinical and Laboratory Standards Institute, 2022.
14. Kigozi BK, Kharod GA, Bukenya H, Shadomy SV, Haberling DL, Stoddard RA, et al. Investigating the etiology of acute febrile illness: a prospective clinic-based study in Uganda. BMC Infect Dis. 2023;23:411. doi:10.1186/s12879-023-08335-4.

15. Netongo PM, Tchoutang AM, Eric MB, Tepa A, Nzuno MC, Kamdem SD. Malaria and typhoid fever coinfection among febrile patients attending a health facility in Yaoundé, Cameroon: a cross-sectional study. J Parasitol Res. 2025;2025:9977704. doi:10.1155/japr/9977704.
16. Singh SK, Arora R, Menon AS, Gupta A, Vashisht R, Prasad R, et al. Clinical features and etiological profile of acute undifferentiated febrile illness in Western Maharashtra: a 2024 monsoon observational study. J Clin Infect Dis Soc. 2025;3:45-51. doi:10.4103/CIDS.CIDS_1_25.
17. Moses A, Atting I, Inyang O. Evidence of overlapping infections of dengue, malaria and typhoid in febrile patients attending a tertiary health facility in Uyo, South-South Nigeria. J Adv Med Med Res. 2016;17:1-9. doi:10.9734/BJMMR/2016/27668.
18. Moise IK, Roy SS, Nkengurutse D, Ndikubagenzi J. Seasonal and geographic variation of pediatric malaria in Burundi: 2011 to 2012. Int J Environ Res Public Health. 2016;13:425. doi:10.3390/ijerph 13040425.
19. Saad NJ, Lynch VD, Antillón M, Yang C, Crump JA, Pitzer VE. Seasonal dynamics of typhoid and paratyphoid fever. Sci Rep. 2018;8:6870. doi:10.1038/s41598-018-25234-w.
20. Ali MK, Sultana S. Antimicrobial sensitivity patterns of Salmonella typhi in children. Bangladesh J Med Sci. 2016;15:416-8. doi:10.3329/bjms.v15i3.30198.
21. Carey ME, Dyson ZA, Ingle DJ, Amir A, Aworh MK, Chattaway MA, et al. Global diversity and antimicrobial resistance of typhoid fever pathogens: insights from a meta-analysis of 13,000 Salmonella Typhi genomes. Elife. 2023;12. doi:10.7554/eLife.85867.
22. Alhomoud F, Almahasnah R, Alhomoud FK. "You could lose when you misuse" – factors affecting over-the-counter sale of antibiotics in community pharmacies in Saudi Arabia: a qualitative study. BMC Health Serv Res. 2018;18:915. doi:10.1186/s12913-018-3753-y.

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AR: Concept, design, preparation, data entry, analysis, drafting and final approval.
MHB: Design, preparation and final approval.
TML: Design, preparation, drafting and final approval.
MAN: Data collection and final approval.