Prevalence, probability, and outcomes of typhoidal/non-typhoidal *Salmonella* and malaria co-infection among febrile patients: a systematic review and meta-analysis

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The geographical overlaps of malaria parasites and *Salmonella* spp. can lead to co-infection of these two pathogens, especially in the tropics where malaria is endemic. Moreover, few literatures suggested that malaria infection was associated with *Salmonella* bacteremia. Therefore, this study quantified pooled prevalence of typhoidal/non-typhoidal *Salmonella* (NTS) and probability of typhoidal/NTS and malaria co-infection among febrile patients. The systematic review protocol was registered at PROSPERO (CRD42021252322). Studies on co-infection of typhoidal/NTS and malaria were searched in PubMed, Scopus, and Web of Science. The risk of bias of the included studies was assessed using the checklist for analytical cross-sectional studies developed by the Joanna Briggs Institute. Meta-analyses on the following criteria were performed: (1) pooled prevalence of typhoidal/NTS and malaria co-infection among febrile patients, (2) pooled prevalence of typhoidal/NTS among malaria patients, (3) pooled prevalence of malaria infections among patients with *Salmonella* spp. infection, and (4) probability of typhoidal/NTS and malaria co-infection among febrile patients. Additionally, the case fatality rate and mean difference of malarial parasitemia between typhoidal/NTS and malaria co-infection and *Plasmodium* monoinfection were also determined. The subgroup analyses of typhoidal/NTS, regions (Africa and Asia), countries, time (publication year), characteristics of participants, and diagnostic tests for identifying *Salmonella* spp. were also conducted. A sensitivity test was performed to determine the robustness of the study outcomes. Publication bias among the included studies was evaluated using the funnel plot and Egger's test. All analyses were performed using Stata version 15 (StataCorp LLC, Texas, USA) with a p-value < 0.05 indicating statistical significance. Eighty-one studies that met the eligibility criteria were included in the analyses. Of the 73,775 study participants, 4523 had typhoidal/NTS and malaria co-infections. The pooled prevalence rates of typhoidal/NTS and malaria co-infection among febrile patients were 14% (95% confidence interval [CI], 9–19%; I², 99.4%; 2971/17,720 cases) and 1% (95% CI 1–1%; I², 89.9%; 252/29,081 cases) using the Widal test and culture methods for identifying *Salmonella* spp., respectively. The pooled prevalence rates of typhoidal/NTS infection among patients with malaria were 31% (95% CI 23–39%; I², 99.5%; 3202/19,208 cases) and 3% (95% CI 2–3%; I², 86.8%; 407/40,426 cases) using the Widal test and culture methods for identifying *Salmonella* spp., respectively. The pooled prevalence rates of

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are classified into six serotypes, which are differentiated based on their antigenicity. Several enterica antigens. The Widal test is widely used in numerous countries where trained technicians and laboratory conducted using the Widal test. This test measures the antibody titers specific for countries are travelers returning from endemic areas. In developing countries, especially in Southeast Asia and Africa, NTS is endemic and is a global burden, contrary to typhoidal 12,14. Poor water quality, and household behaviors, including poor hygiene and consumption of unsafe food and untreated water, increase the risk of fecal–oral enteric infections, including typhoidal and NTS. The most recent meta-analysis revealed that typhoid transmission16. Among children in Africa, NTS is a leading cause of bacteremia, whereas typhoid fever has a relatively low burden. Another study demonstrated that typhoid fever is more common in older children with a period of fever, whereas non-typhoidal bacteremia frequently develops in younger children of poorly educated women or women with low socioeconomic status. People 11,12. Typhoid fever is an important cause of morbidity and mortality worldwide, with an estimated 16–33 million cases and 500,000 to 600,000 deaths annually. Typhoid is endemic in developing countries, especially Africa, whereas developed countries have a much lower incidence. The majority of patients in developed countries are travelers returning from endemic areas. In developing countries, especially in Southeast Asia and Africa, NTS is endemic and is a global burden, contrary to typhoidal Salmonella. The recent meta-analysis revealed that household behaviors, including poor hygiene and consumption of unsafe food and untreated water, increase the risk of typhoid transmission. Among children in Africa, NTS is a leading cause of bacteremia, whereas typhoid fever has a relatively low burden. Another study demonstrated that typhoid fever is more common in older children with a period of fever, whereas non-typhoidal bacteremia frequently develops in younger children of poorly educated women or women with low socioeconomic status.

For the diagnosis of typhoidal Salmonella infection, especially from blood, bacteriological culture is the gold standard. The sensitivity of the culture method depends on the blood volume, antibiotic treatment, affected individual, disease duration, and presence of bacteremia. Blood cultures have a sensitivity of 40–80%. Moreover, they are most sensitive in the first week of infection as circulating bacterial concentrations peak at that time. Stool and rectal swab cultures have lower sensitivity than blood cultures. However, sensitivity can be enhanced by culturing from three specimens or performing multiple cultures from a single stool specimen. Culture methods are less frequently employed in developing countries because of the high cost and requirements for good laboratory facilities and highly trained professionals. Serological diagnoses of infections are conducted using the Widal test. This test measures the antibody titers specific for Salmonella O (somatic) and H (flagella) antigens. The Widal test is widely used in numerous countries where trained technicians and laboratory facilities are limited. Other useful methods for the diagnosis of Salmonella infection include enzyme-linked immunosorbent assay (ELISA), which detects IgM and IgG antibodies against Salmonella surface molecules, and molecular methods, such as nested multiplex polymerase chain reaction (PCR) and real-time PCR, which target Salmonella virulence genes. The real-time PCR test is highly specific and sensitive and has faster turnaround times than culture methods.
The geographical overlaps of malarial parasites and typhoidal/NTS can lead to co-infection of these two pathogens, especially in the tropics where malaria is endemic. The overlap in the clinical symptoms of malaria and non-malaria febrile illness or co-infection of these two pathogens may lead to the misdiagnosis of one disease. Previous studies conducted in Africa demonstrated that bacteremia caused by NTS was associated with malaria parasitemia, recent malaria, anemia, severe malarial anemia, jaundice, and hypoglycemia. Previous studies also demonstrated that NTS infection is associated with more severe anemia and malaria compared with typhoidal Salmonella or other bacteremia infections. Another study demonstrated that NTS infections were associated with previous antimalarial treatment and malarial complications (severe anemia, jaundice, and hypoglycemia). Furthermore, a systematic review demonstrated a higher case fatality rate in children who were co-infected with NTS compared with those infected with malaria alone; however, the study had limitations on high heterogeneity between studies, inclusion of recent malaria infection, use of antigen-based rapid diagnostic tests (RDTs), study design, quality of microbiological data, and publication bias, making the meta-analysis potentially misleading. To the best of our knowledge, meta-analyses determining the association between malaria and typhoid/non-typhoid fever have not been well conducted, and information is not updated. Therefore, the present study aimed to quantify the pooled prevalence, probability, and outcome of typhoidal/NTS and malaria co-infection among febrile patients who were suspected of having these two diseases.

Methods

Protocol and registration. The protocol of systematic review was registered at PROSPERO (CRD42021252322) and conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) statement.

Search strategy. Potentially relevant articles in PubMed, Web of Science, and Scopus were searched using the combined search terms presented in Supplementary Table S1. The relevant search terms were retrieved from Medical Subject Headings to ensure the inclusion of all relevant studies. The searches were conducted from inception to April 27, 2021. Searches were limited to the English language, but the year of publication was not limited. Additional searches were performed by reviewing the reference lists of the included studies and Google Scholar to ensure that all potentially relevant studies were included in the meta-analysis.

Eligibility criteria. Observational studies in the English language that reported concurrent malaria and typhoidal/NTS infection were included in the study. Studies reporting data that could not be extracted, case-control studies, experimental studies, animal studies, case reports, and case series were excluded.

Study selection and data extraction. Potentially relevant articles were selected by two authors (MK, WM) using the eligibility criteria. First, the duplicates from the three databases were removed. Second, the remaining studies were screened for titles and abstracts, and any non-related studies were excluded. Third, the full texts of the remaining studies were examined, and any non-related studies were excluded with reasons. Then, the remaining studies were included in the systematic review and meta-analysis. Any disagreement on the study selection between the two authors was resolved by reaching a consensus after the discussion. Data extraction was performed by two authors (MK, WM) using the pilot standardized datasheets. The following information was obtained from each study: first author names, publication year, study sites (country and region), year the study was conducted, study design, characteristics of participants including age and sex, number of co-infections, number of malaria cases, number of typhoid/non-typhoid cases, number of case fatality in co-infection and Plasmodium monoinfection, diagnostic test for malaria, and diagnostic test for typhoid (best diagnostic test). Any disagreement on data extraction between the two authors was resolved by a third author (PW) for the final decision.

Risk of bias. The risk of bias of the included studies was evaluated using the checklist for analytical cross-sectional studies developed by the Joanna Briggs Institute. The checklist is comprised of eight categories (yes/no/unclear/not applicable answers) based on the design, conduct, and analysis. Studies with yes answers in all eight categories were considered to have low risk of bias (high quality), whereas those that complied with four to six categories were considered to have a moderate risk of bias (moderate quality). Any study that complied with less than four categories was considered to have a high risk of bias (low quality) and thus excluded from the present study. The risk of bias was evaluated by two authors (MK, WM). If the two authors disagreed on the risk of bias assessment, a third author (PW) was responsible for the final decision.

Outcomes. The outcomes of this study were as follows: (1) pooled prevalence of typhoidal/NTS and malaria co-infection among febrile patients, (2) pooled prevalence of typhoidal/NTS infection among patients with malaria, (3) pooled prevalence of malaria infection among patients with Salmonella spp. infection, (4) comparison of typhoidal/NTS infection among patients with severe and non-severe malaria, (5) association between malaria and typhoidal/NTS infections, (6) case fatality rate among patients with typhoidal/NTS and malaria co-infection, and (7) difference in mean parasitemia level between patients with typhoidal/NTS and malaria co-infection and those with Plasmodium spp. monoinfection.

Data synthesis. The pooled prevalence rate of typhoidal/NTS and malaria co-infection among febrile patients, typhoidal/NTS infection rate among patients with malaria, malaria infection rate among patients with Salmonella spp. infection, case fatality rate among patients with typhoidal/NTS and malaria co-infection, and
comparison of typhoidal/NTS infection rates among patients with severe and non-severe malaria were estimated using random-effect models, assuming heterogeneity of the included studies. The results of the individual studies are presented in the forest plots as the point estimates (prevalence in percentage) and 95% confidence interval (CI). The association between *Plasmodium* spp. and *Salmonella* spp. infections was determined using the random-effects model and expressed as odds ratio with 95% CI. The difference in mean parasitemia level between patients with typhoidal/NTS and malaria co-infection and *Plasmodium* spp. monoinfection was estimated using the random-effects model and expressed as weighted mean difference (WMD) with 95% CI. The heterogeneity among the included studies was assessed using Cochran’s Q and I² statistics. Cochran’s Q < 0.05 or I² > 50% indicated substantial heterogeneity among the included studies. If no substantial heterogeneity existed, the fixed-effects model was employed to estimate the effect size (pooled prevalence or pooled odds ratio). The subgroup analysis of typhoidal/NTS, regions (Africa and Asia), countries, time (publication year), characteristics of participants, and diagnostic tests for identifying *Salmonella* spp. were conducted to explore the source(s) of heterogeneity among the overall effect estimate. Sensitivity analyses of the probability of *Plasmodium* spp. and *Salmonella* spp. co-infection were performed using the random- and fixed-effects models after excluding outliers.

**Publication bias.** Publication bias among the included studies was evaluated using a funnel plot between the effect size (ES) and standard error of the ES (seES). A funnel plot with asymmetrical distribution indicated publication bias. Egger’s test was employed if the funnel plot asymmetry was caused by the small study effect. A contour-enhanced funnel plot was also utilized to find the possible causes of funnel plot asymmetry among the included studies. The significance of contour-enhanced funnel plots (p < 0.01) indicated that the cause of funnel plot asymmetry might be more likely other factors such as heterogeneity, selection bias, and quality of the included studies than publication bias.

**Results**

**Search results.** A total of 550 studies were retrieved from the three databases (168 from PubMed, 234 from Scopus, and 148 from Web of Science). After removal of 245 duplicated studies, the titles and abstracts of 305 studies were screened. After excluding 232 unrelated studies, 73 were retained for full-text examination. A total of 30 studies were excluded for the following reasons: 11 studies had no malaria and typhoid co-infection cases, 9 studies had no typhoidal cases, data could not be extracted from 4 studies, 3 were case–control studies, 1 was an experimental study, 1 was an animal study, and 1 study was a case report. Finally, 43 studies were included. Thirty-eight studies from additional searches of reference lists and Google Scholar were included. Thus, 81 studies met the eligibility criteria and thus included in the qualitative and quantitative analyses (Fig. 1).

**Characteristics and quality of the included studies.** The characteristics of the included studies are presented in Table 1. A total of 76 studies were cross-sectional or retrospective studies, whereas 5 were prospective studies. All studies were published between 1987 and 2021. In Africa, 61 studies (75.3%) were conducted; in Asia, 19 studies (23.4%); and in Europe, 1 study. The African studies were conducted in Nigeria (30/61, 49.2%)36–48, Cameroon (5/61, 8.2%)35,40,73,92,93, Ghana (5/61, 8.2%)31,53,56,76,85, Kenya (5/61, 6.2%)28,29,70,72,80, Tanzania (4/61, 6.56%)36,39,44,46, Malawi17,27,30, Burkina Faso34,80, Mozambique29, Sierra Leone36, the Democratic Republic of the Congo39, Ethiopia36, Gabon36, and Gambia31, and one study was conducted in Burkina Faso, Ethiopia, Ghana, Guinea-Bissau, Kenya, Madagascar, Senegal, South Africa, Sudan, and Tanzania42. The Asian studies were conducted in India (12/19, 63.2%)30,69–71,73,81,84,104–106, Pakistan (4/19, 21.1%)32,65,70,102, Myanmar36,38, and Vietnam36. One study was conducted in Sweden36.

Among the 81 studies included in the analysis, 49 studies (60.5%) enrolled febrile patients, 5–41,43–47,55–53,55,57,58,62,64–68,70–77,81,83,84,87,89,90,92,93,95,98,102–105, 8 studies enrolled pregnant women28,31,36,38,39,41–43,45–48,50–52,57,59,61,65–68,70,71,73–78,81–84,86–108, 6 studies enrolled patients with severe malaria30,54,63,70,80, 5 studies enrolled typhoid/non-typhoid-positive patients17,20,30,35,36,38,39,41–43,45–48,50–53,56,58,60,63–65,68,70,71,73–78,81–84,86–108, and 1 study enrolled children with pathogenic bacteria29. Co-infections with malaria and typhoidal/NTS were reported in 4,523 cases from 73,775 total patients enrolled in the 81 included studies. Co-infections with malaria and typhoidal *Salmonella* spp., including *S. typhi* and *S. paratyphi*, were reported in 3813 cases from 56 studies31,36,38,39,41–43,45–48,50–52,57,59,61,65–68,70,71,73–78,81–84,86–108. Co-infections with malaria and typhoidal *Salmonella* spp., including *S. typhi* and *S. paratyphi*, were reported in 707 cases from 18 studies17,27–31,44,53,56,60,62,69,72,79,80,84,85,93. Co-infections with malaria and NTSc were reported in 707 cases from 18 studies17,27–31,44,53,56,60,62,69,72,79,80,84,85,93. Co-infections with malaria and NTS were reported in 13 studies30,31,35,37,40,49,54,55,58,63,64,84,93.

*Salmonella* spp. infection was identified using blood cultures (39/81, 48.1%)17,20,27–31,37,38,44,47,49,50,54,56–58,50,54,62,63,66,68,89,71,72,74–77,81–83,85,89,90,104–106,108, Widal test (27/81, 33.3%)36,40,41,43,45,46,48,54,55,58,61,62,64–68,70–77,80–84,93,97,100–104,108, stool cultures (5/81, 6.17%)94,96,107, RDTs (4/81, 4.94%)35,42,73,91, and molecular methods (3/43, 6.98%)39,55,64. One study35 employed both blood and stool cultures. Some studies used combinations of methods to identify *Salmonella* spp. infection. However, only a definitive method was demonstrated in this qualitative analysis. For the identification of malaria, *Plasmodium* spp. infections were identified via microscopy alone (52/81, 64.2%)17,20,27–31,37,38,44,47,49,50,54,56–58,50,54,62,63,66,68,89,71,72,74–77,81–83,85,89,90,104–106,108, microscopy/RDT (16/81, 19.8%)20,41,44,49,54,56,61,66,68,71,74,78,88,91,100–104,108, RDT alone (5/81, 6.17%)17,37,73,76,101, molecular method35,56, microscopy/RDT/molecular method56,70, not specified4,61,68,70, and ELISA39.

Among the 81 studies included in the present study, 30 (37%) were rated as low risk of bias, whereas 51 studies had a moderate risk of bias (51/81, 63%). Studies with a high risk of bias were removed during the study selection (Supplementary Table S2).
Prevalence of typhoidal and NTS and malaria co-infections among febrile patients. The pooled prevalence rate of typhoidal/NTS and malaria co-infections among febrile patients was estimated from 50 studies. The studies were divided into four groups based on diagnostic tests for *Salmonella* spp. The results indicated that the pooled prevalence rates of typhoidal/NTS and malaria co-infections among febrile patients were 14% (95% CI 9–19%; I², 99.4%) using the Widal test, 1% (95% CI 0–2%; F₂, 81.6%) using blood culture, 1% (95% CI 0–2%; I², 81.6%) using RDTs, 1% (95% CI 0–1%; F₂, 0%) using a molecular method, 7% (95% CI 3–10%; F₂, 81.2%) using stool cultures, and 6% (95% CI 4–9%) using a combination of blood and stool cultures (Fig. 2).

When *Salmonella* spp. infections were detected using the Widal test, the highest prevalence rate of co-infections was noted in Cameroon (51%; 95% CI 45–58%) and Nigeria (21%; 95% CI 11–31%; F₂, 98.3%), whereas lower prevalence rates were detected in Sierra Leone (19%; 95% CI 18–20%), Ethiopia (6%; 95% CI 4–11%), Pakistan (6%; 95% CI 4–7%; F₂, 0%), Ghana (5%; 95% CI 3–8%; F₂, 99.7%), India (0%; 95% CI 0–1%; F₂, 99.7%), and Tanzania (4%; 95% CI 2–6%) (Fig. 3).

Among the studies using blood culture for the identification of *Salmonella* spp. infections, the highest prevalence of co-infection was reported in Burkina Faso, Ethiopia, Ghana, Guinea-Bissau, Kenya, Madagascar, Senegal, South Africa, Sudan, and Tanzania (5%; 95% CI 3–7%). Contrarily, lower prevalence was reported in Nigeria (2%; 95% CI 1–4%; F₂, 82%), India (1%; 95% CI 0–1%; F₂, 87.3%), Tanzania (1%; 95% CI 1–2%), Ghana (0%; 95% CI 0–1%), and Kenya (0%; 95% CI 0–1%) (Fig. 4).

Among the studies using the Widal test for the identification of *Salmonella* spp. infections, the highest prevalence of co-infections was noted in the studies that enrolled participants in all age groups (95% CI 20%; 95% CI 14–25%; F₂, 97.1%). The prevalence of co-infections was 4% in children (95% CI 2–6%; F₂, 99.7%), 8% in the not specified (NS) age group (95% CI 1–15%), and 4% in adults (95% CI 3–5%; F₂, 99%) (Fig. 5) when *Salmonella* spp. infections were detected using the Widal test. Among the studies using blood cultures for the identification of *Salmonella* spp. infections, the prevalence of co-infections was 1% in all age groups (95% CI 1–2%; F₂, 91.1%), 1% in the NS group (95% CI 0–1%; F₂, 46.1%), and 1% in children (95% CI 0–1%; F₂, 89.2%) (Fig. 6).

Subgroup analysis of typhoidal/NTS infection, regions (Africa and Asia), and time (publication year) was performed using the data from studies using blood culture for typhoidal/NTS identification. Results showed that the prevalence rates of malaria and...
| Author                  | Study site   | Year conducted | Study design     | Participants | Age     | No. | | | Salmo... with typhoid | Salmo... with non-typhoid | All malaria cases | Malaria w/o typhoid | Typhoid and other malaria | Test for typhoid | Test for typhoid |
|-------------------------|--------------|----------------|------------------|--------------|---------|-----| | | | | | | | | |
| Alhaji et al. (2019)    | Nigeria      | 2016           | Cross-sectional  | 508 febrile patients | 1–40 years | 246:264 | 115 | 115 | 6 | 278 | 163 | 40 | Microscopy | Widal test |
| Ackah-Baidoe et al.     | Ghana        | 2018           | Cross-sectional  | 512 febrile children | 0–4 months (33) 1–15 years | 38 years (3.8) | 147:136 | 14 | 14 | 145 | 179 | S. typhi and S. paratyphi A/B | Microscopy, RDT, IFA | Rapid diagnosis test |
| Ali et al. (2020)       | Nigeria      | 2020           | Cross-sectional  | 510 febrile patients | 0–24 months (25) 24–48 months (22) 49–72 months (7) 90–120 months (2) 120–160 months (2) | 230:221 | 117 | 117 | 65 | 418 | 274 | Microscopy | Widal test |
| Almeida et al. (2017)   | Guinea       | 2016           | Cross-sectional  | 543 febrile patients | 0–5 years (2), 5–10 years (3), 10–15 years (22), 15–20 years (15), 20–40 years (9) | 16 | 16 | 10 | 147 | 91 | 56 | Microscopy | Blood culture |
| Almeida et al. (2016)   | Guinea       | 2016           | Cross-sectional  | 510 febrile patients | NS | NS | 9 | 9 | 5 | 5 | S. typhi (2), S. paratyphi (1). | Microscopy | Blood culture |
| Ali et al. (2018)       | Tanzania     | 2015           | Cross-sectional  | 749 febrile patients | Mean, 3 years; range, 3–24 | 62:47 | 7 | 7 | 6 | 6 | Molecular method | Molecular method |
| Anjorin et al. (2018)   | Cameroon     | 1997–1998      | Cross-sectional  | 552 febrile children | Median, 5 years; range, 2–9 years | 77:73 | 8 | 8 | 65 | 76 | Male median age: 60 (2–81) males: 40 (10–120), females: 25 (10–70), | Microscopy | Widal test |
| Asinu et al. (2010)     | Nigeria      | 2010           | Cross-sectional  | 504 pregnant women having influenza like illness | Mean, 28; 3 years; range, 18–45 | 14 | 14 | 14 | 34 | NS | Rapid diagnostic test |
| Aung et al. (2013)      | Myanmar      | 2014           | Cross-sectional  | 537 febrile children | <5 years | NS | 12 | 12 | 202 | 173 | 9 | Microscopy | Blood culture |
| Basnet et al. (2011)    | Nepal        | 2011           | Cross-sectional  | 500 children with fever |<5 years | NS | 8 | 6 | 78 | 77 | NS | Microscopy | Blood culture |
| Bedjaoui et al. (2010)  | India        | 2010           | Cross-sectional  | 736 febrile patients | <5 years | NS | 8 | 6 | 78 | 77 | NS | Microscopy | Blood culture |
| Bhattacharya et al.     | India        | 2008           | Cross-sectional  | 577 febrile patients | Mean, 24; 7 years; range, 17–48 | 178:164 | 14 | 14 | 25 | 23 | Microscopy | Blood culture |
| Biggs et al. (2018)     | Tanzania     | 2008–2017      | Cross-sectional  | 4099 febrile children | Median 1.57 years; range, 0.2–5.14 | 1978:1649 | 13 | 13 | 2160 | 2142 | S. typhi (61), Salmonella enterica (199) | Microscopy, RDT | Blood culture |
| Bhime et al. (2009)     | Ethiopia     | 2013           | Cross-sectional  | 208 febrile patients | 24.14±15.6 years; range, 0–48 | 120:88 | 13 | 13 | 73 | 66 | 20 | Microscopy | Widal test |
| Biruk et al. (2008)     | Kenya        | 1998–2002      | Cross-sectional  | 348 non-typhoidal Salmonella | Median 15 months (0–27) | 14 | 14 | 14 | 0 | 112 | ELISA | Blood culture |
| Biruk et al. (2008)     | Malawi       | 1998–2003      | Cross-sectional  | 1994 Salmonella | Median 2 years (2.5–5.5) | 244:234 | 170 | 170 | 244 | 244 | Microscopy | Blood culture |
| Chitsungo et al. (2015) | Zambia       | 2015           | Cross-sectional  | 197 febrile patients | 3–14 years | 189:181 | 13 | 13 | 98 | 98 | 4 | Microscopy | Blood culture |
| Chukwuma et al. (2014)  | Nigeria      | 2013–2015      | Cross-sectional  | 598 pregnant women | 5 | 5 | 5 | 5 | 5 | 5 | Microscopy | Blood culture |
| Edel et al. (2010)      | Nigeria      | 2010–2013      | Cross-sectional  | 510 febrile patients | 10–40 years | 63:57:80 | 11 | 11 | 61 | 61 | 30 | Microscopy | Blood culture |
| Ekhoosh et al. (2017)   | Nigeria      | 2017           | Cross-sectional  | 272 febrile patients | 1 to 16 years | 120:138 | 28 | 28 | 282 | 179 | 8 | Microscopy | Blood culture |
| Ehesan et al. (2010)    | Nigeria      | 2010           | Cross-sectional  | 272 febrile patients | NS | NS | 2 | 2 | 143 | 143 | 28 | Microscopy | Blood culture |
| Enos et al. (2008)      | Ghana        | 2008           | Cross-sectional  | 25 children with fever | NS | NS | 10 | 10 | 23 | 23 | 13 | Microscopy | Blood culture |
| Finer et al. (2013)     | Nigeria      | 2010           | Cross-sectional  | 25 malaria cases | NS | NS | 1 | 1 | 25 | 25 | 2 | Microscopy | Blood culture |
| Foley et al. (2009)     | Democratic Republic of the Congo | 2012          | Cross-sectional  | 154 S. typhi positive, 148 S. non-typhoidal salmonella | Median 16 months (0–39 months) | 27:25 | 3 | 3 | 18 | 18 | 0 | Microscopy | RDT | Blood culture |
| Gobarran et al. (2008)  | Malawi       | 1994–1999      | Cross-sectional  | 214 non-typhoidal salmonella | <6 months | 32 | 32 | 92 | 92 | 0 | Microscopy | Blood culture | Continued
| Author                          | Study site          | Year conducted | Study design            | Participants | Age | Sex (M:F) | All co-infection | Salmoella spp. with typhoid | Salmonella spp. with non-typhoid | All malaria cases | Malaria without typhoid | Typhoid without malaria | Test for residents | Test for typhoid |
|--------------------------------|---------------------|----------------|-------------------------|--------------|-----|-----------|-------------------|-----------------------------|-----------------------------|------------------|---------------------|----------------------|-------------------|------------------|
| Shinkaya et al. (2019)**       | Burkina Faso        | 2014           | Cross-sectional study   | 260 Malaria cases | Median 19 (10–65 years) | 140:140 | 61 | 91 | 6 | 289 | 192 | NS | Microscopy | Widal test |
| Igboh et al. (2010)**          | Nigeria             | 2003           | Cross-sectional study   | 236 Febrile Patients | NS           | NS        | 3 | 3 | 164 | 160 | 1 | Microscopy | Widal test |
| Igboh et al. (2012)**          | Nigeria             | 2012           | Cross-sectional study   | 236 Febrile patients | NS           | 11:11 | 45 | 98 | 45 | 140 | Microscopy | Widal test |
| Jakar et al. (2012)**          | Pakistan            | 2017           | Cross-sectional study   | 546 Febrile patients | 3–18 (15) | 11–20 (16) | 74:70 | 6 | 6 | 28 | 11 | 86 | Microscopy | Widal test |
| Nkeng et al. (2015)**          | Nigeria             | 2014           | Cross-sectional study   | 51,000 Febrile patients | 1–19 years | 2456 | 1841 | 6 | 101 | 11 | 104 | Microscopy | Widal test |
| Knecht et al. (2009)**         | Nigeria             | 2013–2014      | Cross-sectional study   | 75,000 Febrile patients | 0–88 years | 6213 | 1222 | 0 | 780 | 618 | NS | Microscopy | Widal test |
| Karlsson et al. (2009)**       | India               | 2003           | Cross-sectional study   | 700 Malaria cases | 0–88 years | 621 | 132 | 0 | 780 | 618 | NS | Microscopy | Widal test |
| Osu et al. (2016)**            | Nigeria             | 2007–2012      | Cross-sectional study   | 876 Febrile patients | ≤15 years | 26 | 77 | 1563 | 2580 | 0 | Microscopy | Widal test |
| Adewale et al. (2017)**        | Nigeria             | 2014           | Cross-sectional study   | 114 Patients with typhoidal/non-typhoidal salmonella | 15 | 5 | 50 | 15 | NS | 5 | Microscopy | Widal test |
| Kohla et al. (2016)**          | Benin               | 2012–2013      | Cross-sectional study   | 731 Serum malaria | Median 19 (10–86) | 143:143 | 19 | 12 | 11 | 731 | 478 | 9 | Microscopy | Widal test |
| Ugbab et al. (2016)**          | Nigeria             | 2016           | Cross-sectional study   | 216 Febrile patients | 3–58 years | 110:110 | 1 | 1 | 48 | 30 | 9 | Microscopy | Widal test |
| Ugbab et al. (2017)**          | Nigeria             | 2015           | Retrospective study     | 607 Febrile patients | 1–75 years | 375:375 | 136 | 136 | 0 | 235 | 97 | 30 | Microscopy | Widal test |
| Njolle et al. (2020)**         | Nigeria             | 2016           | Cross-sectional study   | 426 Pregnant women | 21–30 years | 129 | 12 | 0 | 120 | 111 | 10 | Microscopy | Widal test |
| Momah et al. (2020)**          | Ghana               | 2016           | Cross-sectional study   | 418 Febrile patients | ≤16 years | 262:198 | 4 | 4 | 125 | 105 | 0 | Microscopy | Widal test |
| Murti et al. (2010)**          | Tanzania            | 2008–2009      | Cross-sectional study   | 126 Children with parasitic bacillae | 2 months to 14 years | 14 | 3 | 31 | 0 | 5 | 5 | 218 Microscopy | Widal test |
| Nsip et al. (2019)**           | Cameroon            | 2018           | Cross-sectional study   | 206 Febrile patients | 9–88 years | 12 | 12 | 0 | 186 | 174 | 14 | Microscopy | Widal test |
| Shinkaya et al. (2015)**       | Ghana               | 2015–2016      | Cross-sectional study   | 771 Malaria cases | ≤15 years | 1808:864 | 31 | 31 | 37 | 771 | 739 | 9 | Microscopy | Widal test |
| Njolle et al. (2018)**         | Cameroon            | 2015           | Cross-sectional study   | 306 Febrile patients | 10 months–60 years | 30:30 | 12 | 12 | 4 | 12 | 12 | 5 | Microscopy | Widal test |
| Njolle et al. (2018)**         | Nigeria             | 2016           | Cross-sectional study   | 708 Pregnant women | NS           | NS       | 136 | 136 | 0 | 132 | 124 | NS | Microscopy | Widal test |
| Njolle et al. (2010)**         | Nigeria             | 2007           | Cross-sectional study   | 254 Febrile patients | 0–78 years | 125:125 | 14 | 14 | 5 | 14 | 14 | 5 | Microscopy | Widal test |
| Njolle et al. (2014)**         | Nigeria             | 2014–2015      | Cross-sectional study   | 47 Adults with F. hepatitis | Adults | NS | 4 | 4 | 47 | 47 | 0 | Microscopy | Widal test |
| Oluwadare et al. (2010)**      | Nigeria             | 2005           | Cross-sectional study   | 308 Febrile patients | All age groups | 146:146 | 127 | 127 | 180 | 180 | 0 | Microscopy | Widal test |
| Obiara et al. (2005)**         | Nigeria             | 1997–1998      | Cross-sectional study   | 278 Febrile patients | 10–60 years | 150:150 | 15 | 15 | 68 | 68 | 22 | Microscopy | Widal test |
| Onyeo et al. (2007)**          | Nigeria             | 2015           | Cross-sectional study   | 178 Pregnant women | 10–45 years | 170 | 170 | 0 | 172 | 172 | 0 | Microscopy | Widal test |
| Onyeo et al. (2007)**          | Nigeria             | 2015           | Cross-sectional study   | 208 Healthy individuals | 1–68 years | 52:46 | 18 | 18 | 54 | 54 | 11 | Microscopy | Widal test |
| Onyeo et al. (2015)**          | Nigeria             | 2015           | Cross-sectional study   | 208 Febrile patients | 1–75 years | 113:113 | 2 | 2 | 282 | 282 | 0 | Microscopy | Widal test |
| Okoh et al. (2015)**           | Nigeria             | 2015           | Cross-sectional study   | 208 Students | 10–30 years | 180:180 | 3 | 3 | 18 | 18 | 30 | Microscopy | Widal test |
| Okoh et al. (2018)**           | Nigeria             | 2017           | Cross-sectional study   | 317 Children with malaria | Mean 12.2 month (25) | 101 | 101 | 954 | 954 | 0 | 954 | 954 | Microscopy | Widal test |
| Okoh et al. (2018)**           | Nigeria             | 2015           | Cross-sectional study   | 294 Pregnant women | ≥20 to 60 years | 290 | 0 | 0 | 16 | 8 | 78 | Microscopy | Widal test |
| Onyeo et al. (2015)**          | Nigeria             | 2015           | Cross-sectional study   | 250 Pregnant women | ≥20 to 60 years | 147 | 147 | 68 | 68 | 68 | 68 | Microscopy | Widal test |
| Onyeo et al. (2015)**          | Nigeria             | 2015           | Cross-sectional study   | 284 Pregnant women | ≥20 to 60 years | 116 | 116 | 55 | 55 | 55 | 55 | Microscopy | Widal test |

Continued
| Author               | Study site       | Year conducted | Study design    | Participants | Age               | Sex (M:F) | All co-infections | Salmonella spp. with typhoid | Salmonella spp. with non-typhoid | All malaria cases | Malaria without typhoid | Typhoid without malaria | Test for malaria | Test for typhoid |
|----------------------|------------------|----------------|-----------------|--------------|-------------------|-----------|-------------------|-------------------------------|----------------------------------|------------------|------------------------|------------------------|----------------|----------------|
| Park et al. (2014)   | Malawi           | 1996–1997      | Cross-sectional | 27 Patients  | 0–70               | 10:17     | 3                 | 0                            | 0                               | 0                | 0                      | 0                      | Microscopy       | RDT, Blood culture |
| Walsh et al. (2014)  | Nigeria          | 2010–2012      | Cross-sectional | 24 Patients  | 0–60               | 12:16     | 1                 | 2                            | 0                               | 0                | 0                      | 0                      | Microscopy       | RDT, Blood culture |
| Sur et al. (2006)    | India            | 2004           | Prospective     | 36 Patients  | ≤ 15               | 18:18     | 2                 | 0                            | 0                               | 0                | 0                      | 0                      | Microscopy       | RDT, Blood culture |
| Shaikh et al. (2012) | Sweden           | 1995–2009      | Cross-sectional | 243 Patients | 0–70               | 130:113   | 1                 | 2                            | 0                               | 0                | 0                      | 0                      | Microscopy       | RDT, Blood culture |
| Quak et al. (2013)   | India            | 2014–2015      | Cross-sectional | 100 Patients | 0–60               | 50:50     | 10                | 2                            | 0                               | 0                | 0                      | 0                      | Microscopy       | RDT, Blood culture |
| Sale et al. (2016)   | India            | 2014–2015      | Cross-sectional | 126 Patients | 0–60               | 65:61     | 1                 | 2                            | 0                               | 0                | 0                      | 0                      | Microscopy       | RDT, Blood culture |
| Raja et al. (2016)   | India            | 2014–2015      | Cross-sectional | 200 Patients | 0–60               | 100:100   | 1                 | 2                            | 0                               | 0                | 0                      | 0                      | Microscopy       | RDT, Blood culture |
| Popoola et al. (2014)| Kenya            | 2005–2006      | Cross-sectional | 120 Patients | 0–60               | 60:60     | 1                 | 2                            | 0                               | 0                | 0                      | 0                      | Microscopy       | RDT, Blood culture |
| Park et al. (2014)   | Nigeria          | 2010–2012      | Cross-sectional | 24 Patients  | 0–60               | 12:12     | 1                 | 2                            | 0                               | 0                | 0                      | 0                      | Microscopy       | RDT, Blood culture |
| Park et al. (2014)   | India            | 2014–2015      | Cross-sectional | 126 Patients | 0–60               | 65:61     | 1                 | 2                            | 0                               | 0                | 0                      | 0                      | Microscopy       | RDT, Blood culture |
| Park et al. (2014)   | India            | 2014–2015      | Cross-sectional | 126 Patients | 0–60               | 65:61     | 1                 | 2                            | 0                               | 0                | 0                      | 0                      | Microscopy       | RDT, Blood culture |
| Park et al. (2014)   | India            | 2014–2015      | Cross-sectional | 126 Patients | 0–60               | 65:61     | 1                 | 2                            | 0                               | 0                | 0                      | 0                      | Microscopy       | RDT, Blood culture |
| Park et al. (2014)   | India            | 2014–2015      | Cross-sectional | 126 Patients | 0–60               | 65:61     | 1                 | 2                            | 0                               | 0                | 0                      | 0                      | Microscopy       | RDT, Blood culture |

Table 1. Characteristics of the included studies. ELISA enzyme-linked immunosorbent assay, NS not specified, RDT rapid diagnostic test.
Figure 2. The pooled prevalence of typhoidal/NTS and malaria co-infection among febrile patients detected using diagnostic tests for Salmonella spp. ES proportion estimate (multiply 100 units for interpreted as prevalence estimate), CI confidence interval.
The pooled prevalence rate of typhoidal/NTS infection among malaria patients was estimated from 57 studies. The studies were divided into groups based on diagnostic tests for *Salmonella* spp. The pooled prevalence rates of typhoidal/NTS infection among patients with malaria were 31% (95% CI 23–39%; I², 99.5%) using the Widal test, 5% (95% CI 0–10%; I², 86.7%) using RDTs, 3% (95% CI 2–3%; I², 86.8%) using blood culture, 2% (95% CI 1 to 5%; I², 58.7%) using molecular methods, 12% (95% CI 5–19%; I², 86%) using stool cultures, and 27% (95% CI 17–39%) using a combination of blood and stool cultures.

### Prevalence of typhoidal/NTS infections among patients with malaria.

The pooled prevalence rate of typhoidal/NTS infection among malaria patients was estimated from 57 studies. The studies were divided into groups based on diagnostic tests for *Salmonella* spp. The pooled prevalence rates of typhoidal/NTS infection among patients with malaria were 31% (95% CI 23–39%; I², 99.5%) using the Widal test, 5% (95% CI 0–10%; I², 86.7%) using RDTs, 3% (95% CI 2–3%; I², 86.8%) using blood culture, 2% (95% CI 1 to 5%; I², 58.7%) using molecular methods, 12% (95% CI 5–19%; I², 86%) using stool cultures, and 27% (95% CI 17–39%) using a combination of blood and stool cultures.

Among the studies using the Widal test for the identification of *Salmonella* spp. infections, the highest prevalence rate of typhoidal/NTS among patients with malaria was reported in Cameroon (90%; 95% CI 83–94%) and...
Figure 4. Pooled prevalence of typhoidal/NTS and malaria co-infection using blood cultures for the identification of *Salmonella* spp. infection stratified by countries. ES proportion estimate (multiply 100 units for interpreted as prevalence estimate), CI confidence interval, NS not specified.

Nigeria (37%; 95% CI 20–54%; I², 98.7%), whereas lower prevalence rates were reported in Burkina Faso (32%; 95% CI 27–38%), Pakistan (28%; 95% CI 8–47%; I², 89.9%), Sierra Leone (24%; 95% CI 23–25%), Ethiopia (18%; 95% CI 11–28%), Tanzania (13%; 95% CI 8–21%), Ghana (12%; 95% CI 6–19%; I², 99.9%), and India (1%; 95% CI 0–1%) (Fig. 12). Among the studies using hemoculture for the identification of *Salmonella* spp. infections, the highest prevalence rate of typhoidal/NTS among patients with malaria was reported in Nigeria (8%; 95% CI 1–2%; I², 89.3%), Tanzania (2%; 95% CI 2–3%), Ghana (1%; 95% CI 1–2%; I², 97.9%), Kenya (1%; 95% CI 1–1%; I², 98.5%), and Sweden (1%; 95% CI 0–1%) (Fig. 13).

Among the studies using the Widal test for the identification of *Salmonella* spp. infections, the prevalence rates of typhoidal/NTS among patients with malaria in all age groups were 38% (95% CI 29–48%; I², 98.5%), whereas lower prevalence rates were reported in Burkina Faso (32%; 95% CI 27–38%), Pakistan (28%; 95% CI 8–47%; I², 89.9%), Sierra Leone (24%; 95% CI 23–25%), Ethiopia (18%; 95% CI 11–28%), Tanzania (13%; 95% CI 8–21%), Ghana (12%; 95% CI 6–19%; I², 99.9%), and India (1%; 95% CI 0–1%) (Fig. 12). Among the studies using blood culture for the identification of *Salmonella* spp. infections, the prevalence rates of typhoidal/NTS among malarial patients were 8% in all age groups (95% CI 4–12%; I², 89%), whereas lower prevalence rates were reported in Myanmar (6%; 95% CI 1–11%; I², 98.4%), India (6%; 95% CI 3–10%; I², 89.3%), Tanzania (2%; 95% CI 2–3%), Ghana (1%; 95% CI 1–2%; I², 97.9%), Kenya (1%; 95% CI 1–1%; I², 98.5%), and Sweden (1%; 95% CI 0–1%) (Fig. 13).

Among the studies using blood culture for the identification of *Salmonella* spp. infections, the prevalence rates of typhoidal/NTS among patients with malaria in all age groups were 38% (95% CI 29–48%; I², 89.5%), 12% in children (95% CI 7–17%; I², 99.9%), 20% in the NS age group (95% CI 11–51%; I², 97.7%), and 11% in adults (95% CI 1–20%; I², 93.5%) (Fig. 14). Among the studies using blood culture for the identification of *Salmonella* spp. infections, the prevalence rates of typhoidal/NTS among malarial patients were 8% in all age groups (95% CI 5–11%; I², 91.3%), 1% in the NS age group (95% CI 1–4%; I², 69.2%), 3% in adults (95% CI 1–7%; I², 48%), and 2% in children (95% CI 1–3%; I², 87.8%) (Fig. 15). Subgroup analysis of age (≤ 3 years and 0–15 years) of NTS infection among patients with malaria was performed using the data of five studies. Results showed that the prevalence rates of NTS infection among patients with malaria were 2% in patients aged 0–15 years (95% CI 1–4%; I², 90.5%) and 1% in patients aged ≤ 3 years (95% CI 1–2%; I², 95.3%) (Fig. 16). Subgroup analysis of typhoidal/NTS, regions (Africa and Asia), and time (publication year) was performed using the data from studies using blood culture for typhoidal/NTS.
Results showed that the prevalence rates of typhoidal and NTS infection among patients with malaria were 6% (95% CI 3–8%; I², 86.9%) and 2% (95% CI 1–2%; I², 87.7%) (Fig. 17). Subgroup analysis of regions showed that the prevalence rates of typhoidal/NTS among patients with malaria were 2% in Africa (95% CI 1–3%; I², 87.5%), 6% in Asia (95% CI 3–9%; I², 86.7%), and 1% in Europe (95% CI 0–1%) (Fig. 18). Subgroup analysis of time showed that the prevalence rate of typhoidal Salmonella infection among patients with malaria was highest (17%) in 2016 (95% CI 1–33%; I², 95.6%), 8% in 2012 (95% CI 3–18%) and 7% in 2018 (95% CI 0–14%; I², 98.5%). The low prevalence of typhoidal Salmonella infection among patients with malaria was demonstrated in 2016 (13%), 2003 (2%), 2013 (2%), and 2009 (1%) (Fig. 19). Subgroup analysis of time showed that the prevalence rates of NTS infection among patients with malaria were highest in 2011 (4%), 2015 (3%), and 2014 (2%) and low in 2014 (2%), 2002 (1%), 2012 (1%), and 2016 (1%) (Fig. 20).

Comparison of typhoidal/NTS infections among patients with severe and non-severe malaria. The pooled prevalence rates of typhoidal/NTS infections among patients with severe and non-severe malaria were estimated using data from 24 studies that enrolled patients with non-severe malaria28,37,38,44,50,53,56–58,60,66,68,69,71,77,78,81,83,89,98,104–106,108 and 6 studies that enrolled patients with severe malaria. All 30 studies employed the blood culture method to identify Salmonella spp. infections. The pooled prevalence rates of typhoidal/NTS infection were 2% in patients with severe malaria (95% CI 1–3%; I², 91.5%) and 3% in patients with non-severe malaria (95% CI 2–3%; I², 86.8%) (Fig. 21).

Prevalence of malaria infections among patients with typhoidal/NTS infections. The pooled prevalence rate of malaria infections among patients with typhoidal Salmonella spp. infection was estimated from three studies20,31,49. The pooled prevalence rate of malaria infection in patients with typhoidal Salmonella
Overall ($I^2 = 89.88\%, p = 0.00$); Heterogeneity between groups: $p = 0.000$

**Subtotal ($I^2 = 89.21\%, p = 0.00$)**

- Singh et al., 2014
- Krumkamp et al., 2016
- Tabu et al., 2012
- Verma et al., 2014

**Subtotal ($I^2 = 46.05\%, p = 0.10$)**

- Sur et al., 2006
- Orok et al., 2016
- Edet et al., 2016
- Alhassan et al., 2012

**Children (<15 years)**

- Singh et al., 2014
- Krumkamp et al., 2016
- Tabu et al., 2012
- Verma et al., 2014

**Adults**

- Singh et al., 2014

**NS**

- Akinyemi et al., 2015
- Igbereghu et al., 2009
- Raja et al., 2016
- Samatha et al., 2015
- Tabu et al., 2012
- Verma et al., 2014

**Subtotal ($I^2 = 46.05\%, p = 0.10$)**

- Singh et al., 2014
- Krumkamp et al., 2016
- Tabu et al., 2012
- Verma et al., 2014

**Overall ($I^2 = 89.88\%, p = 0.00$)**

- 0.01 (0.00, 0.02) 45.46

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**Figure 6.** Pooled prevalence of typhoidal/NTS and malaria co-infection using blood cultures for the identification of *Salmonella* spp. infection stratified by age groups. ES proportion estimate (multiply 100 units for interpreted as prevalence estimate), CI confidence interval, NS not specified.

Spp. was 17% in children (95% CI 6–29%; $I^2$, 33.3%) (Fig. 22). The pooled prevalence rate of malaria infection among patients with NTS, which was estimated from six studies17,20,27,29,31,49, was 43% in children (95% CI 32–53%; $I^2$, 89.1%) (Fig. 23).

**Probability of *Plasmodium* spp. and *Salmonella* spp. co-infections.** The probability of *Plasmodium* spp. and *Salmonella* spp. co-infections was estimated from 46 studies35–38,40,41,43–47,50–53,57,59,61,64–66,68,70,71,73,75,76,81,82,84,87,90–93,95–97,99–104,106,108, which reported the following parameters: total number of *Plasmodium* spp. and *Salmonella* spp. co-infections, total number of malaria, total number of malaria without typhoid, and total number of febrile patients without malaria/typhoid. *Plasmodium* spp. and *Salmonella* spp. co-infections in all age groups occurred by chance ($p = 0.126$; odds ratio, 1.51; 95% CI 0.89–2.58; $I^2$, 95.7%), whereas *Plasmodium* spp. and *Salmonella* spp. co-infections in children did not ($p < 0.0001$; odds ratio, 0.36; 95% CI 0.23–0.58; $I^2$, 73.9%). No association between *Plasmodium* spp. and *Salmonella* spp. infections was observed in the NS age group ($p = 0.24$; odds ratio, 0.40; 95% CI 0.09–1.85; $I^2$, 86%) or adults ($p = 0.799$; odds ratio, 1.14; 95% CI 0.41–3.16; $I^2$, 94%). Overall, *Plasmodium* spp. and *Salmonella* spp. co-infection occurred by chance ($p = 0.987$; odds ratio, 1.00; 95% CI 0.68–1.49; $I^2$, 95.2%) (Fig. 24). A significantly higher odds ratio of co-infection was reported in Nigeria7,59,82,90,97,101, Cameroon84, India105, and Pakistan102, whereas a significantly lower odds ratio of co-infection was found in Nigeria63,67,70, Ghana63,8, Tanzania8, Kenya8, and India81,7.

**Outcomes of malaria and typhoidal/NTS co-infections.** A limited number of studies reported clinical outcomes of patients with co-infections (Table S2). Five studies35,40,43,79,80,83 reported outcomes of co-infection. Among those studies, three studies35,40,83 reported outcomes of malaria and NTS co-infections, and one study35
Figure 7. Pooled prevalence of typhoidal/NTS and malaria co-infection using blood cultures for the identification of Salmonella spp. infection stratified by typhoidal/NTS infection. ES proportion estimate (multiply 100 units for interpreted as prevalence estimate), CI confidence interval, NS not specified.

reported outcomes of malaria and typhoid co-infections. The case fatality rate in patients with malaria and NTS co-infections was 16% (95% CI 9–24%; $I^2$, 89.1%; three studies), while one study$^{29}$ reported the case fatality rate in patients with malaria and typhoidal Salmonella co-infections at 33% (95% CI 6–79%) (Supplementary Fig. S1). The difference in malarial parasitemia between co-infections and Plasmodium spp. monoinfection was estimated by two studies$^{79,80}$. Results showed a higher mean of malarial parasitemia in patients with co-infections than those with Plasmodium spp. monoinfection (p, 0.023; WMD, 7926.7 parasites/µL of blood (95% CI 1091–14,762.3 parasites/µL of blood; $I^2$, 0%, two studies) (Supplementary Fig. S2). The study by Bassat et al.$^{79}$ also showed a lower mean hematocrit in all, 0.54–1.10; $I^2$, 93.6%) (Supplementary Fig. S3). However, the use of a fixed-effects model in the meta-analysis showed a lower rate of respiratory distress in patients with co-infections (4/12, 33.3%) than those with Plasmodium spp. monoinfection (542/1328, 40.8%). The study by Bassat et al.$^{79}$ also showed a lower mean hematocti in patients with co-infections (22.1 ± 9.3%, 12 cases) than those with Plasmodium spp. monoinfection (23.4 ± 8.4%, 1328 cases).

Sensitivity test. After excluding outliers$^{40,82,104}$, the probability of Plasmodium spp. and Salmonella spp. co-infection was estimated from 43 studies$^{35–39,41,43–47,50–53,57,59,61–66,68,70,71,73,75,76,81,84,87,90–93,95–97,99–100,106,108}$. Overall, Plasmodium spp. and Salmonella spp. co-infection occurred by chance (p, 0.148; odds ratio, 0.77; 95% CI 0.54–1.10; $I^2$, 93.6%) (Supplementary Fig. S3). However, the use of a fixed-effects model in the meta-analysis indicated that Plasmodium spp. and Salmonella spp. co-infection did not occur by chance (p < 0.0001; odds ratio, 0.82; 95% CI 0.76–0.88; $I^2$, 93.6%) (Supplementary Fig. S4).

Publication bias. Publication bias among the 43 included studies used for determining the probability of Plasmodium spp. and Salmonella spp. co-infection was evaluated using a funnel plot and Egger’s test. The funnel plot exhibited an asymmetrical distribution of ES, and the seES was far from the middle line (no effect) (Supplementary Fig. S5). Egger’s test demonstrated no small study effect (p, 0.379; coefficient, 1.62; standard error,
Overall ($I^2 = 89.88\%, p = 0.00$); Heterogeneity between groups: $p = 0.006$

| Study                        | ES (95% CI) | Weight (%) |
|------------------------------|-------------|------------|
| Africa                       |             |            |
| Akinyemi et al., 2007        | 0.07 (0.04, 0.11) | 59         |
| Akinyemi et al., 2015        | 0.03 (0.01, 0.07) | 73         |
| Alhassan et al., 2012        | 0.01 (0.01, 0.03) | 2.77       |
| Biggs et al., 2014           | 0.01 (0.01, 0.02) | 8.04       |
| Edet et al., 2016            | 0.11 (0.06, 0.19) | 17         |
| Igbeneghu et al., 2009       | 0.00 (0.00, 0.02) | 5.28       |
| Krumkamp et al., 2016        | 0.00 (0.00, 0.01) | 9.50       |
| Mbuh et al., 2003            | 0.00 (0.00, 0.03) | 4.45       |
| Nwuzo et al., 2009           | 0.06 (0.03, 0.09) | 0.74       |
| Mbuh et al., 2003            | 0.00 (0.00, 0.03) | 3.46       |
| Park et al., 2016            | 0.05 (0.03, 0.07) | 1.54       |
| Tabu et al., 2012            | 0.00 (0.00, 0.01) | 9.39       |
| Subtotal ($I^2 = 87.54\%, p = 0.00$) | 0.01 (0.01, 0.02) | 46.67 |

| Asia                         |             |            |
| Bhattacharya et al., 2013    | 0.00 (0.00, 0.00) | 9.80       |
| Raja et al., 2016            | 0.02 (0.01, 0.07) | 7.99       |
| Sharma et al., 2016          | 0.01 (0.00, 0.02) | 5.87       |
| Singh et al., 2014           | 0.02 (0.01, 0.02) | 7.59       |
| Snehanshu et al., 2014       | 0.03 (0.01, 0.06) | 1.22       |
| Sur et al., 2006             | 0.00 (0.00, 0.00) | 9.75       |
| Vats et al., 2018            | 0.01 (0.00, 0.03) | 3.38       |
| Verma et al., 2014           | 0.01 (0.01, 0.02) | 5.46       |
| Subtotal ($I^2 = 87.32\%, p = 0.00$) | 0.01 (0.00, 0.01) | 52.33 |

| Heterogeneity between groups: $p = 0.006$ | Overall ($I^2 = 89.88\%, p = 0.00$): | 0.01 (0.01, 0.01) | 100.00 |

Figure 8. Pooled prevalence of typhoidal/NTS and malaria co-infection using blood cultures for the identification of *Salmonella* spp. infection stratified by regions. ES proportion estimate (multiply 100 units for interpreted as prevalence estimate), CI confidence interval, NS not specified.

1.82; t, 0.89). A contour-enhanced funnel plot analysis revealed missing studies in the significant areas ($p < 0.01$) (Supplementary Fig. S6), indicating that the funnel plot asymmetry was likely due to factors such as heterogeneity, selection bias, and quality of the included studies rather than publication bias.

Discussion

The present meta-analysis revealed a high prevalence of malaria and typhoidal/NTS co-infections among febrile patients detected using the Widal test (14%) and a low prevalence of malaria and typhoidal/NTS co-infections among febrile patients detected using blood cultures (1%). Moreover, the meta-analysis demonstrated that the prevalence of typhoidal/NTS infection among patients with malaria using the Widal test was high (31%), whereas the prevalence of typhoid/non-typhoid using blood culture was low (3%). A high prevalence of malaria infections among patients with typhoidal *Salmonella* spp. infections (17%) and NTS (43%) was also detected. The highest prevalence of co-infections detected using the Widal test was observed in Cameroon15, followed by Nigeria37,57,83 and Sierra Leone5, compared with Ghana, India, Ethiopia, Tanzania, and Pakistan. In using blood cultures, the gold standard method for the identification of *Salmonella* spp., the results indicated that the highest prevalence of co-infection was reported in Nigeria37,57,83 compared with India, Tanzania, Ghana, and Kenya. Based on these results, typhoid/non-typhoid and malaria co-infection among febrile patients frequently occurred in Nigeria. In 2020, Nigeria accounted for the most malaria cases (27%) and malaria-related deaths (23%) worldwide1. Moreover, typhoid fever is a major disease in Nigeria due to increased urbanization, insufficient water supply, movement of immigrant workers, poor processing of human waste, and overuse of antibiotics. Due to the co-endemicity of these two pathogens, the possibility of co-infection might increase in this country.

Using the data from studies performing blood culture to identify typhoidal/NTS infection, the subgroup analysis of typhoidal/NTS infection demonstrated low prevalence of malaria and typhoid co-infections among febrile patients (1%) and low prevalence of typhoid among patients with malaria (6%). Moreover, the low prevalence of malaria and NTS co-infections among febrile patients (1%) and NTS infection among patients with malaria (2%)
was observed. The highest prevalence of malaria and typhoid co-infections among febrile patients was reported in Nigeria, suggesting that malaria and typhoid are indeed halo-endemic in this area\(^5\). In the meta-analysis of typhoid among patients with malaria, the highest prevalence of typhoid among patients with malaria was noted in Nigeria\(^38,57\). These results suggested an increasing episode of persistent fever among patients with *S. typhi* and *P. falciparum* infections in Nigeria. For NTS infection among patients with malaria, the prevalence was highest in Kenya, and NTS infection was the most common bacteremia in children with malaria\(^28\). The high rate of bacteremia in patients with malaria in Nigeria might be due to the high prevalence of NTS infections and malnutrition\(^28\).

Using the data from studies performing blood culture to identify typhoidal/NTS infection, the subgroup analysis of regions demonstrated that the prevalence of malaria and typhoidal/NTS co-infections were 1% in both Africa and Asia. However, the prevalence of typhoidal/NTS among patients with malaria was higher in Asia (6%) than those of Africa (2%). The difference in the prevalence of typhoidal/NTS co-infections between two regions might be caused by the heterogeneity of the prevalence estimates between two regions or real difference caused by environmental factors. For example, studies in India suggested that malaria and typhoid are endemic because of poor hygiene and environmental factors\(^104,108\). In Africa, although the pooled prevalence of typhoidal/NTS infection among patients with malaria was lower than those in Asia; the results of individual studies were heterogenous. For example, the high prevalence of typhoidal/NTS infection among patients with malaria were reported by four studies conducted in Nigeria\(^37,38,57,83\), while a lower prevalence was reported by other studies included in the meta-analysis.

**Figure 9.** Pooled prevalence of typhoidal and malaria co-infection using blood cultures for the identification of *Salmonella* spp. infection stratified by time (publication year). ES proportion estimate (multiply 100 units for interpreted as prevalence estimate), CI confidence interval, NS not specified.

| Study | ES (95% CI) | Weight (%) |
|-------|-------------|------------|
| 1. year 2003 Mboh et al., 2003 | 0.00 (0.00, 0.03) | 5.67 |
| 2. year 2006 Sur et al., 2006 | 0.00 (0.00, 0.00) | 15.34 |
| 3. year 2009 Igbeneghu et al., 2009 Nwuzo et al., 2009 Subtotal (*I^2 = 98.99%, p = 0.00*) | 0.00 (0.00, 0.02) 0.06 (0.03, 0.09) 0.01 (-0.00, 0.01) | 6.93 0.83 7.76 |
| 4. year 2012 Alhassan et al., 2012 | 0.01 (0.01, 0.03) | 3.34 |
| 5. year 2013 Bhattacharya et al., 2013 | 0.00 (0.00, 0.00) | 15.45 |
| 6. year 2014 Singh et al., 2014 Shehanthu et al., 2014 Verma et al., 2014 Subtotal (*I^2 = 83.15%, p = 0.00*) | 0.03 (0.01, 0.06) 0.01 (0.01, 0.02) 0.01 (-0.00, 0.02) | 1.39 7.22 23.34 |
| 7. year 2015 Akinyemi et al., 2015 Samatha et al., 2015 Subtotal (*I^2 = 91.58%, p = 0.00*) | 0.03 (0.01, 0.07) 0.01 (0.00, 0.02) 0.01 (0.00, 0.01) | 0.83 7.88 8.71 |
| 8. year 2016 Edet et al., 2016 Orok et al., 2016 Raju et al., 2016 Sharma et al., 2016 Subtotal (*I^2 = 72.80%, p = 0.01*) | 0.11 (0.06, 0.19) 0.01 (0.00, 0.03) 0.02 (0.01, 0.07) 0.02 (0.01, 0.02) 0.02 (0.00, 0.03) | 0.19 4.26 0.89 10.89 16.24 |
| 9. year 2018 Vats et al., 2018 | 0.01 (0.00, 0.03) | 4.15 |
| Heterogeneity between groups: p = 0.023 Overall (*I^2 = 84.84%, p = 0.00*) | 0.01 (0.00, 0.01) | 100.00 |
Using the data from studies performing blood culture to identify typhoidal/NTS infection, the subgroup analysis of time (year of publication) showed that the prevalence of malaria and typhoid co-infections among febrile patients, and typhoidal Salmonella infections among patients with malaria was highest in 2016, while lower prevalence was reported in before and after 2016. In 2016, three studies conducted in Nigeria and India reported the highest prevalence rates of typhoid among patients with malaria. The peak of typhoid among patients with malaria in 2016 was different from those of NTS infections among patients with malaria. The subgroup analysis showed that the peak prevalence rate of NTS infection among patients with malaria was highest in 2011, lower in 2012–2016, and 2001. These results indicated that the prevalence of NTS might decreased with time in 2011–2016, while the prevalence of typhoid among patients with malaria might not depend on time, which are needed to be further investigated.

Using the data from studies performing blood culture to identify typhoidal/NTS infection, the subgroup analysis of age of patients demonstrated that the prevalence rate of typhoidal/NTS infection among patients with malaria was higher in adults (3%) compared to that in children (2%). The previous study showed that peaks of NTS infection occurred in children aged < 2 years and adults aged 25–40 years, while the lower rate of NTS infection occurred in children aged less than 12 years old, and the proportion of hospitalization was decreased with age. These age groups were supported by the subgroup analysis of age that the prevalence of typhoidal/NTS was higher in adults than in children. Nevertheless, as the limitation of age information in studies reported typhoidal/NTS co-infections among febrile patients, the subgroups analysis of age might not represent the exact difference in the prevalence of typhoidal/NTS co-infections between adults and children.

The present meta-analysis demonstrated a wide gap in prevalence of malaria and typhoid/non-typhoid co-infections among febrile patients as measured by the Widal test and blood culture in analysis. The high rate of typhoid/non-typhoid and malaria co-infections detected using the Widal test and low rate of co-infections detected using blood cultures might be due to the lack of differentiation between Salmonella species/serotypes by the Widal test and cross-reactivity with other Enterobacteriaceae. Moreover, false-positive Widal tests have been reported in patients with malaria and other infections. The malaria Plasmodium may share similar strong immunogenic antigens with the typhoidal Salmonella (S. typhi); thus, Plasmodium infections could induce the generation of antibodies against S. typhi antigens, leading to cross-reactivity and false-positive results. Furthermore, malaria loading strongly correlated with Salmonella antibody titers in numerous studies. This cross-reaction of typhoidal/NTS antibodies with malarial antigen leads to overdiagnosis of typhoid fever. The Widal test also generates false-negative results if patients are tested during the early phase of typhoid fever.

Figure 10. Pooled prevalence of NTS and malaria co-infection using blood cultures for the identification of Salmonella spp. infection stratified by time (publication year). ES proportion estimate (multiply 100 units for interpreted as prevalence estimate), CI confidence interval, NS not specified.

| Study | ES (95% CI) | Weight (%) |
|-------|-------------|------------|
| 1. year 2012 | Tabu et al., 2012 | 0.00 (0.00, 0.01) | 34.99 |
| 2. year 2014 | Biggs et al., 2014 | 0.01 (0.01, 0.02) | 29.56 |
| 3. year 2016 | Krumkamp et al., 2016 | 0.00 (0.00, 0.01) | 35.45 |
| Overall (P² = 92.31%, p = 0.00) | | 0.01 (0.00, 0.01) | 100.00 |
Figure 11. Prevalence of typhoidal/NTS infection among patients with malaria detected using diagnostic tests for *Salmonella* spp. ES: proportion estimate (multiply 100 units for interpreted as prevalence estimate), CI: confidence interval.
The high prevalence of typhoid fever may also be due to poor interpretation of the Widal test when diagnosing typhoid fever. Nevertheless, in Africa and other territories, the Widal test is the most common diagnostic tool used for typhoid fever, owing to its low cost, ease of performance, and minimal training and equipment requirements. Of note, false-positive results of the Widal tests in febrile patients suspected of having *Salmonella* spp. infection may lead to incorrect treatment for malaria parasites. Thus, careful interpretation of the Widal test for the diagnoses of typhoid fever in resource-poor countries is required, as the overdiagnosis of typhoid fever can lead to unnecessary treatment of patients with antibiotics, microbial resistance, and poor outcome. The use of Widal test alone for the diagnosis of typhoid fever will cause misdiagnoses.

Using blood cultures alone to identify *Salmonella* spp. infection may underestimate *Salmonella* spp. infections, as blood culture has a lower sensitivity compared with the Widal test. Negative blood culture test results may be noted in patients with acute disease before the antibody response. Based on the results of this study, the Widal test should not be used alone but in combination with blood/stool cultures. Therefore, a combination of the Widal test and blood and stool cultures is an excellent choice for diagnosing *Salmonella* spp. infection among febrile patients or patients with malaria. Although the high laboratory expenses for combination testing are difficult to overcome, the use of more than one diagnostic method to identify *Salmonella* spp. infections among patients with malaria is important to prevent incorrect treatment and misdiagnoses of malaria and *Salmonella* spp. infections and typhoidal/NTS infection among patients with malaria using the Widal test for the identification of *Salmonella* spp. infection stratified by countries. ES proportion estimate (multiply 100 units for interpreted as prevalence estimate), CI confidence interval.

**Figure 12.** Prevalence of typhoidal/NTS infection among patients with malaria using the Widal test for the identification of *Salmonella* spp. infection stratified by countries. ES proportion estimate (multiply 100 units for interpreted as prevalence estimate), CI confidence interval.

| Study                        | ES (95% CI) | % Weight |
|------------------------------|-------------|----------|
| Nigeria                      |             |          |
| Abah et al., 2019            | 0.41 (0.36, 0.47) | 4.78    |
| Agwu et al., 2009            | 0.28 (0.24, 0.32)  | 4.84    |
| Enabulele et al., 2016       | 0.03 (0.01, 0.06)  | 4.89    |
| Eze et al., 2011             | 0.12 (0.04, 0.39)  | 4.35    |
| Igharo et al., 2012          | 0.49 (0.39, 0.59)  | 4.52    |
| Mike et al., 2017            | 0.58 (0.52, 0.65)  | 4.76    |
| Odkamnoro et al., 2017       | 0.67 (0.60, 0.73)  | 4.74    |
| Onyido et al., 2014          | 0.20 (0.11, 0.33)  | 4.47    |
| Oshikhoumamie et al., 2021   | 0.50 (0.24, 0.76)  | 2.80    |
| Salie et al., 2020           | 0.41 (0.32, 0.50)  | 4.80    |
| Subtotal (I^2 = 98.68%, p = 0.00) | 0.37 (0.20, 0.54)  | 44.75   |
| Ghana                        |             |          |
| Afoakwaah et al., 2011       | 0.25 (0.12, 0.45)  | 3.97    |
| Anabire et al., 2018         | 0.11 (0.06, 0.19)  | 4.75    |
| Subtotal (I^2 = 99.85%, p = 0.00) | 0.12 (0.06, 0.19)  | 8.72    |
| Cameroon                     |             |          |
| Ammah et al., 1999           | 0.90 (0.83, 0.94)  | 4.79    |
| India                        |             |          |
| Bhalla et al., 2019          | 0.00 (0.00, 0.02)  | 4.90    |
| Katyar et al., 2020          | 0.16 (0.13, 0.18)  | 4.88    |
| Subtotal (I^2 = 99.85%, p = 0.00) | 0.01 (0.00, 0.01)  | 9.79    |
| Ethiopia                     |             |          |
| Birhanie et al., 2014        | 0.18 (0.11, 0.28)  | 4.63    |
| Tanzania                     |             |          |
| Chipwaza et al., 2015        | 0.13 (0.08, 0.21)  | 4.74    |
| Burkina Faso                 |             |          |
| Ibrahim et al., 2019         | 0.32 (0.27, 0.38)  | 4.79    |
| Pakistan                     |             |          |
| Jalani et al., 2019          | 0.46 (0.26, 0.66)  | 3.57    |
| Sajid et al., 2017           | 0.32 (0.22, 0.44)  | 4.45    |
| Shalik et al., 2018          | 0.12 (0.09, 0.15)  | 4.87    |
| Subtotal (I^2 = 89.90%, p = 0.00) | 0.28 (0.08, 0.47)  | 12.90   |
| Sierra Leone                 |             |          |
| Kargbo et al., 2014          | 0.24 (0.23, 0.25)  | 4.90    |
| Heterogeneity between groups: p = 0.000 | 0.31 (0.23, 0.39)  | 100.00  |
susceptibility. Increased free iron from hemolysis may also promote the survival of other acute febrile illnesses. Infections caused by typhoidal Salmonella, including *S. typhi* and *S. paratyphi*, and the associated serious complications require treatment with antibiotics, including chloramphenicol, cefixime, amoxicillin, trimethoprim/sulfamethoxazole, azithromycin, aztreonam, and cefotaxime, to prevent severe illness and death. NTS infections do not usually require treatment with antibiotics. However, complications, such as septicemia and meningitis, require treatment with ciprofloxacin, ceftriaxone, and ampicillin, according to the WHO. Presently, antibiotic resistance of *Salmonella* species is an emerging threat, so reliable diagnostic tests and appropriate treatments for typhoid/non-typhoid fever are important.

The present meta-analysis demonstrated that *Salmonella* spp. bacteremia developed in approximately 2% of patients with severe malaria. This occurrence was not much different from the *Salmonella* spp. bacteremia pooled prevalence of 3% in patients with non-severe malaria. Several mechanisms have been suggested to elucidate why patients with malaria may be predisposed to *Salmonella* spp. infection and bacteremia. First, immunosuppression occurs during malaria infection and treatment. Second, malaria can lead to hemolysis, which may predispose patients to infection with Gram-negative bacteria, such as typhoidal *Salmonella* spp. Third, changes in iron storage metabolism from malaria-induced hemolysis cause neutrophil dysfunction and increased susceptibility. Increased free iron from hemolysis may also promote the survival of *Salmonella* spp. Fourth, the sequestration of parasitized red blood cells in the intestine causes reduced blood flow in the mucosal gut barrier, which increases intestinal susceptibility to bacterial infection. The high rate of NTS bacteremia

| Study | ES (95% CI) | % Weight |
|-------|-------------|----------|
| Nigeria | 0.15 (0.09, 0.23) | 1.19 |
| Akinyemi et al., 2007 | 0.44 (0.19, 0.73) | 0.06 |
| Alhassan et al., 2012 | 0.08 (0.03, 0.18) | 1.02 |
| Edet et al., 2016 | 0.27 (0.16, 0.42) | 0.33 |
| Igbenoghnu et al., 2009 | 0.01 (0.00, 0.03) | 7.73 |
| Mbu et al., 2003 | 0.02 (0.00, 0.09) | 3.64 |
| Nwuzo et al., 2009 | 0.42 (0.27, 0.59) | 0.21 |
| Onok et al., 2016 | 0.01 (0.00, 0.04) | 7.37 |
| Subtotal (p² = 88.95%, p = 0.00) | 0.08 (0.04, 0.12) | 21.53 |
| Myanmar | 0.08 (0.01, 0.24) | 0.88 |
| Aung et al., 2018 | 0.06 (0.02, 0.14) | 1.60 |
| Nyen et al., 2015 | 0.06 (0.01, 0.11) | 2.23 |
| Subtotal (p² = 98.42%, p = 0.00) | 0.02 (0.02, 0.03) | 8.90 |
| India | 0.02 (0.01, 0.08) | 4.07 |
| Bhattacharya et al., 2013 | 0.20 (0.06, 0.51) | 0.10 |
| Raja et al., 2016 | 0.01 (0.01, 0.03) | 7.59 |
| Samatha et al., 2015 | 0.23 (0.18, 0.29) | 1.60 |
| Sharma et al., 2016 | 0.01 (0.00, 0.04) | 7.45 |
| Singh et al., 2014 | 0.14 (0.06, 0.29) | 0.46 |
| Shenhu et al., 2014 | 0.03 (0.01, 0.09) | 3.19 |
| Sur et al., 2006 | 0.10 (0.03, 0.25) | 0.54 |
| Vats et al., 2018 | 0.06 (0.03, 0.10) | 24.99 |
| Tanzania | 0.02 (0.02, 0.03) | 8.90 |
| Biggs et al., 2014 | 0.01 (0.01, 0.02) | 7.88 |
| Nigeria | 0.03 (0.02, 0.04) | 17.07 |
| Krukamp et al., 2016 | 0.01 (0.01, 0.02) | 17.07 |
| Nielsen et al., 2015 | 0.19 (0.18, 0.20) | 17.07 |
| Subtotal (p² = 98.47%, p = 0.00) | 0.01 (0.01, 0.02) | 17.07 |
| Kenya | 0.01 (0.01, 0.01) | 9.40 |
| Oundo et al., 2002 | 0.04 (0.03, 0.06) | 6.79 |
| Were et al., 2011 | 0.01 (0.01, 0.01) | 16.18 |
| Subtotal (p² = 98.47%, p = 0.00) | 0.01 (0.01, 0.01) | 16.18 |
| Sweden | 0.01 (0.00, 0.01) | 9.09 |
| Sandlund et al., 2012 | 0.03 (0.02, 0.03) | 100.00 |

**Figure 13.** Prevalence of typhoidal/NTS infection among patients with malaria detected using blood cultures for the identification of *Salmonella* spp. infection stratified by countries. ES proportion estimate (multiply 100 units for interpreted as prevalence estimate), CI confidence interval.
Figure 14. Prevalence of typhoidal/NTS infection among patients with malaria using the Widal test for the identification of *Salmonella* spp. infection stratified by age groups. ES proportion estimate (multiply 100 units for interpreted as prevalence estimate), CI confidence interval, NS not specified.

is well described in patients with malaria-related severe anemia\(^{121}\). Severe anemia and hemolysis increase the iron level in the blood and tissues; therefore, pathogens can be actively transported, and iron acquisition is easier\(^{121}\). Based on our results, the increased risk of typhoidal *Salmonella* bacteremia in patients with severe malaria might reflect the high rate of parasite sequestration and vital organ dysfunction. Moreover, bacteremia cannot be excluded from patients with severe malaria; severe malaria is difficult to distinguish from bacterial sepsis\(^{56,85}\). Therefore, the WHO guidelines for malaria recommend that children with severe falciparum malaria in high-transmission areas should receive empirical broad-spectrum antibacterial therapy. However, empirical antibiotics should not be administered to adults with severe malaria unless there is clear evidence of bacterial infection\(^{122}\). In the low-transmission areas, WHO suggests that physicians should determine whether patients should receive antibiotics depending on the patient’s condition or parasitemia levels, but patients with severe malaria should not be routinely treated with antibiotics\(^{122,123}\). In addition to the WHO guidelines, two studies conducted in Myanmar\(^{56,78}\) stated that “clinicians should have a lower threshold for commencing empirical antibacterial therapy in adults diagnosed with falciparum malaria in these locations than is presently recommended.”

The present meta-analysis revealed that typhoidal/NTS and malaria co-infection did not occur by chance or that there was an association
between typhoid/non-typhoid and malaria co-infection in some way. Further studies are required to investigate this association.

The present meta-analysis of case fatality rate of patients with co-infection demonstrated the high rate of mortality (16%) without heterogeneity among the three included studies. These three studies enrolled patients with severe malaria and co-infected with NTS and indicated that both diseases facilitate the higher fatality rate than those of the malaria or NTS infection alone. Moreover, the meta-analysis of two studies showed a higher mean parasitemia level in patients with malaria and co-infected with NTS compared to those with malaria alone (without heterogeneity, 0%), but it is important to note the limitation in the number of included studies in the analysis. Therefore, there is a need to investigate if co-infection of malaria and NTS leads to poor outcome or demonstrated the association of both diseases.

This study had several limitations. First, most included studies were cross-sectional studies that determined the prevalence of typhoidal/non-typhoidal Salmonella spp. and malaria co-infection. Therefore, data were not available to determine the differences between co-infected patients and mono-infected patients. Second, the number of studies evaluating the occurrence of Salmonella spp. bacteremia in patients with severe malaria was limited; therefore, the pooled prevalence of Salmonella spp. bacteremia in patients with severe malaria might not represent all patients with severe malaria. Third, the heterogeneity among the included studies used to determine the probability of typhoidal/non-typhoidal Salmonella spp. and malaria co-infection was high; therefore, the association between typhoidal/non-typhoidal Salmonella spp. and malaria co-infection should be carefully interpreted with the results from the sensitivity test. Compared with the previous systematic review, the present study excluded studies with recent malaria infection; most included studies used microscopy rather than RDTs for malaria detection; and there was no publication bias among the included studies.

Figure 15. Prevalence of typhoidal/NTS infection among patients with malaria using blood cultures for the identification of Salmonella spp. Infection stratified by age groups. ES proportion estimate (multiply 100 units for interpreted as prevalence estimate), CI confidence interval, NS not specified.
In conclusion, whether typhoidal/non-typhoidal *Salmonella* spp. and malaria co-infection occurred by chance or not, healthcare providers must provide support to patients with nonspecific clinical symptoms of malaria or typhoidal/non-typhoidal diseases. In the present study, malaria associated with typhoidal/NTS infection in children and the high case fatality rate among few co-infected patients were highlighted. Future prospective longitudinal studies using the appropriate and confirmatory diagnosis for *Salmonella* spp. infections are highly recommended to ensure the real prevalence of co-infection and highlight the outcome of co-infection for providing adequate treatment of co-infections in febrile patients who live in areas where malaria is endemic like tropical Africa or India.
| Study                              | ES (95% CI)          | Weight (%) |
|-----------------------------------|----------------------|------------|
| Typhoid/NTS                       |                      |            |
| Akinyemi et al., 2007             | 0.15 (0.09, 0.23)    | 1.19       |
| Nyein et al., 2015                | 0.06 (0.02, 0.14)    | 1.60       |
| Subtotal (P^2 = 99.43%, p = 0.00) | 0.10 (0.05, 0.14)    | 2.79       |
| Typhoid                           |                      |            |
| Akinyemi et al., 2015             | 0.44 (0.19, 0.73)    | 0.08       |
| Ahassan et al., 2012              | 0.08 (0.03, 0.18)    | 1.02       |
| Aung et al., 2018                 | 0.05 (0.01, 0.24)    | 0.63       |
| Bhattacharya et al., 2013         | 0.02 (0.01, 0.08)    | 4.07       |
| Edet et al., 2016                 | 0.27 (0.16, 0.42)    | 0.33       |
| Igbenehu et al., 2009             | 0.01 (0.00, 0.03)    | 7.73       |
| Mbuh et al., 2003                 | 0.02 (0.00, 0.09)    | 3.64       |
| Nwuzo et al., 2009                | 0.42 (0.27, 0.59)    | 0.21       |
| Okok et al., 2016                 | 0.01 (0.00, 0.04)    | 7.37       |
| Raja et al., 2016                 | 0.20 (0.06, 0.51)    | 0.10       |
| Samatha et al., 2015              | 0.01 (0.01, 0.03)    | 7.59       |
| Sharma et al., 2016               | 0.23 (0.18, 0.29)    | 1.60       |
| Singh et al., 2014                | 0.01 (0.00, 0.04)    | 7.45       |
| Snehanshu et al., 2014            | 0.14 (0.06, 0.29)    | 0.46       |
| Sur et al., 2006                  | 0.03 (0.01, 0.09)    | 3.19       |
| Vats et al., 2018                 | 0.10 (0.03, 0.25)    | 0.54       |
| Subtotal (P^2 = 86.93%, p = 0.00) | 0.06 (0.03, 0.08)    | 45.97      |
| NTS                               |                      |            |
| Biggs et al., 2014                | 0.02 (0.02, 0.03)    | 8.90       |
| Krunkamp et al., 2016             | 0.01 (0.01, 0.02)    | 9.19       |
| Nielsen et al., 2015              | 0.03 (0.02, 0.04)    | 7.88       |
| Oundo et al., 2002                | 0.01 (0.01, 0.02)    | 9.40       |
| Sandlund et al., 2012             | 0.01 (0.00, 0.01)    | 9.09       |
| Were et al., 2011                 | 0.04 (0.03, 0.06)    | 6.79       |
| Subtotal (P^2 = 87.67%, p = 0.00) | 0.02 (0.01, 0.02)    | 51.25      |
| Heterogeneity between groups: p = 0.000 |                                      |            |
| Overall (P^2 = 86.84%, p = 0.00)  | 0.03 (0.02, 0.03)    | 100.00     |

**Figure 17.** Prevalence of typhoidal/NTS infection among patients with malaria using blood cultures for the identification of *Salmonella* spp. infection stratified by typhoidal/NTS infection. ES proportion estimate (multiply 100 units for interpreted as prevalence estimate), CI confidence interval, NS not specified.
| Study                | ES (95% CI) | Weight (%) |
|---------------------|-------------|------------|
| Africa              |             |            |
| Akinyemi et al., 2007 | 0.15 (0.09, 0.23) | 1.19 |
| Akinyemi et al., 2015 | 0.44 (0.19, 0.73) | 0.06 |
| Alhassan et al., 2012 | 0.08 (0.03, 0.18) | 1.02 |
| Biggs et al., 2014 | 0.02 (0.02, 0.03) | 8.90 |
| Edet et al., 2016 | 0.27 (0.16, 0.42) | 0.53 |
| Igbeneghu et al., 2009 | 0.01 (0.00, 0.03) | 7.73 |
| Krumkamp et al., 2016 | 0.01 (0.01, 0.02) | 9.19 |
| Mbu et al., 2003 | 0.02 (0.00, 0.09) | 3.64 |
| Nielsen et al., 2015 | 0.03 (0.02, 0.04) | 7.88 |
| Nwuzo et al., 2009 | 0.42 (0.27, 0.59) | 0.21 |
| Okoko et al., 2016 | 0.01 (0.00, 0.04) | 7.57 |
| Oundo et al., 2002 | 0.01 (0.01, 0.01) | 9.40 |
| Were et al., 2011 | 0.04 (0.03, 0.06) | 6.79 |
| Subtotal (I^2 = 87.53%, p = 0.00) | 0.02 (0.01, 0.03) | 63.68 |
| Asia                |             |            |
| Aung et al., 2018 | 0.05 (0.01, 0.24) | 0.63 |
| Bhattacharya et al., 2013 | 0.02 (0.01, 0.08) | 4.07 |
| Nyin et al., 2015 | 0.06 (0.02, 0.14) | 1.60 |
| Raja et al., 2016 | 0.20 (0.06, 0.51) | 0.10 |
| Samatha et al., 2015 | 0.01 (0.01, 0.03) | 7.59 |
| Sharma et al., 2016 | 0.23 (0.18, 0.29) | 1.60 |
| Singh et al., 2014 | 0.01 (0.00, 0.04) | 7.45 |
| Snehanshu et al., 2014 | 0.14 (0.06, 0.29) | 0.46 |
| Sur et al., 2006 | 0.03 (0.01, 0.09) | 3.19 |
| Vats et al., 2018 | 0.10 (0.03, 0.25) | 0.54 |
| Subtotal (I^2 = 86.73%, p = 0.00) | 0.08 (0.03, 0.09) | 27.23 |
| Europe              |             |            |
| Sandlund et al., 2012 | 0.01 (0.00, 0.01) | 9.09 |
| Heterogeneity between groups: p = 0.000 | 0.03 (0.02, 0.03) | 100.00 |
| Overall (I^2 = 86.84%, p = 0.00) | 0.03 (0.02, 0.03) | 100.00 |

**Figure 18.** Prevalence of typhoidal/NTS infection among patients with malaria using blood cultures for the identification of *Salmonella* spp. infection stratified by regions. *ES* proportion estimate (multiply 100 units for interpreted as prevalence estimate), *CI* confidence interval, *NS* not specified.
**Figure 19.** Prevalence of *Salmonella* spp. infection among patients with malaria using blood cultures for the identification of *Salmonella* spp. infection stratified by time (publication years). *ES* proportion estimate (multiply 100 units for interpreted as prevalence estimate), *CI* confidence interval, *NS* not specified.
**Figure 20.** Prevalence of NTS infection among patients with malaria using blood cultures for the identification of *Salmonella* spp. infection stratified by time (publication years). *ES* proportion estimate (multiply 100 units for interpreted as prevalence estimate), *CI* confidence interval, *NS* not specified.
### Table 1

| Study                | ES (95% CI)     | % Weight |
|----------------------|-----------------|----------|
| Non-severe malaria   |                 |          |
| Akinyemi et al., 2007| 0.15 (0.09, 0.23)| 0.71     |
| Akinyemi et al., 2015| 0.44 (0.19, 0.73)| 0.03     |
| Atahassen et al., 2012| 0.08 (0.03, 0.18)| 0.61     |
| Aung et al., 2018    | 0.02 (0.01, 0.24)| 0.38     |
| Bhattacharyya et al., 2013| 0.02 (0.01, 0.08)| 2.61  |
| Biggs et al., 2014   | 0.02 (0.02, 0.03)| 6.42     |
| Edet et al., 2016    | 0.27 (0.16, 0.42)| 0.19     |
| Igbinighi et al., 2009| 0.01 (0.00, 0.03)| 5.41     |
| Krumkamp et al., 2016| 0.01 (0.01, 0.02)| 6.69     |
| Mbuh et al., 2003    | 0.02 (0.00, 0.09)| 2.31     |
| Nielsen et al., 2015 | 0.03 (0.02, 0.04)| 5.53     |
| Nwuzo et al., 2009   | 0.42 (0.27, 0.59)| 0.13     |
| Nyen et al., 2015    | 0.06 (0.02, 0.14)| 0.97     |
| Ondu et al., 2002    | 0.01 (0.00, 0.04)| 5.11     |
| Raja et al., 2016    | 0.20 (0.06, 0.51)| 0.06     |
| Samatha et al., 2015 | 0.01 (0.01, 0.03)| 5.29     |
| Sandund et al., 2012 | 0.01 (0.00, 0.01)| 6.59     |
| Sharma et al., 2016  | 0.23 (0.18, 0.29)| 0.97     |
| Singh et al., 2014   | 0.01 (0.00, 0.04)| 5.18     |
| Snehanshu et al., 2014| 0.14 (0.06, 0.29)| 0.27     |
| Sur et al., 2006     | 0.03 (0.01, 0.09)| 2.00     |
| Vats et al., 2018    | 0.10 (0.03, 0.28)| 0.52     |
| Were et al., 2011    | 0.04 (0.03, 0.06)| 4.64     |
| Subtotal (I² = 86.84%, p = 0.00) | 0.03 (0.02, 0.03)| 69.30 |

| Severe malaria       |                 |          |
| Bassat et al, 2009  | 0.01 (0.00, 0.01)| 6.64     |
| Berkley et al., 1999 | 0.01 (0.00, 0.02)| 6.47     |
| Bronzan et al, 2007 | 0.03 (0.02, 0.04)| 6.09     |
| Evans et al., 2004  | 0.43 (0.26, 0.63)| 0.09     |
| Matha et al., 2014  | 0.06 (0.03, 0.06)| 4.76     |
| Phu et al., 2020    | 0.00 (0.00, 0.01)| 6.65     |
| Subtotal (I² = 91.49%, p = 0.00) | 0.02 (0.01, 0.03)| 30.70 |

### Figure 21

Pooled prevalence of typhoidal/NTS infection among patients with severe and non-severe malaria.

ES proportion estimate (multiply 100 units for interpreted as prevalence estimate), CI confidence interval.
Figure 22. Pooled prevalence of malaria infection among patients with typhoidal Salmonella spp. ES proportion estimate (multiply 100 units for interpreted as prevalence estimate), CI confidence interval.

| Study               | ES (95% CI) | Weight |
|---------------------|-------------|--------|
| Falay et al., 2016  | 0.31 (0.14, 0.56) | 20.31  |
| Mabey et al., 1987  | 0.11 (0.05, 0.23) | 57.59  |
| Mtove et al., 2010  | 0.21 (0.08, 0.48) | 22.10  |
| Overall (I^2 = 33.31%, p = 0.22) | 0.17 (0.06, 0.29) | 100.00 |

Figure 23. Pooled prevalence of malaria infection among patients with NTS. ES proportion estimate (multiply 100 units for interpreted as prevalence estimate), CI confidence interval.

| Study               | ES (95% CI) | Weight |
|---------------------|-------------|--------|
| Brent et al., 2006  | 0.33 (0.26, 0.40) | 17.64  |
| Falay et al., 2016  | 0.54 (0.45, 0.63) | 16.65  |
| Graham et al., 2000 | 0.37 (0.31, 0.44) | 17.91  |
| Mabey et al., 1987  | 0.42 (0.31, 0.54) | 15.67  |
| Mtove et al., 2010  | 0.69 (0.54, 0.80) | 14.64  |
| Walsh et al., 2000  | 0.25 (0.18, 0.33) | 17.49  |
| Overall (I^2 = 89.05%, p = 0.00) | 0.43 (0.32, 0.53) | 100.00 |
### Data availability

All data related to the present study in this manuscript are available.

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Author contributions

M.K., P.W. and W.M. carried out the study design, study selection, data extraction, and statistical analysis; and drafted the manuscript. W.K.K., K.U.K. and P.R. participated in approving the manuscript. All authors read and approved the final manuscript.
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Competing interests
The authors declare no competing interests.

Additional information
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