Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 July 2021 doi:10.20944/preprints202107.0515.v1

DIVERSITY IN TYPHOID DIAGNOSTIC PROTOCOLS AND RECOMMENDATION FOR COMPOSITE REFERENCE STANDARD

Bernard Egwu Igiri [a,*], Stanley Irobekhian Reuben Okoduwa [a,b,#], Shaibu Ahmed Munirat [c], Iquo Bassey Otu-Bassey[d], Abdullahi Bashir,[c], Otori Mercy Onyiyioza[c]

[a] Directorate of Research and Development, Nigerian Institute of Leather and Science Technology, Zaria, Nigeria
[b] Department of Biochemistry, Babcock University, Ilishan-Remo, Nigeria
[c] Department of Microbiology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria
[d]Department of Medical Laboratory Sciences, University of Calabar, Calabar

***Correspondence**: B.E. Igiri, Medical Microbiologist/Parasitologist, egwubernard2@gmail.com; Tel.: +234-706-859-7797
 # Co-Correspondence: Dr. S.I.R. Okoduwa, Medical Biochemist/Toxicologist, Department of Biochemistry, School of Basic Medical Sciences, Babcock University, Ilishan-Remo, Nigeria. okoduwas@babcock.edu.ng, siroplc@gmail.com, Tel: +234-803-295-5882

ABSTRACT

Typhoid fever is a major public health burden which causes substantial global morbidity and mortality due to lack of decisive diagnostic protocols. The capacity of commonly use diagnostic test to validate the absence of typhoid fever is controversial. This study explores to evaluate new techniques for typhoid diagnosis and proposed a harmonised suitable standardized composite reference to be adopted. Published peer-reviewed articles indexed in PubMed, MEDLINE and Google scholar were reviewed for hospital-based studies. This study reveals new typhoid diagnostic techniques such as proteomics, serology, Rapid Diagnostic tests (RDTs), transcriptomics, genomics, and metabolomics. 34.4% of the studies use prospective study design. The study result establishes that, Widal test has a moderate diagnostic accuracy with average percentage sensitivity (52.9%), specificity (54%), positive predictive value (PPV) (56.8%) as well as negative predictive value (NPV) (55.6%) when compared with 29.4%, 28%, 29.5%, and 27.8% of Typhidot respectively. The findings showed a statistically significant difference on the

© 2021 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 July 2021 doi:10.20944/preprints202107.0515.v1

sensitivity between Widal and Typhidot t (40) = 2.639, p = 0.012 at p<0.05 using independent sample *t*-test. When there is no perfect reference standard that has an optimal diagnostic accuracy, the need for a harmonised suitable standardized composite reference is essential. Hence, this study recommends that, peripheral blood culture with established sensitivity of 60% and Widal test with average sensitivity of 52.9% be adopted as a consensus composite reference standard for typhoid fever diagnosis in other to improve confidence in prevalence estimates.

Keywords: Typhoid fever; Diagnostic; Metabolomics; Composite reference standard; Accuracy; Sensitivity.

1. INTRODUCTION

Enteric fever (EF), commonly called typhoid fever (TF) is a systemic infectious disease caused by *Salmonella* Typhi and *Salmonella* Paratyphi, which if left untreated could cause substantial ill health and death. The disease causes serious public health challenge, with approximately 12,000,000 to 27,000,000 cases annually (Kumar *et al.,* 2005; Alba *et al.,* 2016). The estimated death varies between 129,000 to 223,000 annually (WHO 2015; Stanaway *et al.,* 2019). Typhoid fever causes overwhelming affliction in low and middle income countries, with a world-wide projection of 11.9 million cases and 120 000–220 000 mortality yearly (Buckle *et al*., 2012; Mogasale *et al.,* 2014). The prevalence of TF has been reported to be high in preschool children and infants (Crump *et al.,* 2004; Crump *et al.,* 2008; Mogasale *et al.,* 2014; Britto *et al.,* 2017). In Africa, it has been observed that the burden of the disease is significantly high (Mogasale *et al.,* 2014; Antillón *et al*., 2017).

Lack of suitable diagnostic test as well as effective antibiotic treatment could result in TF death (Dutta *et al.,* 2014). In perspective to the absence of decisive diagnostic protocols, the disease

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 July 2021 doi:10.20944/preprints202107.0515.v1

could lead to fatal complications such as intestinal perforations (Haques *et al.,* 1999). Research findings for the best enteric fever diagnostic techniques are being carried out by many research groups globally, with elusive results (Baker *et al* 2010). The diagnostic accuracy of typhoid fever is a major challenge. The gap in TF diagnosis has led to under-diagnosis, imprecise disease burden estimates, inaccurate, and over-diagnosis that could result in inappropriate and excessive intake of antibiotics (Wlekidan *et al.,* 2015; Igiri *et al.,* 2018). Various diagnostic techniques have been employed, including blood and bone marrow culture that were recommended as reference standard diagnostic tests (Vallenas *et al*., 1985; WHO 2003). The most sensitive and specific test for enteric fever is bone marrow culture, though, invasive with medical complications, requiring technical expertise and specialized equipment (Mogasale *et al.,* 2016).. Blood culture is the most frequently used techniques among culture based methods with limited resources for proper culturing in many setting (Storey *et al.,* 2015). Blood and bone marrow culture sensitivity has been quantified at 40-80% and >80% respectively (Storey *et al.,* 2015; Mogasale *et al.,* 2016).

Rapid and accurate enteric fever diagnosis is significant and could reduce mortality, ill health and control the spread of the ailment (Zaki and Karande 2011; Ugochukwu *et al*.,2013; Goay *et al.,* 2016; Sandro 2017). Widal test is widely used due to its readily availability and affordability, but lack sensitivity and specificity and is not very reliable (Parry *et al* 2011; Andrews and Ryan 2015; Maude *et al* 2015; Darton *et al* 2017). However, this cheap and quick Widal test is the reason for the extensive report on the incidence of typhoid fever in different areas of Nigeria (Igiri *et al.,* 2018). Novel diagnostic techniques for enteric fever are in progress that could mitigate this deficit. New point-of-care technologies such as rapid magneto-DNA nanoparticle assay, proteomics, genomics, Loop Mediated Isothermal Amplification, Typhidot, Polymerase Chain Reaction (PCR), transcriptomic, and metabolomic has been establish (Kantele *et al*., 2013; Charles *et al.,* 2014; Darton *et al.,* 2015; Park *et al*., 2016; Blohmke *et al.,* 2016; Darton *et al.,* 2017; Sharma *et*

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 July 2021 doi:10.20944/preprints202107.0515.v1

al., 2018). Hemagglutination and enzyme linked immunosorbent assay (ELISA) are serological tests used for *Salmonella enterica* serova Typhi H and O antigens with poor sensitivity and specificity (Lateef *et al.,* 2000).

. The use of gold standard test with imperfect diagnostic accuracy could bring about newer and better technique (Whiting *et al.,* 2013). The development of a composite reference standard (CRS) is alternative technique to improve diagnostic accuracy in the absence of a perfect reference test (Alonzo and Pepe 1999). Composite reference standard is the combination of more than one imperfect technique aimed at increasing diagnostic accuracy. This study discussed current biomarkers of enteric fever diagnosis and presented the diagnostic performance of typhoid fever test. However, based on the results of these findings, the study proposed a harmonised CRS for adoption as suitable standardized composite reference which would help to improved confidence in the prevalence estimates of typhoid fever infections.

2. NEW BIOMARKERS OF ENTERIC FEVER DIAGNOSIS

2.1 Proteomics

Serological markers that are available for enteric fever test are characterized with low sensitivity and specificity. In Nigeria, studies on children with acute enteric fever based on proteome microarray tested IgA, IgM and IgG ELISAs using S. Typhi LPS and hemolysin E (HlyE) proteins (Huw *et al.,* 2016; Felgner *et al.,* 2017). This study recognized putative sero-diagnostic biomarkers such as invasion (SipC), proteins targeted toward host cell attack (HlyE) and bacterial cell surface (OmpA) (Darton *et al*., 2017). Other studies of S. Typhi proteome arrays have shown HlyE as a suitable sero-diagnostic biomarker based on IgG and IgA responses (Liang *et al.,* 2013; Charles *et al.,* 2014; Huw Davies *et al.,* 2016; Darton *et al*., 2017). OmpA is also seen as a valuable biomarker from proteomic screening (Charles *et al.,* 2014). Salmonella Typhi proteome array screening has

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 July 2021 doi:10.20944/preprints202107.0515.v1

identified N-acetylmuramoyl-L-alanine amidase (t2002, STY0927) as a new putative biomarkers which is use in the catabolism of peptidoglycans.

During vaccination and cholera infection, antibody in Lymphocyte Supernatant assay (ALS) is used (Chang and Sack 2001), tuberculosis (Raqib *et al.,* 2003) and influenza (Hallilley *et al.,* 2010). This has been used to detect S. Typhi antibodies in infected cohorts (Sheikh *et al*., 2009). Antibody in Lymphocyte Supernatant assay is applied to translate new sero-diagnostic markers through immune-profiling to detect immune-dominant antigens (Charles *et al.,* 2014; Darton *et al.,* 2017). Putative protein targets that should be considered for future diagnostic development in RDTs are IgA, S. Typhi LPS and IgG for better diagnostic results in resource-limited settings (Darton *et al.,* 2017).

2.2 Metabolomics

Metabolomics is a new technique of scientific research, which was developed to identify and measure minute quantities of small chemicals in complex biological material using cutting-edge mass spectrometry (Zurfluh *et al.,* 2018). Progress has been made to validate the diagnosis of enteric fever by using diagnostic metabolites in plasma from patients (Nasstrom *et al.,* 2014). This technique is useful to several infectious diseases, detecting signatures of urinary tract infections, inflammatory disease and viral infections (Capati *et al*., 2017; French *et al.,* 2018; Shrinet *et al* 2016). Furthermore, it is could predict disease outcomes, progression, and even onset, as was recorded in tuberculosis (Weiner *et al.,* 2018). This technique was used to produce metabolites signatures on plasma samples by 2-dimensional gas chromatography with time of-flight mass spectrometry from 50 typhoid fever patients (25 with S. Typhi and 25 with S. Paratyphi A) against 25 febrile controls (Nasstrom *et al*., 2014). It has been identified and validated that significant and reproducible metabolomics signatures could separate typhoid and other febrile illness with asymptomatic controls in a Nepali patient cohort (Nasstrom *et al.,* 2014; Nasstrom *et al.,* 2017)

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 July 2021 doi:10.20944/preprints202107.0515.v1

This diagnosis compares the metabolites profiles of healthy and infected persons with typhoid and paratyphoid. Threshold is determined to single out those with acute typhoid fever (McKinnon and Abdool 2014)

2.3 Transcriptomics

Transcriptomics require the measurement of gene expression in a given cellular compartment at a specific time. The transcript mRNA is measured and quantity by calculating the amount of mRNA transcribed from genomic DNA using microarrays and RNAseq high-throughput molecular technology. Profiles can be produced from a small quantity of biological sample, such as whole blood (Nakaya *et al.,* 2016). Therefore, transcriptomic has been used to generate detailed insights into the human transcriptional response to several diseases (Berry *et al.,* 2010; Mejias *et al.,* 2013; Subramaniam *et al.,* 2015; Hoang *et al.,* 2010; Barton *et al.,* 2017; Blohmke *et al.,* 2016) and other alternative immune perturbations (Vahey *et al.,* 2010; Nakaya *et al.,* 2011; Blohmke *et al.,* 2017; Li *et al.,* 2017). The human challenge model has been the source of various gene expression datasets, which were generated in a highly controlled setting, allowing the interrogation of the human transcriptional responses to S. Typhi and S. Paratyphi A (Barton *et al.,* 2017; Blohmke *et al.,* 2016; Blohmke *et al.,* 2019). This techniques use the response of the host as a possible biomarkers that are specific to identify typhoid fever patients. It confirms that gene expression profiles are specific enough to possibly differentiate diseases with similar clinical presentation (Gliddon *et al.,* 2018). This is a powerful tool to detect new diagnostic biomarkers for diseases and could represent the future of molecular diagnostics for typhoid fever.

2.4 Genomics

This technique has allowed for *S.* Typhi genome sequencing and assisted in the recognition of specific gene targets. DNA markers that are specific for *S.* Typhi have been identified using the

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 July 2021 doi:10.20944/preprints202107.0515.v1

genome database and nucleic acid sequence alignment tools (BLASTn). The primer sensitivities and specificities designed for amplifying specific gene sequences were authenticated using *S.* Typhi, non-Typhi *Salmonella* and non-*salmonella* clinical isolates (Goay *et al*., 2016).

2.5 Other Typhoid Diagnostic Techniques

Several other techniques aside the new approaches such as molecular detection of bacteria genes using PCR has being the focus of research towards developing an improved typhoid test. PCR technique is a non-culture based techniqus designed to identify the flagellin genes of *S*. Typhi, *S.* Paratyphi A and other specific targets (Zhou *et al.,* 2016; Levy *et al.,* 2008). A PCR-based technique has the capacity to identify bacterial DNA and do not recover living cell. Nevertheless, any pathogen-directed test for S. Typhi/S. Paratyphi A is limited by the low concentration of bacteria in the blood as well the inherent inhibitors in the sample (Nga *et al.,* 2010). Laboratory methods to lyse red blood cells have shown increasing yield (Boyd *et al.,* 2015), and extracting DNA from samples could generate increase yield, thus improving sensitivity and specificity (Zhou and Pollard 2012).

Loop-mediated isothermal amplification (LAMP) is a method for the amplification of nucleic acid at a constant temperature, targeting specific *S.* Typhi gene (Fan *et al.,* 2015; Frickmann *et al.,* 2019).

An attractive approach for typhoid fever diagnostics is the detection of an antibody response signifying recent infection using serum and plasma. The most promising targets identified through serological response are (IgG) and (IgA) to hemolysin E and S. Typhi–specific lipopolysaccharide (LPS) (Davies *et al*., 2016), IgM and/or IgG to hemolysin E (Liang *et al.,* 2013), and IgA to *S.* Typhi–specific LPS (Darton *et al.,* 2017).

Widal test (WT) measures agglutinating antibodies against lipopolysaccharide (LPS) (O) and flagellar (H) antigens of *S.* Typhi in the sera of typhoid fever patient (WHO 2019). This technique

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 July 2021 doi:10.20944/preprints202107.0515.v1

is unreliable with low sensitivity and specificity (Maude *et al.,* 2015). Rapid diagnostic tests (RDTs) could detect antigens or antibodies with Typhidot-M® and TUBEX$^{T\ M}$ as the current commercial RDTs for typhoid fever (Thriemer *et al.,* 2013; Baker *et al.,* 2010). *Salmonella* Typhi antibodies specific to LPS antigens are detected by Enterocheck WB techniques in a lateral flow format similar to ICT. Negative result is indicative of the absence of pink to pink-purple coloured band in the test area (Mogasale *et al*., 2016; Darton *et al.,* 2017). Blood culture technique is considered the reference standard for enteric fever diagnosis (Paolucci *et al*., 2010; Martiny *et al.,* 2013). The sensitivity of culture specimens differs considerably depending on the type and volume of fluid analysed, prior antimicrobial use, age of the infected person and period of ailment. Bone marrow culture is obtained through aspirate of the iliac crest or sternum with a sensitivity of approximately 90% when the culture is over 4 days (Crump *et al.,* 2015; Gilman *et al.,* 1975; Darton *et al.,* 2017).

3. RESEARCH METHODOLOGY

3.1 Research design

The analysis of the research design was a quantitative method with a survey approach which involves five respective steps: identification of studies; selection of articles; quality assessment of studies; extraction of data; and data analysis.

3.2 Search strategy for identification of relevant articles

A search strategy was designed to identify all articles in English that assessed enteric fever diagnostic protocols among humans. Published peer-reviewed articles of diagnostic protocol for enteric fever were identified from Google scholar, PubMed, and MEDLINE databases. The search words were enteric fever, novel typhoid diagnostic, cultural diagnostic test, diagnostic protocol of typhoid, rapid diagnostic test and widal test for trials in progress.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 July 2021 doi:10.20944/preprints202107.0515.v1

3.3 Search outcome

In all, three hundred and ninety two (392) articles were identified and screened (Figure 1). 355 articles were excluded after reviewing the articles based on the following: duplicates 94, misleading titles and abstract 102, articles without Widal and rapid diagnostic test 91, articles that do not meet quality criteria 68.

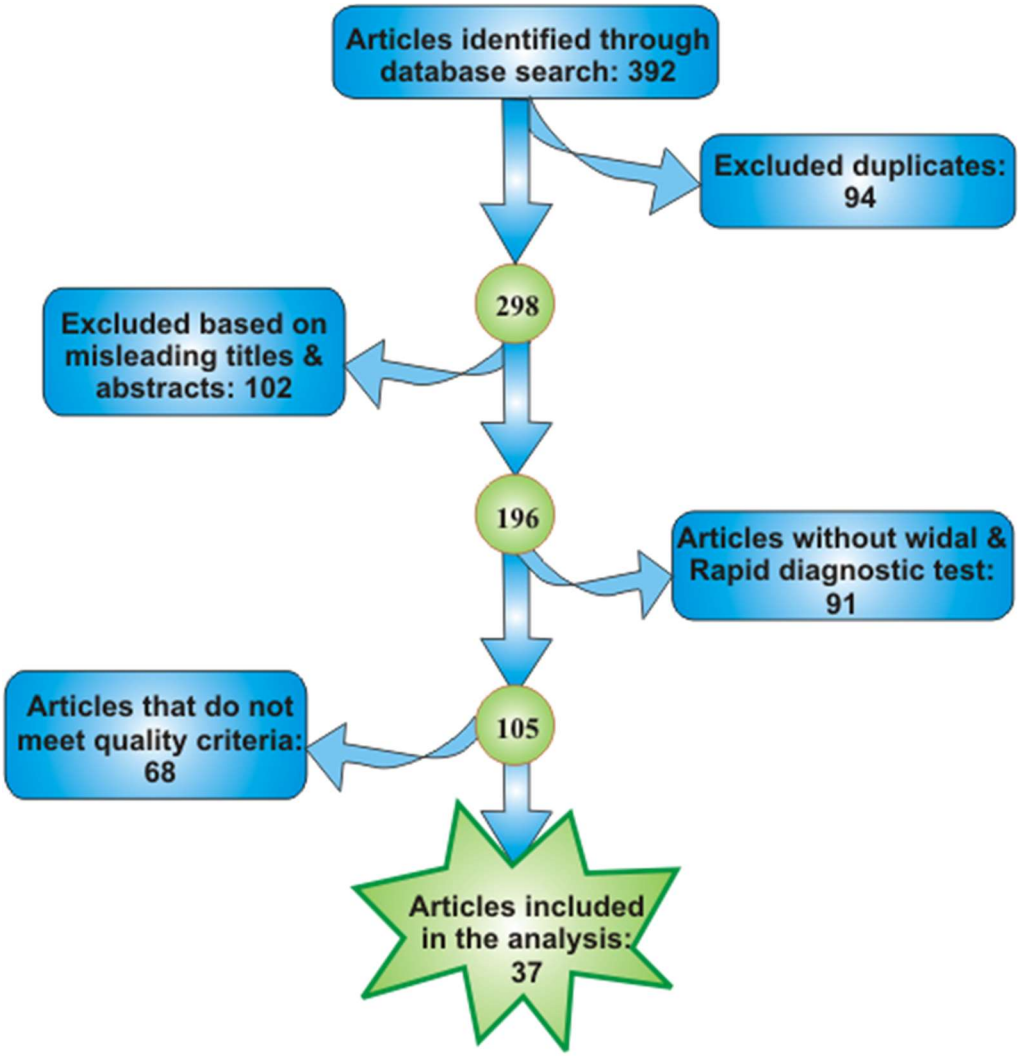

Figure 1: Flowchart of published peer-reviewed journals detected through databases and included in the analysis.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 July 2021 doi:10.20944/preprints202107.0515.v1

3.4 Inclusion and exclusion criteria

Studies in English language were included without restrictions on country, age, demographics, or time. Cross-sectional studies, paired comparative study, prospective cohort studies, and retrospective studies were included. Studies that do not meet quality criteria were excluded.

3.5 Ethics statement

All studies included were those approved by an institutional ethics review committee in addition obtained informed consent from all their participants. Ethics approval was not requisite for the present study as it is based on secondary data generated from the primary source.

3.6 Statistical analysis

The data obtained from the literature were entered into Microsoft Excel and Statistical Package for the Social Science (SPSS), version 25.0 for windows (SPSS Inc., Chicago, Illinois, USA) and were used for descriptive analysis of the data. The results are presented as percentages. Independent sample *t*-test was used to explore differences in the mean percentage sensitivity of diagnostic tests. The level of statistical significance was set at $P < 0.05$.

4. RESULTS

Results of electronic search

The results of the synthesis from the studies collected by the literature review are presented in the Table 1. Of the 392 searched articles, 37(9.4%) articles met the inclusion criteria with 11,525 participants (Table 1). Exactly 27 studies use widal test, 5 use TUBEX, 15 use Typhidot (Typhidot, Typhidot-M, and TyphiRapid-Tr02), 1 use Diazo test, 2 use Enterocheck WB®, 2 use Multiplex PCR, 1 use ONSITE Typhoid Combo, 1 Multi-test dipstick, and 2 Panbio ELISA. Studies that used the gold standard for comparison were 31. All of the diagnostic tests were developed to

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 July 2021 doi:10.20944/preprints202107.0515.v1

identify *S.* Typhi infection and Paratyphi. 34.4% of the studies in this review used prospective study design.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 July 2021 doi:10.20944/preprints202107.0515.v1

Table 1: Diversity of Typhoid fever diagnostic protocols and accuracy values

Diagnostic tests	Study design	Sample size (11,525)	Gold Standard	Country	Prev (%)	Sen (%)	Spec (%)	PPV (%)	NPV (%)	Citations
Widal	Cross-sectional	372	Stool culture	Ethiopia	56.2	80	44.5	3.8	98.8	Deksisa & Gebremedhin 2019
Widal	Cross-sectional	95	Stool culture	Ethiopia	68.4	84.2	35.5	24.6	90	Ameya *et al.,* 2017
Widal	Randomized design	125	Stool Culture	Nigeria	17.6	78.3	93.6	68.2	98.1	Minjibir *et al* 2020
Widal	Cross sectional	158	Stool Culture	Tanzania	81	81.5	18.3	10.1	89.7	Mawazo *et al* 2019
Widal	Prospective study	810	Blood and stool culture	Nigeria	NR	49.1	90.7	46.2	91.6	Ohanu *et al.* 2019

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 July 2021 doi:10.20944/preprints202107.0515.v1

Widal	Cross-sectional	91	Stool culture	Nigeria	62.5	71.4	66.7	83.3	50	Ramyi *et al.*, 2013
Widal	NR	71	PCR	India	NR	50	50	92.8	39.5	Ambati *et al.,* 2007
Widal	Comparative study	112	Stool culture	Cameroon	57.1	40.9	32.4	6.44	28.1	Wam *et al.,* 2019
Widal	Cross-sectional	158	Stool and blood culture	NR	81	81.5	18.3	10.1	89.7	Mawazo *et al.,* 2019
Widal	Retrospective study	100	Blood Culture	India	NR	71.4	47.3	9.25	95.7	Lalremruata *et al.,* 2014
Widal	Prospective study	270	Blood culture	Ethiopia	32.6	71.4	68.4	5.7	98.9	Andualem *et al.,* 2014
Widal	Prospective study	163	Blood culture	India	54	65.4	89.8	NR	NR	Maheshwari *et al.*, 2016
Widal	Cross-sectional	271	Blood culture	Nigeria	45.76	35	51	17	73	Enabulele &Awunor 2016

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 July 2021 doi:10.20944/preprints202107.0515.v1

TUBEX	Comparative study	139	Blood culture	Tanzanian	NR	79	89	NR	NR	Ley *et al.,* 2011
TUBEX	Retrospective study	970	Blood culture	Pakistan	1.86	41.9	96	31.6	97.3	Khan *et al.*, 2017
Typhidot	Retrospective study	42	Blood culture	NR	NR	92.7	NR	97.4	NR	Garg *et al*., 2018
Typhidot	Cross-sectional	211	Blood culture	NR	95.9	95.9	26.5	30.3	96	Hussain *et al*., 2018
Typhidot	Prospective study	500	NR	Cambodia	63.6	63.6	82.9	26.4	95.9	Ameya *et al.,* 2017
Typhidot	Retrospective study	145	Blood Culture	NR	32.4	26.7	61.5	7.4	87.9	Mehmood *et al.,* 2015
Multiplex PCR	Prospective study	680	Blood culture	Bangladesh	20	95	92.9	NR	NR	Ambati *et al*., 2007
Enterocheck	Prospective study	145	Blood Culture	NR	NR	85.5	88.6	51.1	97.7	Baker *et al.*, 2010

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 July 2021 doi:10.20944/preprints202107.0515.v1

Widal	Prospective	80	Blood		62.5	71	62	91	31	
Widal	study		culture	India	NR	71	62	91	31	Aziz & Haque 2012
PCR	Comparative		Blood			100	86.5			
Widal	study	76	culture	NR	NR	88.6	62.5	NR	NR	El-Sayed *et al.,* 2015
Widal	Comparative		Blood		45.7	34.1	42.8			
Typhidot	study	105	culture	India	74.3	92.6	37.5	NR	NR	Narayanappa *et al.,* 2010
Typhidot			Blood		97.36	96	89.5	95		
Widal	NR	124	culture	NR	73.68	72	87	87	NR	Khoharo 2011
Widal	Comparative		Blood		35	86.7	89.5	79.5	93.4	
Typhidot	study	140	culture	Egypt	36.5	93.3	90.6	82.3	96.6	Salama & Said 2019
Widal	Comparative	100	NR	India	48.1	NR	NR	94	NR	
Widal	study				26			52		Patel & Trivedi 2017
Widal	Comparative		Blood		57	57	83			
Typhidot	study	-	culture	New Delhi	79	79	87.5	NR	NR	Sherwal *et al.,* 2004

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 July 2021 doi:10.20944/preprints202107.0515.v1

ONSITE Typhoid Combo	NR	136	NR			100	94.4	63.2	100	
TUBEX				Zimbabwe	NR	100	94.1	63.2	100	Tarupiwa *et al*., 2015
Typhidot			Blood and bone marrow		70	70	77	89		
Widal	NR	97		Pakistan	54	55	81	89	NR	Ahmed & Mansurali 1999
Widal	Prospective		Blood			68	86	94	57	
Typhidot	study	100	culture	India	NR	85	100	100	75	Goay *et al*., 2016
Typhidot	Comparative		Blood			97.3	88.1	97.4	87.8	
Enteroscreen	study	2699	culture	India	NR	98.2	92	96.2	82.3	Prasad *et al*., 2015
Widal	Comparative		Blood			84.1	52.7	25.3	94.5	
ELISA	study	1371	culture	Nepal	NR	95.5	94.7	77.7	99.1	Adhikari *et al.,* 2015
Typhidot M						90	100	100	92.1	
Diazo test	Prospective study	145	Blood culture	India	NR	86.7	85.7	83.9	88.2	Beig *et al*., 2010
Widal						40	91.4	80	NR	

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 July 2021 doi:10.20944/preprints202107.0515.v1

Panbio ELISA	Prospective study	144	NR	NR	84	78	80	68.4	87.4	
Widal						98	76.6	69	98.6	
Typhidot						98	76.6	98.6	69	Anagha *et al.,* 2012
TUBEX	NR	500	Blood culture	New Guinea	15	51.1	88.3	0.31	0.95	Siba *et al.*, 2012
Typhidot					23	70	80.1	0.26	0.96	
TyphiDot Rapid (TR-02)					22	89.4	85	0.38	0.99	
Widal					8	51.1	95.8	0.56	0.95	
Multi-test Dipstick	Prospective study	80	NR	Vietnam	NR	89	50	85	60	
Typhidot						79	89	96	59	
TUBEX						78	94	98	59	
Widal						64	76	88	48	Olsen *et al.,* 2004

2

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 July 2021 doi:10.20944/preprints202107.0515.v1

Sensitivity, Specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV)

The percentage sensitivity, specificity, PPV, and NPV of the data obtained are presented in Table 1. The average sensitivity of 52.9% (figure 2), average specificity of 54% (figure 3), average PPV of 56.8% (figure 4), and average NPV of 55.6% (figure 5) was determine in Widal test while 29.4%, 28%, 29.5%, and 27.8% was determine in Typhidot respectively. There was a statistical evidence to demonstrate that there was a significant different between Widal test and Typhidot. The diagnostic accuracy of Widal was moderate compared to others.

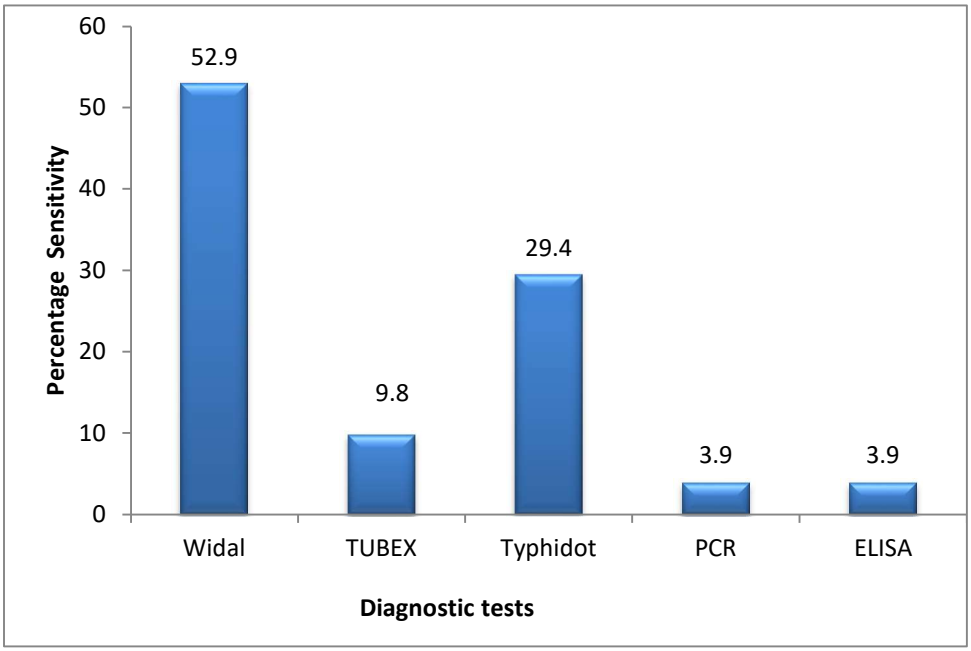

Figure 2: The mean percentage sensitivity of typhoid fever diagnostic techniques

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 July 2021 doi:10.20944/preprints202107.0515.v1

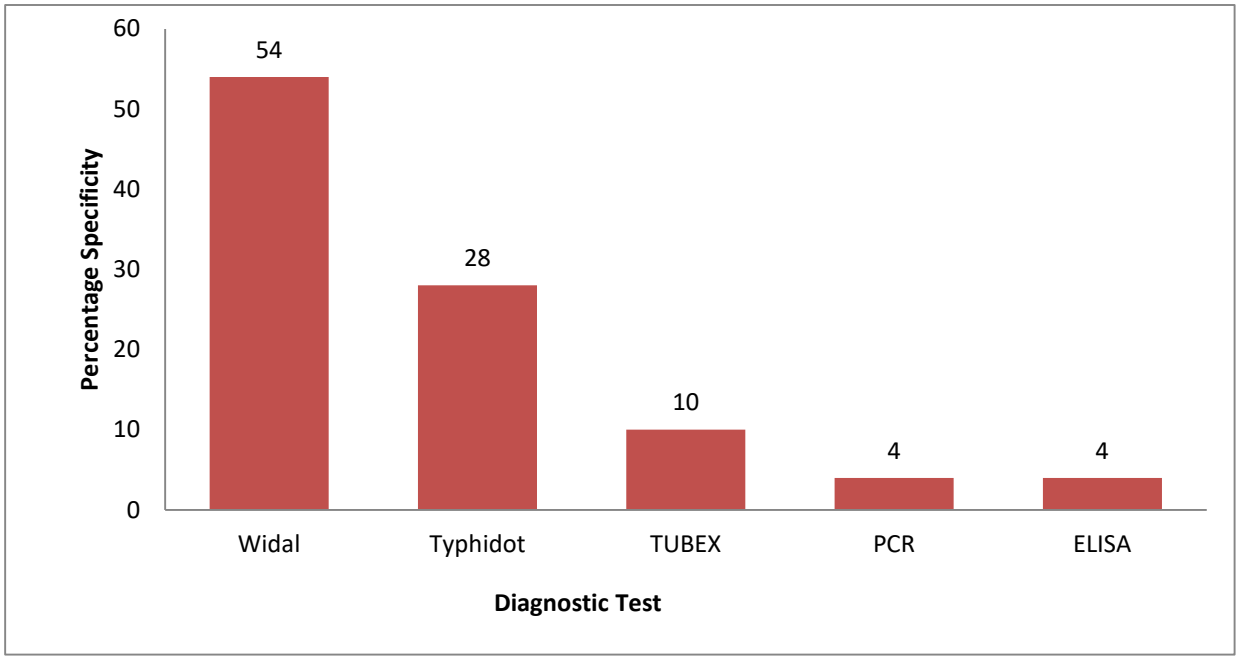

Figure 3: Mean percentage specificity of typhoid fever diagnostic techniques

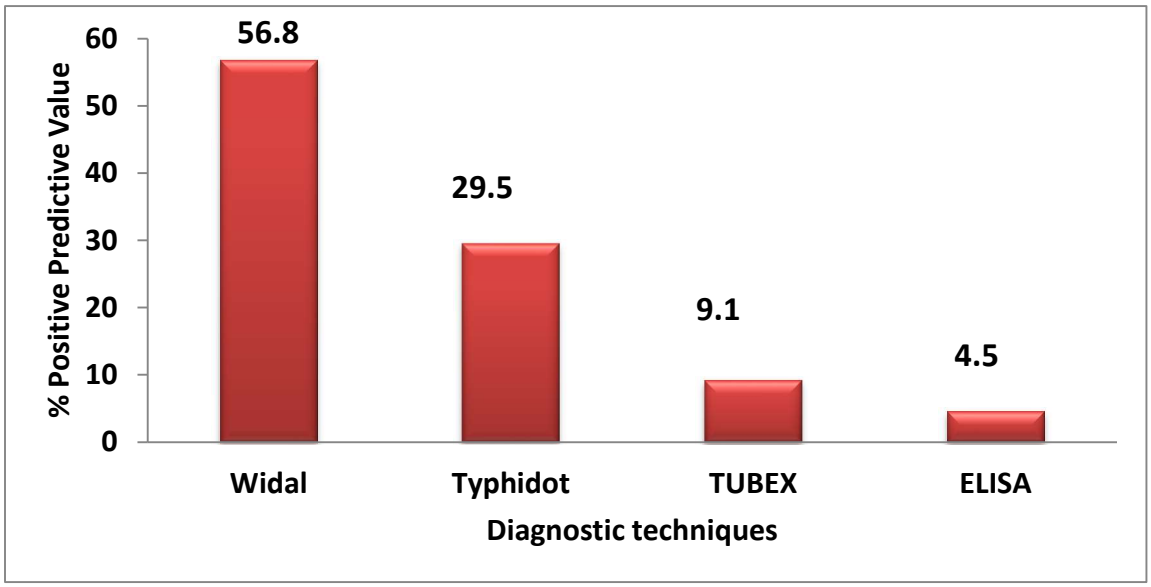

Figure 4: Mean percentage positive predictive value of typhoid fever diagnostic technique

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 July 2021 doi:10.20944/preprints202107.0515.v1

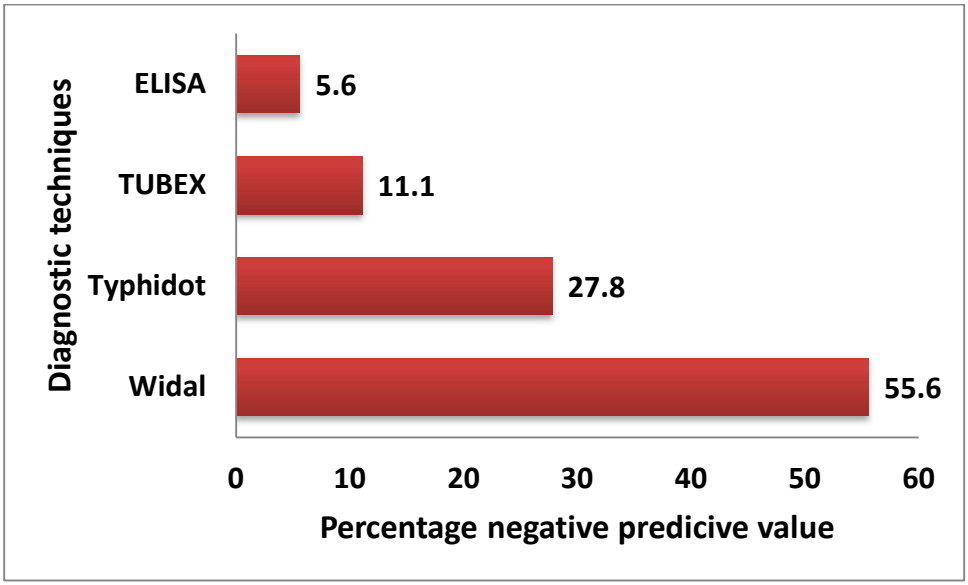

Figure 5: Mean percentage negative predictive value of typhoid fever diagnostic techniques

5. Discussion

This study reveals a moderate average diagnostic accuracy of Widal test in the diagnosis of TF. A clinically diagnosis requires high values for both sensitivity and specificity. TF diagnosis actually begins with the evaluation of clinical signs and symptoms. Different diagnostic tools are used for enteric fever diagnosis and include cultures from blood, bone marrow, urine and stool. Recently, diagnostic tools such as proteomics, transcriptomics, metabolomics and genomics have been exploited to detect biomarkers unique to *S.* Typhi (Sharma *et al.,* 2018). The sensitivity of any diagnostic test is the percentage of patients that are positive and are appropriately identified with the ailment. However, specificity is the percentage of patients that are properly diagnosed as negative. The probability that those confirmed positive by the techniques are actually having the disease is positive predictive value (PPV) while the probability that those confirm negative by the techniques are actually not infected is negative predictive value (NPV).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 July 2021 doi:10.20944/preprints202107.0515.v1

Several gold standard techniques have been used to assess typhoid diagnostics, with blood culture being the most used techniques. The lack of perfect gold standards is a major challenge with assessing the accuracy of typhoid diagnostics. Blood cultures, which has a near perfect specificity, is only 50–65% sensitive. Storey *et al*. (2015) reveal that no single test has adequately good performance but recommended that some existing techniques could be useful as composite reference standard.

The study reveals that the combinations of existing sensitive and specific techniques could surmount the limitations of accuracy that is intrinsic in single test. The application of composite reference standard could enhance better estimates for test performance based on combinations of tests. The purpose of this study is to evaluate typhoid diagnostic accuracy and the urgent need for the best components of a typhoid CRS in other to establish a standardized composite reference as novel typhoid protocol. This will enable careful comparison of diagnostic accuracy data across studies, which is often difficult because of varying study designs and reference standards. To authenticate the diagnostic accuracy of a new test, results from several studies is required. Furthermore, the use of CRS will enhance confidence in prevalence estimates, which could help guide typhoid vaccination efforts. A new CRS for typhoid may still be imperfect compared to diagnostic truth, but there is much to gain from the approval of a standardized composite reference. There is an urgent need for better diagnostic tests to detect enteric fever in order to improve disease burden estimates and potentially accelerate the adoption of new typhoid vaccines (Crump and Mintz 2010; DeRoeck *et al.,* 2005). In order to achieve this, standardization, and broad approval of a single reference standard based on a composite reference are required.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 July 2021 doi:10.20944/preprints202107.0515.v1

6. Challenges and future outlook

Identifying real typhoid patients with negative blood culture is a significant constrain in enteric fever diagnosis (Moore *et al.,* 2014). Indistinguishable clinical symptoms and the absence of a reliable gold standard test complicate typhoid diagnosis. About 20mL to 30mL of blood is needed for the detection of blood related infections and this poses a challenge for elderly and neonatal patients (Mancini *et al.,* 2010). Additionally, lack of funding support, research capacity, and lack of institutional infrastructure are challenges facing TF diagnosis and other infectious diseases, especially in Nigeria. Absence of perfect test leads to unwise antibiotic use, resulting to complications, morbidity as well as drug resistance.

A diagnostic technique requiring small blood volumes with quick and correct detection is thus required. Furthermore, the quality of a reliable future evaluation of enteric fever diagnosis can be improved by the use of composite reference standard CRS, which will enhance assurance in prevalence estimates and avoid inappropriate intake of antibiotics by patients that do not need it. Future research should employ a prospective cohort design with CRS as a diagnostic tool. From our outlook, metabolite profiles are possible future solution for diagnosing typhoid fever, but there are many challenges in making these indicative chemical signatures routinely accessible. The use of CRS as upgraded reference standard for typhoid fever diagnosis will improve the actual worldwide burden of typhoid widespread.

7. Limitations and strengths

Limitations in this study arise from the variety of diagnostic technique used by several investigators, as well as the way those tests were conducted. As a result, many articles could not be included in the analysis. Also, this analysis was based on published literature only. Regardless of these limitations, the strength of this study is the thorough review of published peer review

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 July 2021 doi:10.20944/preprints202107.0515.v1

articles assessing the current trend and performance of various diagnostic protocols for the detection of enteric fever.

8. Conclusions and recommendations

In conclusion, it is a real possibility to eliminate typhoid fever, but novel diagnostics is necessary and should be factored into future trajectory for the disease control. This study found a diverse evidence of typhoid diagnostics with a substantial degree of variation in typhoid fever testing. The principle finding of this study reveals that the diagnostic performance of Widal test was moderate at a sensitivity of 52.9%. The combination of imperfect tests to determine typhoid fever disease status is a transparent technique to deal with the challenge of imperfect reference standard However, the study proposes the combinations of peripheral blood culture and Widal test as a composite reference standard which will possibly improve confidence in prevalence estimate.

Conflict of interest: The authors declare that there are no conflicts of interest.

Authors' contributions: Author BEI got the concept. The study design and data analysis was performed by S.I.R.O. and B.E.I. Author B.E.I, I.B.O and S.A.M participated in the drafting of the manuscript, which was critically reviewed for important intellectual content by S.I.R.O, A.B, and O.M.O. Interpretation of data obtained was done by S.A.M, S.I.R.O, I.B.O and A.B. The statistical analysis was performed by B.E.I and O.M.O. All authors read and approved the final version of the revised manuscript for publication.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 July 2021 doi:10.20944/preprints202107.0515.v1

References

Storey H. L, Huang Y, Crudder C, Golden A, de los Santos T, Hawkins K (2015). A Meta-Analysis of Typhoid Diagnostic Accuracy Studies: A Recommendation to Adopt a Standardized Composite Reference. *PLoS ONE*. 10(11):e0142364–2.

Adhikari A, Rauniyar R, Raut PP, Manandhar KD, Guta BP. Evaluation of Sensitivity and Specificity of ELISA against Widal test for Typhoid Diagnosis in Endemic Population of Kathmandu. BMC Infect Dis 2015; 15:523

Ahmed Z, Mansurali N. Rapid Serologic Diagnosis of Pediatric Typhoid Fever in an Endemic Area: A Prospective Comparative Evaluation of two dot-enzyme Immunoassays and the Widal Test. Amer J Trop Med Hyg 1999;61(4):654-7.

Alba S, Bakker MI, Hatta M, Scheelbeek PFD, Dwiyanti R, Usman R, Tandiriang N, Anur M, Sultan AR, Sabir M, Yasir Y. Risk Factors of Typhoid Infection in the Indonesian Archipelago. *PLoS One* 2016;11

Alonzo T A, Pepe M S. Using a combination of reference tests to assess the accuracy of a new diagnostic test. *Stat Med* 1999; 18, 2987–3003

Ambati SR, Nath G, Das BK. Diagnosis of Typhoid Fever by Polymerase Chain Reaction,

Ameya G, Atalel E, Kebede B, Yohannes B. Comparative Study of Widal test against Stool [Culture for Typhoid Fever Suspected Cases in Southern Ethiopia. *Path Lab Med Int* 2017;9:1.

Anagha K, Deepika B, Shahriar R, Sanjeev K. The Easy and Early Diagnosis of Typhoid Fever. *J Clin Diagnost Research,* 2012; 6(2):198-9..

Andrews JR, and Ryan ET. Diagnostics for Invasive Salmonella Infections; Current Challenges and Future Directions. *Vacine* 2015;33(3):C8-15

Andualem G, Tamrat A, Nigatu K, Gebre-Selassie S, Mihret A, Alemayehu H. A Comparative Study of Widal test with Blood Culture in the Diagnosis of Typhoid Fever in Febrile Patients. *BMC Research Notes* 2014;7:653

Antillón M, Warren J L, Crawford F W, et al. The burden of typhoid fever in low- and middle-income countries: a meta-regression approach. *PLoS Negl Trop Dis* 2017; 11

Archibald LK, Reller LB. Clinical Microbiology in Developing Countries. *Emerging Infect Dis* 2001;7:302-305

Aziz T, Haque SS. Role of Widal test in the Diagnosis of Typhoid Fever in Context to other

Baker S, Favorov M, Dougan G. Searching for the elusive typhoid diagnostic. *BMC Infect Dis* 2010; 10: 45.

Barton A.J, Hill J, Pollard A.J, Blohmke C.J. Transcriptomics in human challenge models. *Front Immunol* 2017; 8:1839.

Beig FK, Ahmad F, Ekram M, Shukla I. Typhidot M and Diazo test vis-à-vis Blood Culture and Widal test in the early Diagnosis of Typhoid Fever in Children in a Resource Poor Setting, *Brazilian J Infect Dis* 2010;14(6).

Berry MP, Graham CM, McNab FW, et al. An interferon-inducible neutrophildriven blood transcriptional signature in human tuberculosis. *Nature* 2010; 466:973–7.

Blohmke CJ, Darton TC, Jones C, Suarez NM, Waddington CS, Angus B, Zhou L, Hill J, Clare S, Kane L, Mukhopadhyay S. Interferon-driven alterations of the Host's amino acid Metabolism in the Pathogenesis of Typhoid Fever. *J Exper Med* 2016;213: 1061-77.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 July 2021 doi:10.20944/preprints202107.0515.v1

Blohmke C. J, Muller J, Gibani M. M, et al. Diagnostic host gene signature for distinguishing enteric fever from other febrile diseases. *EMBO Mol Med* 2019; 11: e10431.

Blohmke C.J, Darton T.C, Jones C, et al. Interferon-driven alterations of the host's amino acid metabolism in the pathogenesis of typhoid fever. *J Exp Med* 2016; 213:1061–77.

Blohmke C.J, Hill J, Darton T.C, et al. Induction of cell cycle and NK cell responses by live-attenuated oral vaccines against typhoid fever. *Front Immunol* 2017; 8:1276.

Boyd M. A, Tennant S. M, Melendez J. H, et al. Adaptation of red blood cell lysis represents a fundamental breakthrough that improves the sensitivity of Salmonella detection in blood. *J Appl Microbiol* 2015; 118:1199–209.

Britto C, Pollard AJ, Voysey M, Blohmke CJ. An appraisal of the clinical features of pediatric enteric fever: systematic review and meta-analysis of the age-stratified disease occurrence. *Clin Infect Dis* 2017; 64:1604–11.

Buckle GC, Walker CLF, Black RE. Typhoid Fever and Paratyphoid Fever: Systematic Review to Estimate Global Morbidity and Mortality for 2010. *J Glob Health* 2012;2(1).

Capati A, Ijare OB, Bezabeh T. Diagnostic applications of nuclear magnetic resonance- based urinary metabolomics. *Magn Reson Insights* 2017; 10:1178623X17694346.

Chang H. S, Sack D.A. Development of a novel in vitro assay (ALS assay) for evaluation of vaccine-induced antibody secretion from circulating mucosal lymphocytes. *Clin. Diagn. Lab. Immunol*, 2001; 8, 482–488.

Charles R.C, Liang L, Khanam F, Sayeed M. A, Hung C, Leung D.T, Baker S, Ludwig A, Harris J.B, LaRocque R.C, et al. Immunoproteomic analysis of antibody in lymphocyte supernatant in patients with typhoid fever in Bangladesh. *Clin. Vaccine Immunol*. CVI 2014;21, 280–285.

Charles RC, Liang L, Khanam F, Sayeed MA, Hung C, Leung DT, Baker S, Ludwig A, Harris JB, Larocque RC, Calderwood SB. Immunoproteomic Analysis of Antibody-in – Lymphocyte Supernatant (ALS) in Patients with Typhoid Fever in Bangladesh. *Clin Vaccine Immunol* 2014;21(3): 280-5.

Crump JA, Mintz E D. Global Trends in Typhoid and Paratyphoid Fever. *Clin Infect Dis* 2010; 50: 241–246

Crump JA, Sjolund-Karlsson M, Gordon MA, Parry CM. Epidemiology, Clinical Presentation, Laboratory Diagnosis, Antimicrobial Resistance, and Antimicrobial Management of Invasive *Salmonella* Infections. *Clin Microbiol Rev* 2015; 28:901-37.

Crump JA, Luby SP, Mintz ED. The global burden of typhoid fever. *Bull World Health Organ* 2004; 82:346–53.

Crump JA, Ram PK, Gupta SK, Miller MA, Mintz ED. Part I. Analysis of data gaps pertaining to Salmonella enterica serotype Typhi infections in low and medium human S development index countries, 1984–2005. *Epidemiol Infect* 2008; 136:436–48.

Darton T. C, Blohmke C. J, Pollard A. J. Typhoid epidemiology, diagnostics and the human challenge model.*Curr Opin Gastroenterol* 2014; 7–17

Darton T. C, Jones C, Dongol S, Voysey M, Blohmke C. J, Shrestha R, Karkey A, Shakya M, Arjyal A, Waddington C. S, et al. Assessment and Translation of the Antibody-in-Lymphocyte Supernatant (ALS) Assay to Improve the Diagnosis of Enteric Fever in Two Controlled Human Infection Models and an Endemic Area of Nepal. *Front. Microbiol,* 2017; 8

Darton TC, Blohmke CJ, Giannoulatou E, Waddington CS, Jones C, Sturges P, Webster C, Drakesmith H, Pollard AJ, Armitage AE. Rapidly Escalating Hepcidin and Associated

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 July 2021 doi:10.20944/preprints202107.0515.v1

Serum Iron Starvation are features of the Acute Response to Typhoid Infection in Humans. *PLoS Negl Trop Diseases,* 2015; 9(9):e0004029.

Darton TC, Zhou L, Blohmke CJ, Jones C, Waddington CS, Baker S, Pollard AJ. Blood Culture-PCR to Optimise Typhoid Fever Diagnosis after Controlled Human Infection Identifies Frequent Asymptomatic Cases and Evidence of Primary Bacteraemia. *J Infection.* 2017;74(4):358-66

Darton TC, Zhou L, Blohmke CJ, Jones C, Waddington CS, Baker S, Pollard AJ. Blood Culture-PCR to Optimise Typhoid Fever Diagnosis after Controlled Human Infection Identifies Frequent Asymptomatic Cases and Evidence of Primary Bacteraemia. *J Infection.* 2017; 74(4): 358-66.

Darton, T.C, Baker S, Randall, A, Dongol S, Karkey A, Voysey M, Carter M.J, Jones C, Trappl K, Pablo J, et al. Identification of Novel Serodiagnostic Signatures of Typhoid Fever Using a Salmonella Proteome Array. *Front. Microbiol.* 2017; 8.

Davies DH, Jain A, Nakajima R, et al. Serodiagnosis of acute typhoid fever in Nigerian pediatric cases by detection of serum IgA and IgG against hemolysin E and lipopolysaccharide. *Am J Trop Med Hyg* 2016; 95:431–9.

Deksisa T, Gebremedhin EZ. A Cross-Sectional Study of Enteric Fever among Febrile Patients at Ambo Hospital. Prevalence, Risk Factors, Comparison of Widal test and Stool Culture and Antimicrobial Susceptibility Pattern of Isolate. *BMC Infect Dis* 2019;19(1):288.

DeRoeck D, Clemens J D, Nyamete A, Mahoney R T. Policymakers' views regarding the introduction of new-generation vaccines against typhoid fever, shigellosis and cholera in Asia. *Vaccine* 2005; 2762–2774.

Dutta S, Das S, Mitra U, Jain P, Roy I, Ganguly SS, Ray U, Dutta P, Paul DK. Antimicrobial resistance, virulence profiles and molecular subtypes of Salmonella enterica Serovars Typhi and Paratyphi A Blood isolates from Kolkata, India during 2009-2013. *PLoS One* 2014;9(8):e101347.

El-Sayed Ahmed KA, El-Shishtawy M, El-Taweel F. Multiplex PCR for Diagnosis of { Salmonella Enterica Serovar Typhi. *Clin Lab* 2015;61(10):1537-43.

Enabulele O, Awunor SN. Typhoid Fever in a Tertiary Hospital in Nigeria; Another look at { the Widal Agglutination Test as a Preferred Option for Diagnosis. *Nigeria Med J* 2016;57(3):145-9.

Fan Fenxia, Pengcheng Du Biao Kan, Meiying Yan. The Development and Evaluation of a LoopMediated Isothermal Amplification Method for the Rapid Detection of Salmonella enterica serovar Typhi *PLOS ONE* 2015; DOI:10.1371/journal.pone.0124507

Fan F, Du P, Kan B, Yan M. The development and evaluation of a loop-mediated isothermal amplification method for the rapid detection of Salmonella enteric serovar Typhi. *PLoS One* 2015; 10:e0124507.

Felgner J, Jain A, Nakajima R, Liang L, Jasinskas A, Gotuzzo E, Vinetz J.M, Miyajima F, Pirmohamed M, Hassan-Hanga F. et al. Development of ELISAs for diagnosis of acute typhoid fever in Nigerian children. *PLoS Negl. Trop. Dis* 2017; 11, e0005679.

French CD, Willoughby RE, Pan A, et al. NMR metabolomics of cerebrospinal fluid differentiates inflammatory diseases of the central nervous system. *PLoS Negl Trop Dis* 2018; 12:e0007045.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 July 2021 doi:10.20944/preprints202107.0515.v1

Frickmann H, Wiemer D.F, Wassill L, et al. Loop-mediated isothermal amplification-based detection of typ hoid fever on an automated Genie II Mk2 system—a case-control-based approach. *Acta Trop* 2019; 190:293–5.

Garg S, Sankle A, Joshi A, Mehta S. Comparison of Typhidot IgM Test and Blood Culture in Children with Clinically Compatible Enteric Fever. *Int J Contemporary Paediat* 2018;5(6):2129-32.

Gilman RH, Terminel M, Levine MM, Hernandez-Mendoza P, Hornick RB. Relative Efficacy of Blood, Urine, Rectal Swab, Bone-marrow, and Rose-spot Cultures for Recovery of *Salmonella* Typhi in Typhoid Fever. *Lancet* 1975;1(7918):1211-3.

Gliddon H. D, Herberg J. A, Levin M, Kaforou M. Genome-wide host RNA signatures of infectious diseases: discovery and clinical translation. *Immunology* 2018; 153:171–8.

Goay XY, Chin KL, Tan CLL, Yeoh CY, Ja'afar JN, Zaidah AR, Chinni SV, Phua KK. Identification of Five Novel Salmonella Typhi-specific Genes as Markers for Diagnosis of Typhoid Fever Using Single-gene Target PCR Assays. *Biomed Research Int* 2016.

Halliley J. L, Kyu S, Kobie J. .J, Walsh E. E, Falsey A. R, Randall T. D, Treanor J, Feng C, Sanz I, Lee F. E. H. Peak frequencies of circulating human influenza-specific antibody secreting cells correlate with serum antibody response after immunization. *Vaccine* 2010; 28, 3582–3587

Haques A, Ahmed J, Qureshi JA. Early Detection of Typhoid by Polymerase Chain Reaction. *Annal Saudi Med* 1999;19(4):337-40

Hoang LT, Lynn DJ, Henn M, et al. The early whole-blood transcriptional signature of dengue virus and features associated with progression to dengue shock syndrome in Vietnamese children and young adults. *J Virol* 2010; 84:12982–94.

Hussain K, Jamal H, Bashur T, Sanaullah IU, Khoso I. Diagnostics Accuracy of Typhidot in Patients of Typhoid Fever. *Pat Armed Forces Med J* 2018;68(5):1215-8.

Huw D D, Jain A, Nakajima R, Liang L, Jasinskis A, Supnet M, Felgner P.L, Teng A, Pablo J., Molina D.M (2016). Serodiagnosis of Acute Typhoid Fever in Nigerian Pediatric Cases by Detection of Serum IgA and IgG against Hemolysin E and Lipopolysaccharide. *Am. J Trop. Med. Hyg*. 2016; 95, 431–439.

Igiri BE, Inyang-Etoh PC, Ejezie GC, Jimoh O, Sobo MA, Idoko GO. Diagnostic Investigations{and Prevalence of Enteric Fever in Ahmadu Bello University Teaching Hospital Shika-Zaria, Kaduna State, Nigeria. *Clin Microbiol Infect Dis* 2018;3(1):1-5.

Kaforou M, Wright V. J, Levin M. Host RNA signatures for diagnostics: an example from paediatric tuberculosis in Africa. *J Infect* 2014; 69(Suppl 1):S28–31.

Kantele A, Pakkanen SH, Kattunen R, Kantele JM. Head-to-Head Comparison of Humoral Immune Response to Vi Capsular Polysaccharide and *Salmonella* Typhi Ty21a Typhoid Vaccines-a Randomized Trial. *PLoS One* 2013;8(4):e60583.

Keddy KH, Sooka A, Letsoalo ME, Hoyland G, Chaignat CL, Morrissey AB, Crump JA. Sensitivity and Specificity of Typhoid Fever Rapid Antibody Tests for Laboratory Diagnosis at two sub-Saharan African sites. *Bull World Health Organ* 2011;89:640-7.

Khan K, Khalid L, Wahid K, Ali I. Performance of TUBEX ®TF in the Diagnosis of Enteric Fever in Private Tertiary Care Hospital Peshawar, *Pak J Pak Med Asso* 2017;67(5): 661-4.

Khoharo HK. A Comparative Sudy of the Typhidot (Dot-EIA) and Widal Tests in the Blood Culture Positive Cases of Typhoid Fever. *Tropical doctor* 2011;41(3):136-8.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 July 2021 doi:10.20944/preprints202107.0515.v1

Kumar S, Balakrishna K, Singh GP, Batra HV. Rapid Dtection of *Salmonalla* Typhi in Foods by Combination of Immunomagnetic Separation and Polymerase Chain Reaction. *World J Microbiol Biotechnol* 2005;21:625-8.

Lalremruata R, Chadha S, Bhalla P. Retrospective Audit of the Widal test for Diagnosis of Typhoid Fever in Pediatric Patients in an Endemic Region. *J Clin Diagnosis Research JCDR* 2014; 8(5),DC22.

Lateef A, Olopoenpiaa LA, Kingb AL. Widal Agglutination test- 100 Years Later: Still Plagued by Controversy. *Postgrad med J* 2000; 6:80-4.

Levy H, Diallo S, Tennant S. M, et al. PCR method to identify Salmonella enteric serovars Typhi, Paratyphi A, and Paratyphi B among Salmonella isolates from the blood of patients with clinical enteric fever. *J Clin Microbiol* 2008; 46:1861–6.

Ley B, Thriemer K, Ame SM, Mtove GM, Von Seidlein L, Amos B, Ochiai LR. Assessment and Comparative Analysis of a Rapid Diagnostic test (TUBEX ®) for the Diagnosis of Typhoid Fever among Hospitalized Children in Rural Tanzanian, *BMC Infect Dis* 2011;11(1):147.

Li S, Sullivan N. L, Rouphael N, et al. Metabolic phenotypes of response to vaccination in humans. *Cell* 2017; 169:862–77.e17.

Liang L, Juarez S, Nga T.V.T, Dunstan S, Nakajima-Sasaki R, Davies, D.H, McSorley S, Baker S, Felgner P.L. Immune profiling with a Salmonella Typhi antigen microarray identifies new diagnostic biomarkers of human typhoid. *Sci. Rep*.2013; 3, 1043.

Liang L, Juarez S, Nga TVT et al. Immune profiling with a Salmonella Typhi antigen microarray identifies new diagnostic biomarkers of human typhoid. *Sci. Rep*. 2013; 3:1043

Maheshwari V, Kaore NM, Ramnani VK, Sarda S. A Comparative Evaluation of Different Diagnostic Modalities in the Diagnosis of Typhoid Fever using a Composite Reference Standard; a Tertiary Hospital based Study in Central India. *J Clin Diagn Res* 2016;10(10):DC01.

Mancini N, Carletti S, Ghidoli N, Cichero P, Burioni R, Clement M. The Era of Molecular and other Nonculture-based Methods in Diagnosis of Sepsis. *Clin Microbiol Rev* 2010; 23:235-51.

Martiny D, Debaugnies F, Gateff D, Gerard M, Aoun M, Martin C, Konopmiki D. Impact of Rapid Microbial Identification Directly from Positive Blood Cultures using Matrix-assisted Laser Desorption/ionization Time-of-flight Mass Spectrometry on Patient Management. *Clin Microbiol Infect* 2013;19:E568-81

Maude RR, de Jongm HK, Wijedoru L, Fukushima M, Ghose A, Samad R. The Diagnostic Accuracy of Three Rapid Diagnostic tests for Typhoid Fever at Chittagong Medical College Hospital, Chittagong, Bandladesh. *Tropl Med Int Health* 2015; 20(10):1376-84.

Mawazo A, Bwire GM, Matee MIN. Performance of Widal test and Stool Culture in the Diagnosis of Typhoid Fever among Suspected Patients in Dares Salaam, Tanzania, BMC *Research Notes* 2019;12(1):316.

Mawazo A, George M. B, and Mecky I. N. M. Performance of Widal test and stool culture in the diagnosis of typhoid fever among suspected patients in Dar es Salaam, Tanzania *BMC Res Notes* 2019 *BMC Res Note* 12; 316

McKinnon LR, Abdool KQ. Honing in on Enteric Fever. *eLife* 2014;3,e03545

Mehmood K, Sundus A, Naqvi IH, Ibrahim MF, Siddique O, Ibrahim NF. Typhidot A Blessing or a Menace. *Pakistan J Med Sc* 2015;31(2):439.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 July 2021 doi:10.20944/preprints202107.0515.v1

Mejias A, Dimo B, Suarez NM, et al. Whole blood gene expression profiles to assess pathogenesis and disease severity in infants with respiratory syncytial virus infection. *PLoS Med* 2013; 10:e1001549.

Minjibir Abdullahi A., Sani U. Diso, Idris S. Ibrahim, Muhammad S. Abdallah, Muhammad Ali. Comparative Study of Widal test Against Stool Culture in Diagnosis of Typhoid Fever Suspected Cases in Kano, Northern Nigeria. *South Asian Res J Eng Tech* 2020; 2:5

Mogasale V, Maskery B, Ochai RI, Lee JS, Mogasale VV, Ramani ES… & Kim YE. Burden of Typhoid Fever in Low-income and Middle-income Countries: A Systematic, Literature based Update with Risk Factor Adjustment. *Lancet Glob Health* 2014;2(12):e696.

Mogasale V, Ramani E, Mogasale V V, Park J. What proportion of Salmonella Typhi cases are detected by blood culture? A systematic literature review. *Ann Clin Microbiol Antimicrob.* 2016; 15(1):32

Mogasale V, Ramani E, Mogasale VV, Park J. What Proportion of *Salmonella typhi* cases are detected by Blood Culture? A Systematic Literature Review. *Annal of Clin Microbiol Antimicrob* 2016;15(1):32.

Moore CE, Pan-Ngum W, Wijedoru LP, Sona S, Nga TV, Duy PT, Vinh PV, Chheng K. Evaluation of the Diagnostic Accuracy of Typhoid IgM Flow Assay for the Diagnosis of Typhoid Fever in Cambodian Children using a Bayesian Latent Class Model Assuming an Imperfect Gold Standard. *The America J Trop Med Hyg* 2014;90:114-20.

Nakaya H. I, Wrammert J, Lee E. K, et al. Systems biology of vaccination for seasonal influenza in humans. *Nat Immunol* 2011; 12:786–95.

Narayanappa D, Sripathi R, Jagdishkumar K, Rajani HS. Comparative Study of Dot Enzyme Immunoassay (Typhidot-M) and Widal test in the Diagnosis of Typhoid Fever, *Indian Pediatr* 2010;47.

Nasstrom E, Parry CM, Vu Thieu NT, Maude RR, de Jong HK, Fukushima M, Rzhepishevska O. Reproducible Diagnostic Metabolites in Plasma from Typhoid Fever Patients in Asia and Africa. 2017;dio:10.7554/*eLife*.15651.

Nasstrom E, Thieu NTV, Dongol S, Karkey A, Vinh PV, Thanh TH, Basnyat B. *Salmonella* Typhi and *Salmonella* Paratyphi A Elaborate Distinct Systemic Metabolite Signatures during Enteric Fever. *eLife* 2014;3:e03100.

Nga T.V, Karkey A, Dongol S, et al.. The sensitivity of real-time PCR amplification targeting invasive Salmonella serovars in biological specimens. *BMC Infect Dis,* 2010; 10:125.

Ohanu M E, Michael O. I, Uzoamaka M, Obinna D. O, Harish C. G. Typhoid fever among febrile Nigerian patients: Prevalence, diagnostic performance of the Widal test and antibiotic multi-drug resistance. *Malawi Medical Journal* 2019; 31 (3): 184-192

Olsen SJ, Pruckler J, Bibb W, Nguyen T M, Tran MT, Nguyen TM, Sivapalasingam S. Evaluation of Rapid Diagnostic tests for Typhoid Fever. *J Clin Microbiol* 2004;*42*(5):1885-9.

Paolucci M, Landini MP, Sambri V. Conventional and Molecular Techniques for the Early Diagnosis of Bacteraemia. *Int J Antimicrob Agents* 2010; 36: S6-6.

Park K S, Hyun J C, Farhana K, Hakho L, Rasheduzzaman R, Md. Taufiqur B, Amanda B, Jason B. H, Stephen B. C, Edward T. R, Firdausi Q, Ralph W & Richelle C. C. Amagneto-DNA nanoparticle system for the rapid and sensitive diagnosis of enteric fever. *Scientific Reports* 2016; 6:32878

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 July 2021 doi:10.20944/preprints202107.0515.v1

Parry CM, Wijedoru L, Arjyal A, Baker S. The Utility of Diagnostic tests for Enteric Fever in Endemic Locations. *Expert Rev anti- Infective Ther* 2011;9(6):711-25.

Patel RP, Trivedi MB. Study of Diagnostic Efficacy of Widal Slide Agglutination test against Widal tube Agglutination test in Enteric Fever. *Indian J Microbiol Research* 2017;4(3):267-9.

Prasad KL, Oberoi JK, Goel N, Wattal C. Comparative Evaluation of Two Rapid Salmonella-IgM tests and Blood Culture in the Diagnosis of Enteric Fever. *Indian J Med Microbiol* 2015;33(2):237-42.

Ramyi MS, Ihuoma OJ, Ogundeko TO, Ameh JM, Olorundare F, Adeniyi OG, Izam MM. Comparative Study on the use of Widal test and Stool Culture in the Laboratory Diagnosis of Salmonella Infection in Adult and Children in Jos Metropolis, Plateau State, Nigeria. *Int J Sc Research* 2013;2319-7064.

Raqib R, Rahman J, Kamaluddin A.K.M, Kamal S.M.M, Banu, F.A, Ahmed S, Rahim Z, Bardhan P.K, Andersson J, Sack D.A. Rapid diagnosis of active tuberculosis by detecting antibodies from lymphocyte secretions. *J. Infect. Dis*, 2003; 188, 364–370.

Salama R1, Said NM. A Comparative Study of the Typhidot (Dot-EIA) versus Widal test in Diagnosis of Typhoid Fever among Egyptian Patients. *Open J Gastroenterology* 2019;9:91-8.

Sandro C. Typhoid Intestinal Perforation in Developing Countries: Still Unavoidable Deaths? *World J Gastroent* 2017;23(11):1925-31.

Sharma T, Bhatnagar S, Tiwari A. Typhoid Diagnostics: Looking Beneath the Surface. *J Clin Diagnost research*. 2018;12(9): KE01-7.

Sheikh A, Bhuiyan, M.S, Khanam F, Chowdhury F, Saha A, Ahmed D, Jamil K.M.A, LaRocque R.C, Harris J.B, Ahmad M.M, et al. Salmonella enterica serovar Typhi-specific immunoglobulin A antibody responses in plasma and antibody in lymphocyte supernatant specimens in Bangladeshi patients with suspected typhoid fever. *Clin. Vaccine Immunol*, 2009; 16, 1587–1594.

Sherwal BL, Dhamija RK, Randhawa VS, Jais M, Kaintura A, Kumar M. A Comparative Study of Typhidot and Widal test in Patients of Typhoid Fever, *J Indian Academy of Clin Med* 2004;5(3):246.

Shrinet J, Shastri JS, Gaind R, Bhavesh NS, Sunil S. Serum metabolomics analysis of patients with chikungunya and dengue mono/co-infections reveals distinct metabolite signatures in the three disease conditions. *Sci Rep* 2016; 6:36833.

Siba V, Horwood PF, Vanuga K, Wapling J, Sehuko R, Siba PM, Greenhill AR. Evaluation of Serological Diagnostic tests for Typhoid Fever in Papua New Guinea using a Composite Reference Standard. *Clin Vaccine Immunol* 2012;1833-7.

Stanaway JD, Reiner RC, Blacker BF, Goldberg EM, Khalil IA, Troeger CE, Andrews JR, Bhutta ZA, Crump JA, Im J, Marks F. The global burden of typhoid and paratyphoid fevers: a systemic analysis for the global burden burden of disease study group 2017. *Lancet Infect Dis* 2019;19(4):369-81.

Storey HL, Huang Y, Crudder C, Golden A, de los Santos T, Hawkins K. A Meta-Analysis of Typhoid Diagnostic Accuracy Studies: A Recommendation to Adopt a Standardized Composite Reference. *PLoS One* 2015; 10(11):e0142364.

Subramaniam K.S, Spaulding E, Ivan E, *et al.* The T-cell inhibitory molecule butyrophilin-like 2 is up-regulated in mild *Plasmodium falciparum* infection and is protective during experimental cerebral malaria. *J Infect Dis* 2015; 212:1322–31.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 22 July 2021 doi:10.20944/preprints202107.0515.v1

Tarupiwa A, Tapera S, Mtapuri-Zinyowera S, Gumbo P, Ruhanya V, Gudza-Mugabe M, Majuru NX, Chn'ombe N. Evaluation of TUBEX-TF and ONSITE Typhoid IgG/IgM Combo Rapid tests to detect *Salmonella enterica* serovar Typhi Infection during a Typhoid Outbreak in Harare, Zimbabwe, *BMC Research Notes* 2015;8(1):50.test. *Amer J Bioch* 2012;2(1):16-8.

Thriemer K, Ley B, Menten J, Jacobs J, van den Ende JA. A Systematic Review and Meta-analysis of the Performance of Two Point of care Typhoid Fever tests. TUBEX TF and Typhidot, in Endemic Countries. *PLOS ONE* 2013;8(12):e81263.

Ugochukwu I, Amu OC, Nzegwu MA. Ileal Perforation due to Typhoid Fever-Review of Operative Management and Outcome in an Urban Centre in Nigeria. *Int J Surg* 2013;11(3):218-22.

Vahey M.T, Wang Z, Kester K.E, *et al.* Expression of genes associated with immunoproteasome processing of major histocompatibility complex peptides is indicative of protection with adjuvanted RTS,S malaria vaccine. *J Infect Dis*; 2010; 201:580–9.

Vallenas C, Hernandez H, Kay B, Black R, Gotuzzo E. Efficacy of Bone Marrow, Blood, Stool and Duodenal Contents Cultures for Bacteriologic Confirmation of Typhoid Fever in Children. *Pediat Infect Dis* 1985;4:496-8.

Wam EC, Arrey CN, Sama LF, Agyingi LA, Wam AN. Comparative Study on the use of Widal test to Stool Culture in the Laboratory Diagnosis of Typhoid Fever in Holy Family Hospital Akum, North West Region of Cameroon. *The Open Microbiol J* 2019; 13:73-80

Weiner J 3rd, Maertzdorf J, Sutherland JS, et al. GC6-74 Consortium. Metabolite changes in blood predict the onset of tuberculosis. *Nat Commun* 2018; 9:5208.

Whiting P F, Rutjes A W, Westwood M E, Mallett S. A systematic review classifies sources of bias and variation in diagnostic test accuracy studies. *J Clin Epidemiol* 2013; 66:1093–1104.

WHO. 2015. Immunization, Vaccines and Biological Typhoid. World Heal Organ 1-2

Wijedoru L, Mallett S, Parry CM. Rapid Diagnostic tests for Typhoid and Paratyphoid (Enteric) Fever. Cochrane Databases System Reviews, 2017; https://doi.org/10.1002/14651858.

Wlekidan LN, Gebremarian SA, Welderufael AL, Muthupandian S, Haile TD, Dejene TA. Diagnosis and Treatment of Typhiod Fever and Associated Prevailing Drug Resistance in Northern Ethiopia. *Intl J Infect Dis* 2015;35:96-102.

World Health Organisation, WHO. The Diagnosis, Treatment, and Prevention of Typhoid Fever. 2003

World Health Organization. Typhoid vaccines: WHO Position Paper. March, 2018 Recommendation *Vaccine* 2019;37(2): 214-6.

Zaki SA, Karande S. Multidrug-resistance Typhoid Fever: A Review. *The J Infection Develop Count* 2011;5(5):324-37.

Zhou L, Jones C, Gibani M. M, et al. Development and evaluation of a blood culture PCR assay for rapid detection of Salmonella Paratyphi A in clinical samples. *PLoS One* 2016; 11:e0150576.

Zhou L, Pollard A. J. A novel method of selective removal of human DNA improves PCR sensitivity for detection of Salmonella Typhi in blood samples. *BMC Infect Dis* 2012; 12:164.

Zurfluh S, Baumgartner T, Meier MA, et al. The role of metabolomic markers for patients with infectious diseases: implications for risk stratification and therapeutic modulation. *Expert Rev Anti Infect Ther* 2018; 16:133–42.