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The diagnosis of typhoid fever in the Democratic Republic of the Congo

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ABSTRACT

The diagnosis of typhoid fever (TF) in Kinshasa (DR Congo) was assessed by on-site surveys, external quality assessment (EQA) of the Widal test and a microbiological blood culture surveillance study. In 331/536 (61.8%) health facilities, clinicians diagnosed TF by clinical picture and the Widal test. An EQA on the Widal test consisting of three samples revealed correct scores by respectively 27.1%, 65.6% and 3.1% of 125 participating laboratories. Most (80.9% of 152 laboratories) performed <100 Widal tests per month, with a median sample positivity rate of 32.6% (range 0–90.7%). The Widal test was mostly performed on a single sample and by slide agglutination (89.5% and 97.0% respectively); errors in cold chain and procedures were recorded (not making serial dilutions, estimating titres by the intensity of agglutination). Among 293 prescribers, 52.2% and 40.8% requested the Widal test for treatment follow-up and detection of chronic carriers respectively. *Salmonella* Typhi was recovered from the blood in 2.4% of 3820 patients suspected as having TF, with non-typhoid *Salmonellae* and other *Enterobacteriaceae* accounting for the majority of organisms. In conclusion, clinicians rely highly on the Widal test for the diagnosis of TF and the Widal test is poorly performed and interpreted.

1. Introduction

In 2009, 118 727 cases of typhoid fever (TF) were notified in the province of Kinshasa, Democratic Republic of the Congo (DRC).[1] On an estimated population of 10 000 000 inhabitants in Kinshasa, the corresponding incidence of TF for 2008 was 1662/100 000, which is much higher than expected for a country in Africa.[2,3] Likewise, the reported case fatality rate reported in 2009 was 0.03%, which is unexpectedly low.

These data suggest an over-notification of TF in DRC, which we hypothesized to be linked to the fact that diagnosis of TF is mainly based on clinical grounds and serology, in particular the Widal test (see Box 1). To get more insight, we conducted several studies about the diagnosis of TF in Kinshasa. They included three knowledge and practice surveys (among health care centers, laboratories and clinicians respectively), one study on the baseline titre of the Widal test, one external quality assessment (EQA) session about the performance of the Widal test, and one surveillance study of bloodstream infections. These studies were carried out by the National Institute of Biomedical Research (INRB) in Kinshasa, in the scope of a project on laboratory quality management. This paper describes the main findings of these studies.

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2. Materials and methods

2.1. Survey about the criteria used for the diagnosis of typhoid fever

From 24 April to 2 May 2006, an on-site survey was conducted among the health care facilities (HCF) in Kinshasa province participating in the Ministry of Health (MoH) surveillance of communicable diseases. Medical doctors and nursing staff were interviewed about the tools used for diagnosis of TF using a standardised questionnaire.

2.2. Baseline titres for the Widal test in blood donors

The Widal test, described in Box 1, may generate false-positive results in patients infected with non-typhoid *Salmonella* (NTS), other *Enterobacteriaceae* or even in patients with other febrile illnesses such as malaria.[4,5] By consequence, in regions where these infections are endemic, it is important to determine the so-called baseline titres of the Widal test.[6,7] We assessed healthy blood donors recruited at Kingasani Reference Hospital, a 144-bed reference hospital in the township of Kinshasa. Left-over serum samples of adult family blood donors were collected between January and December 2009 and stored at −20 °C until analysis. The tube agglutination Widal test (SPAN Diagnostics Ltd, Udhna, India) was performed according to the manufacturer's instructions. Titres are further mentioned as the reciprocal of the dilution (e.g. 80 or 160).

Box 1. Tools for the diagnosis of typhoid fever (TF)

The definitive diagnosis of TF is based on the isolation of *Salmonella enterica* serovar Typhi from blood, bone marrow, stool, urine or other body fluids by culture or of DNA amplification.[24–26] Diagnosis based on clinical grounds alone is unreliable because the signs and symptoms of TF are nonspecific.

The Widal test detects serum antibodies against lipopolysaccharide O (somatic) and H (flagellar) antigens of *Salmonella* Typhi. In its standard formulation, both an acute and a convalescent serum (serum obtained 7–14 days after the onset of clinical disease) are tested simultaneously (so-called 'paired sera'). Serial two-fold of dilutions or the sera are made in tubes, starting at a dilution of 1/40 (in some laboratories 1/50) and ranging to 1/640. To these tubes, test solutions with particles (mostly latex) coated with O and H antigens are added and the reaction mixture is incubated. If antibodies to O or H are present, they will bind to the antigens coated on the particles which results in the appearance of so-called agglutinations. The highest dilution for which agglutination is still observed represents the so-called titre, reported as the reciprocal of the dilution (e.g. 80 or 160).

The Widal test suffers from serious limitations[4,13,27–29] From a technical standpoint, there is a lack of standardisation of antigens, and storage of reagents at low temperatures is needed. Further, *Salmonella* Typhi shares O and H antigens with other *Salmonella* serotypes and has cross-reacting epitopes with other *Enterobacteriaceae*. False-positive results may also occur with other febrile illnesses such as malaria, schistosomiasis and miliary tuberculosis and meningitis). An overview of the technical and diagnostic aspects of the Widal test can be read in reference 4; a recent update in reference 5.

2.3. External quality assessment of the Widal test among diagnostic laboratories

From 3–16 September 2009, diagnostic laboratories participating in the EQA sessions organised by INRB in Kinshasa were addressed and those that performed the Widal test were included. A panel of three serum samples was submitted; they had been assessed for TO and TH antibodies with the tube agglutination test at INRB (Span Diagnostics Ltd) and at the Institute of Tropical Medicine (Diamondal, Sees, France) respectively. Sample 1 was obtained from a patient with a culture-confirmed bacteremia with *Salmonella* Typhi. The two other serum samples were obtained from healthy blood donors at Kingasani hospital.

Serum samples had been stored at −20 °C for a maximum of 14 days before distribution. They were transported in a cool box (2–8 °C) and delivered on-site after a maximum 7 h transport delay. Participants were asked to report the results as they would do in daily practice.

For Sample 1, titres of 160 or above for both TO and TH were considered as correct. For sample 2, titres of <40 for TO and of ≤40 for TH were considered as correct. As these titres were below the cut-off value of ≥100 previously determined for a similar African setting (Cameroon),[8] the result reported as 'Widal negative' was also considered correct. For Sample 3, we considered titres of ≤40 for TO and of ≥160 for TH as correct.

2.4. Survey among diagnostic laboratories about the use and technique of the Widal test

The EQA session was followed by a knowledge and practice survey about the practice of the Widal test. The laboratory staff were presented with a questionnaire based on a similar one used in Cameroon.[8] This questionnaire addressed the numbers of the technicians performing the Widal test, the numbers of samples processed and their positivity rate and the brand of reagent kits used. In addition, storage conditions of the reagent kits were surveyed; serious problems in electricity supply were defined as at least two power interruptions per week with no backup generator available. The nature and number of samples performed per patient were assessed as well as details about the methodology (slide vs tube technique and testing of serial dilutions).

2.5. Survey among clinicians about the indications and interpretation of the Widal test

In conjunction with the laboratory survey, a survey among the clinicians (doctors and nurses) prescribing the Widal test at the same HCF was conducted. As for the previous study, the questionnaire was designed on the one used in Cameroon.[8] The items surveyed included the practice of requesting the Widal test (indications and number of samples) and the knowledge about the diagnostic value of the Widal test and the causes of false-positive or false-negative

results. In addition, four clinical scenarios with Widal test results expressed as titres were presented and participants were asked for interpretation and action (administration of antibiotics).

2.6. Surveillance of bloodstream infections in the Democratic Republic of the Congo

As part of a microbiological surveillance study and during April 2007 to December 2009, blood cultures were performed in patients suspected of TF or other systemic infections. Patients were recruited from HCF in Kinshasa as well as from hospitals in six out of eleven provinces in DRC (Bandundu, Bas Congo, Equateur, Kasai Occidental, Kinshasa and Oriental province). Blood cultures were sampled according to standard criteria and suspected focus of infection including suspicion of TF was recorded. For children (<14 years old), 1–4 ml of blood was sampled into a pediatric blood culture vial (BacT/ALERT FP, bioMérieux, Marcy L'Etoile, France), for adults, 2×10 ml of blood was inoculated into aerobic blood culture vials (BacT/ALERT FA, bioMérieux). Inoculated vials were shipped to INRB, incubated at 35 °C and daily checked for growth by visual inspection of the chromogenic growth indicator at the bottom of the vials. In addition, a 5% fraction of the blood cultures that did not grow was blindly subcultured as a control. Grown bottles were Gram stained, subcultured MacConkey and 5% Sheep Blood agar (Difco, NJ, USA) and incubated at 35 °C for 24 h. Isolates were identified to the species level by standard biochemical methods at INRB and later on confirmed by Vitek II (Card GN21 341, bioMérieux). Isolates identified as *Salmonella* spp. were further identified to the serotype level using commercial antisera (Sifin, Berlin, Germany) and quality control was done by the Public Health Institute, National Reference Laboratory of *Salmonella* and *Shigella*, Brussels, Belgium.

2.7. Data registration, statistical analysis

Data were anonymously entered in an Excel database (Microsoft Corporation, Redmond, Washington, USA). Differences between proportions (e.g., the referral level of HCFs and the tools they used for the diagnosis of TF and the EQA scores obtained when using particular brands of Widal kits) were calculated by means of the χ^2 test (Stata 11.1, StataCorp LP, College Station, TX, USA). A p-value of <0.05 was considered as significant.

2.8. Ethical issues

All surveyed participants were asked to give consent. For the microbiological surveillance study, ethical approval was granted by the Institutional Review Board of the Institute of Tropical Medicine ITM and the Ethical Committee of Antwerp University, Antwerp, Belgium. Pending the installation and functioning of the ethical committee in Kinshasa, clearance for the studies was obtained by the MoH.

3. Results

3.1. Survey about criteria used for the diagnosis of typhoid fever

A total of 536/573 (93.5%) HCF were reached, representing 33 of 35 Health Zones of Kinshasa province. Nearly two-thirds (331, 61.8%) of HCF declared the use of a combination of clinical symptoms and serology for the diagnosis of TF, and 154 (28.7%) and 43 (8.0%) reported relying uniquely on clinical picture and serology respectively. Blood cultures were only available in a small minority (8, 1.5%) of HCF. There was no relation between the referral level or affiliation (public, private or confessional) and the criteria used for diagnosis. It was noted however that the HCF affiliated to one congregation (representing 30 HCF) had deliberately banned the Widal test.

3.2. Baseline titres of the Widal test in blood donors

A total of 3260 serum samples were collected; donors' ages ranged from 17 to 80 years. The vast majority of blood donors (3140, 96.3%) had TO and TH antibody titres of $\leq$40; 54, 24 and one donor had TH antibody titres of 80, 160 and 320 respectively; another 12 and two donors had TO antibody titres of 80 and 160. Eighteen donors showed combined TO/TH antibody titres of 80 each, and two, one and six donors showed combined TO/TH antibody titres of 80/160, 80/320 and 160/160 respectively.

3.3. External quality assessment of the Widal test among diagnostic laboratories

A total of 164 laboratories participated in the EQA session, of which 144 (87.8%) returned the results within the deadline. Among them, 125 participants gave eligible answers for all three samples. For sample 1, 34 (27.1%) participants reported the correct results in terms of TO and TH titres. If reports expressed as 'positive' were also considered as correct, the ratio of correct results increased to 63 (50.4%). All incorrect results were titres that were too low compared to the reference results. For sample 2, 82/125 (65.6%) participants reported the correct result; among the incorrect results, there were 24 (19.2%) participants who reported either 'positive' or $\geq$160 for both TO and TH (i.e., more than two-fold the reference value). For sample 3, 4 (3.1%) participants reported the correct result in terms of titres. If the results 'negative' for TO and 'positive' for TH were equally considered as correct, this number increased to 32 (25.6%). Thirty-one (24.4%) participants reported 'positive' or TO-titres $\geq$160 (i.e., more than two-fold the reference value). Figure 1 shows the combined correct results for the 125 participants who gave eligible answers for all three samples. When only titres were considered, none of the participants reported correct results for all three samples. If the results reported as 'negative' and 'positive' were also considered, the numbers of participants reporting correct results for all three samples combined was 15 (12.0%). In total, eight different brands of Widal test kits were used; there was no relation between the results for the EQA samples and the brand of Widal test used.

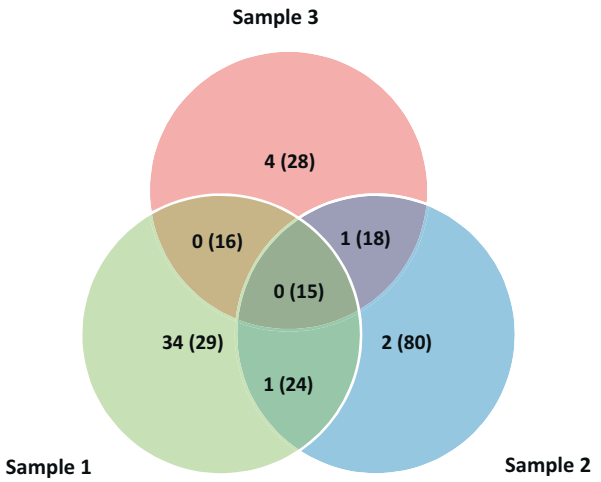

Figure 1. Combined correct scores for the three samples for Widal testing as submitted for external quality assessment. A total of 125 participants gave eligible answers for all three samples. The data represent numbers of participants reporting the correct titre and, in parentheses, the numbers of participants reporting correct answers in terms of 'positive' and 'negative'.

Forty-four of 144 participants (30.5%) reported titres although they declared during the subsequent survey not to perform serial dilutions. When queried for explanation, they declared to estimate titres according to the visible intensity of the slide agglutinations.

3.4. Survey among diagnostic laboratories about the use and technique of the Widal test

Of the 164 HCF addressed, 152 (94.5%) returned completed data regarding workload and positivity rate for the month immediately prior to the survey. The majority (123/152, 80.9%) had processed fewer than 100 samples (Table 1). The median rate of positive Widal tests was 32.6% (range 0–90.7%), with 61 (40.1%) and 28 (18.4%) participants reporting, respectively, more than one-third and one-half of their samples as positive. Reagent kits were not stored in a refrigerator in 29/152 (19.1%) laboratories, and another 91 (59.9%) mentioned serious problems in electricity supply. Most laboratories (147/164, 89.5%) performed the Widal test on a single serum sample. The vast majority (159/164, 97.0%) of laboratories used the slide agglutination technique, and only a few (n=5) used the tube agglutination technique. Two-thirds (110/164, 67.1%) of participants declared not to make serial dilutions.

Table 1
Number of samples processed matched with sample positivity rate (% positive samples/total samples submitted). A total of 152 eligible answers were obtained

	Numbers of samples processed monthly				
Sample positivity rate	<10	10–49	50–99	≥100	Total
≤10	4	17	3	6	30
11–20	4	9	8	5	26
21–40	4	26	12	6	48
41–60	2	13	7	8	30
61–80	4	5	2	3	14
>80	0	1	2	1	4
Total	18	71	34	29	152

3.5. Survey among prescribers about the indications and interpretation of the Widal test

Prescribers included 155 medical doctors and 138 nurses but not all replied to all questions (Table 2). With regard to knowledge, the diagnostic value of the Widal test (orientation to the diagnosis of TF) was correctly scored by most prescribers (254/293, 86.7%), both doctors and nurses. However, 143/274 (52.2%) and 109/267 (40.8%) of prescribers replied to request the Widal test for follow-up of treatment and for the diagnosis of chronic carriers respectively. Likewise, only about one-third and one-half of the participants recognized the causes of, respectively, false-positive and false-negative Widal test results. Overall, knowledge was lower among nurses as compared to doctors, with 43.6% (58/133) of nurses considering the Widal test rather than blood or stool culture as the main diagnostic tool for TF. Two-thirds (217, 66.3%) of prescribers declared to request the Widal test on two samples (one sample at onset and another sample 7–14 days later).

Table 3 lists the results for the interpretation of the Widal test titres. Two-thirds (105, 67.7%) of doctors and about half (76, 55.0%) of nursing prescribers declared themselves to be competent to interpret titres. Apart from the interpretation of the 'TO 80; TH 160' case, the majority of doctors answered correctly. Most of note was the fact that 33/69 nurses declared to interpret the 'TO ≤ 40; TH 160' presentation as an argument in favour of administration of antibiotics.

3.6. Surveillance of bloodstream infections in the Democratic Republic of the Congo

Table 4 gives an overview of the clinically significant organisms recovered from blood cultures. The overall positivity rate of clinically significant organisms was 10.9%, the contamination rate was 9.6%. In 3820 patients suspected of TF and 2857 patients suspected of other clinical foci of bloodstream infection, *Salmonella* Typhi was recovered from 2.4% and 1.2% respectively. Among the significant organisms recovered from patients suspected of TF, NTS ranked first in frequency and other *Enterobacteriaceae* (*Klebsiella* spp., *Escherichia coli*, *Enterobacter* spp. accounted for one-third (117, 32.2%). This pattern of isolates was observed among all participating centers. Among the organisms recovered from patients suspected of other causes of bloodstream infections, other *Enterobacteriaceae* and NTS accounted for 208 (56.7%) and 82 (22.3%) of isolates, and *Salmonella* Typhi was recovered from 33 (9.0%) of patients.

4. Discussion

The present study showed that nearly two-thirds of clinicians in Kinshasa rely on the clinical picture and the Widal test for the diagnosis of TF. Baseline Widal titres above the cut-off values were very low among healthy adult blood

Table 2
Results for the survey about the indications and interpretation of Widal test among prescribing doctors (n = 155) and nurses (n = 138). Data represent numbers of eligible answers ('no answers' are not listed)

Questions	Doctors		Nurses		
	Yes	No	Yes	No	p value[a]
1. When do you request serology Widal test?					
a. confirmation of typhoid fever in a suspected patient	48	99	101	33	<0.001
b. orientation to the diagnosis of typhoid fever in a suspected patient	137	14	117	19	NS
c. follow-up of treatment of typhoid fever	64	78	79	53	NS
d. diagnosis of carriers of typhoid fever	35	102	74	56	<0.001
2. The Widal test can be false-positive in case of					
a. other febrile illnesses such as malaria	82	73	63	75	NS
b. infections with non-typhoid *Salmonella* spp.	80	75	73	65	NS
c. previous immunization against typhoid fever	39	116	22	116	NS
3. The Widal test can be false-negative in case of					
a. the patient is administered antibiotic treatment	79	76	97	38	<0.001
b. the Widal test is performed during the first week of onset of illness	85	70	45	90	<0.001
4. Which among the alternatives below is the mainstay method of diagnosis of typhoid fever? (only one option could be answered)					
a. Blood culture	67		39		NS
b. Stool culture	70		36		NS
c. Widal test	8		58		<0.001
5. How many samples do you request by patient for Widal test?					
a. one	24		49		NS
b. two samples, with the second at least 7 or 14 days after the onset of typhoid fever	90		54		<0.001

[a] NS: not significant.

Table 3
Results for the survey about the interpretation scenario of Widal test results among 181 prescribing doctors (n = 105) and nurses (n = 76) who declared to be familiar with the interpretation of Widal test titres. Data represent numbers of eligible answers ('no answers' are not listed)

Based on clinical presentation a patient is suspected of typhoid fever. He/she has no antibiotics. Below are the results of the Widal tests.	Do you consider these results as an argument in favour of typhoid fever?					Do you consider these results as an argument to administer antibiotics?				
	Doctors		Nurses			Doctors		Nurses		
	Yes	No	Yes	No	p value[a]	Yes	No	Yes	No	p value[a]
1. TO 160; TH 160	73	28	66	9	NS	78	19	64	9	NS
2. TO ≤40; TH 160	10	89	30	40	<0.001	13	80	33	36	<0.001
3. TO 80; TH 160	39	60	57	14	<0.001	42	55	55	16	<0.001
4. TO 640; TH 320	91	12	63	9	NS	91	8	63	9	NS

[a] NS: not significant.

donors. The laboratories' performance of the Widal test as assessed by EQA and survey was poor, as was the knowledge about the indications and interpretation of the Widal test by prescribers. In addition, the results of a blood culture surveillance study showed that organisms other than *Salmonella* Typhi, in particular NTS and *Enterobacteriaceae*, represented the majority of significant organisms recovered from patients suspected of TF.

The present study suffered from limitations inherent to surveys and EQA sessions. Indeed, samples received as part of EQA may be treated differently compared to routine samples and participants may be triggered to the correct answer in the case of multiple choice questions.[9] This was illustrated by the fact that two-thirds of prescribers correctly replied to submit two samples for the Widal test, whereas 9 in 10 laboratories reported to perform the Widal

Table 4
Distribution of clinically significant organisms recovered from blood cultures in patients suspected of typhoid fever and in patients suspected of other causes of bloodstream infection. Data represent numbers (%). Only the first isolate per patient was included

Organisms	Typhoid fever (n = 3820)		Other causes (n = 2857)		Total
	n	%	n	%	
Salmonella Typhi	92	25.3	33	9.0	125
Non-typhoid *Salmonella* spp.	134	36.9	82	22.3	216
Klebsiella species	57	15.7	117	32.0	174
Escherichia coli	35	9.6	39	10.6	74
Enterobacter species	25	6.9	52	14.1	77
Staphylococcus aureus	20	5.5	44	12.0	64
Total	363	100	367	100	730

test only on a single sample. Further, we did not assess the locally purchased Widal kits with samples from patients with microbiologically proven TF, precluding the definition of a clear-cut threshold value of TO and TH titres among the population in Kinshasa. Likewise, the questions about Widal test interpretation were focused on the results of a single sample. As to the actual performance of the Widal test by other laboratories in the country, it must be reminded that the laboratories participating to the EQA sessions organised by INRB probably represent the better ones among the estimated 8266 HCF in DRC. Another limitation was that the survey about the tools used for the diagnosis of TF was carried out in 2006, whereas the other studies were performed in 2009. With regard to the microbiological surveillance study, it should be noted that the blood culture system was read by visual inspection instead of by the dedicated instrument and that the contamination rate was high, which may have decreased its diagnostic yield. Finally, it should be noted that although the present studies shed a light on the diagnosis of TF, they were not designed to measure actual incidence of TF.

Despite being used for more than 100 years and extensively studied, there is surprisingly little known about the factual performance of the Widal test by diagnostic laboratories. In Cameroon, apparent over-diagnosis of TF incited a survey about the performance of the Widal test in 16 diagnostic laboratories: conventional tube agglutination test with serial dilutions was performed by only two of them, with the others performing a rapid slide agglutination test. As observed during site visits, the study in Cameroon revealed that many simple and straightforward laboratory procedures such as shaking the antigen vial and well mixing the antigen/sample suspension tended to be omitted. In addition, 5/14 laboratories surveyed in Cameroon practised the slide agglutination technique but did not make serial dilutions and laboratory staff reported titres based on the intensity of the agglutination observed on the slide.[8] The present study assessed a higher and probably more representative number of laboratories, and pointed to similar errors occurring even at higher frequencies: at the most relaxed criterion, only 12.0% of laboratories reported correct results for the three EQA samples. Although we presently did not assess the diagnostic accuracy of the locally purchased diagnostic kits, there is evidence to ascribe the poor results to other factors than the kit's performance: among the Widal kits used, no particular brand performed better or worse, and the reference results obtained at INRB with locally available Widal kits were in line (although at lower titres) with those obtained at ITM. Apart from procedural errors that may account for bad performance, there were problems with the cold chain (electricity supply) which are indicative for the overall logistic challenges to laboratories in DRC.

Performing the Widal test on a single sample and by agglutination rather than by the test tube technique are less serious errors that may be accepted in view of the local conditions: many patients are not able to present for a second sample, and in most tropical settings, the laboratory organisation is not prepared to serial test samples. Although the tube test appears to be better standardised, there has been no difference in sensitivity and specificity demonstrated between the slide and tube test method.[10] However, in a recent comparative study in Africa, the slide agglutination technique performed worse than the tube agglutination technique, in particular because of its low specificity.[11] There is much controversy about the usefulness of the Widal test in endemic settings, especially when only a single sample is considered.[4] However, a recent study from Tanzania showed that, when carefully selected, the application of the Widal test on a single serum sample may generate adequate specificity and exclusion power – despite an apparent lack of sensitivity.[5]

As for the laboratory performance, there is little information about the knowledge, attitudes and practice of clinicians concerning the Widal test. The above cited survey from Cameroon revealed findings similar to the present ones: overall knowledge about indications and interpretations among prescribers was low, and significantly lower among nurses compared to doctors.[8] The present study illustrated not only poor knowledge about technical issues of the Widal test (such as causes of false-positive and false-negative results), but also misconceptions about its indication, such as treatment follow-up and detection of chronic carriers.

The data from the baseline study and the microbiological surveillance study provide indirect evidence about the burden of TF in DRC. The low prevalence of O and H antibodies among healthy blood donors suggests that TF is relatively uncommon in Kinshasa. This contrasts with the 53% prevalence of O and H antibody titres $\geq$160 among 200 healthy blood donors from Nigeria[12] as well as with the 8% of O antibody titres among 100 healthy blood donors from Malawi.[13]

Typhoid fever is generally considered as endemic in DRC. In addition to sporadic cases, microbiologically confirmed outbreaks have been recorded. For instance, from December 2004 to January 2005, an epidemic of TF in Kinshasa was traced down from the observations of unusually high numbers of patients presenting with intestinal perforations.[14] The present data seemingly contrasts with these high-profile outbreaks but are in line with the downwards revised estimations of the incidence of TF in Africa.[3,15] They also fit the global estimates of the burden of TF as made by Crump and co-workers in 2004,[16] who categorised Africa as a region of medium incidence (10–100/100 000 cases/year) as opposed to high incidence regions (>100/100 000 cases/year) in south-central and south-east Asia. In-depth population-based epidemiological studies with microbiological confirmation are needed to clarify the real incidence of TF and its geographic and demographic distribution, especially when control measures such as improved sanitation, water supply and vaccination are targeted.[17,18] To our knowledge, only one such study has been carried out in Africa, reporting an incidence of 59 cases per 100 000 persons per year for Egypt.[19]

The bloodstream surveillance study showed *Salmonella* Typhi in 2.4% of patients suspected of TF, which is in line with a previous study in Cameroon detecting *Salmonella* Typhi in 2.5% of blood cultures.[20] Of note was the recovery of other organisms (in particular NTS and other *Enterobacteriaceae*) among patients suspected of TF as well as the recovery of *Salmonella* Typhi from patients suspected of

other foci of bloodstream infections: both observations illustrate the low sensitivity and specificity of the clinical diagnosis of TF, which is challenging because its signs and symptoms overlap with those of many other febrile illnesses.[20] Although not specified to culture site (urine, blood or stool samples), the recovery of other organisms in patients suspected of TF has been described before in a study from Nigeria.[21]

Apart from the Widal test, there were no other antigen- or antibody tests in use for the diagnosis of TF, and only a few sites had culture facilities available. In addition, we documented high sample positivity rates for the Widal test and a high reliance on this test among clinicians. As did Ntusebu in Cameroon,[8] we conclude to an over-notification of TF in Kinshasa, DRC.

The consequences of misdiagnosis of TF are numerous and include increased expenditure for the patient, misdiagnosis of other diseases and unnecessary administration of antibiotics with exposure to the side-effects and increasing antibiotic resistance. The development of affordable microbiological facilities with periodic microbiological surveillance studies may be of use to provide incidence rates of culture-proven TF and to document the actual antimicrobial resistance rates of *Salmonella* Typhi.[22,23] In addition, there is an urgent need for training in the use, performance and interpretation of the Widal test and for appropriate diagnostic tools for TF.

5. Conclusion

In conclusion, the present study illustrates the high reliance on the Widal test as well as a poor technical performance and interpretation of the Widal test by laboratories and clinicians. Based on these observations, we conclude that TF is probably over-diagnosed in Kinshasa. There is a need for microbiological surveillance, training and appropriate tools for the diagnosis of TF.

Authors' contributions: OL, SAM, MFP, JJM and JJ conceived and designed the surveys, OL, PM and JJ conceived and designed the external quality assessment study, OL, EB, PM and JJ conceived and designed the baseline Widal titre study and OL, EB, JV and JJ conceived and designed the microbiological surveillance study. Results were analysed by OL, MFP, PM and JJ and interpretations were done by OL, MFP, EB, PM, SAM and JJ. OL, MFP, SAM, EB and PM drafted the manuscript and JM and JV critically revised it. All authors read and approved the manuscript. OL is guarantor of the paper.

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Competing interests: None declared.

Ethical approval: For the microbiological surveillance study, ethical approval was granted by the Institutional Review Board of ITM and the Ethical Committee of Antwerp University, Antwerp, Belgium. Pending the installation and functioning of the ethical committee in Kinshasa, clearance for the studies was obtained by the MoH, 4th Direction.

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