COMPARATIVE STUDY BETWEEN THE WIDAL/FELIX TEST AND STOOL CULTURE IN THE DIAGNOSIS OF SALMONELLOSIS: CASE OF THE URBAN COMMUNE OF DALABA (REPUBLIC OF GUINEA)

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ABSTRACT

Introduction: In human pathology, salmonellosis includes two main types of illness: gastroenteritis and typhoid and paratyphoid fever. *Objective:* Contribute to improving the health care of patients suffering from typhoid fever. *Methods:* This is a comparative, prospective and descriptive study of a cross-sectional type which was carried out over a period of three months, going from April 2 to July 2, 2023. *Results:* From 230 patients received at the laboratory, samples of 5 patients revealed positive antigen reactions (TO+/TH+), i.e. a prevalence of 2.17%, 9 samples reacted only to the TO antigen (TO+/TH-), i.e. 3.91% and 30 patients reacted to the antigen TH (TO-/TH+), i.e. 13.04%. On the other hand, the majority of blood serum samples did not react to any of the antigens (TO-/TH-), i.e. 80.87%. Diagnosis by the Widal/Félix method is the most sensitive with 14 positive cases, or 6% compared to stool culture with 4 positive cases, or 1.74%. Of the 14 positive stool and blood samples (blood culture and stool culture), Salmonella showed sensitivity to Amoxicillin with 50% and resistance with 50%, followed by Ciprofloxacin with 29% sensitivity, 7% sensitivity intermediate, 50% resistance and 14% sensitivity not determined. Erythromycin and Kanamycin reacted with 21% sensitivity, 56 and 50% resistance respectively. Ceftriaxone recorded the lowest sensitivity with 14%. The female gender was the most represented with 64.28% compared to 35.71% for the male gender. Students are the most represented with 28.57%, followed by Drivers and Workers with 21.43% each and Housewives with 14.28%. Butchers and Masons are the least represented with 7.14% each. Singles are the most represented with a prevalence of 57% compared to 42.86% among Marrieds. Patients whose age groups are between 11-20 years and 31-40 years are the most affected by typhoid fever with 28.57% each followed by those aged 41-50 years and 51-60 years with respectively 14 .28% and 7.14%. Patients from the urban commune are the most represented with 64% and those from the sub-prefectures of Dalaba represent only 36%. *Conclusion:* This study showed that typhoid fever remains a major public health problem in the Republic of Guinea and the most reliable diagnosis is culture.

INTRODUCTION

In developing countries where bacterial culture is expensive, Widal and Félix serodiagnosis remains the most accessible diagnostic method and is used for the greatest number of typhoid fever diagnoses. This method would contribute to increasing the number of cases of typhoid fever, thanks to its numerous false positive results. Despite its significant limitations, this test can be useful, particularly in regions which cannot afford other more expensive diagnostic means, provided that the results are interpreted with caution, taking into account the threshold values determined within the the local population (Mohamed Stitou, 2019). Bacterial culture, a real anxiety for populations living in these tropical regions, is one of the main concerns of both the patient and the medical profession. Furthermore, frequent rumors surrounding sudden deaths, unrelated to malaria, have led to an increased demand for "Widal". Often this request is made by the patient directly to the medical analysis laboratory without prior medical consultation. This results in self-medication with excessive consumption of antibiotics (Youssouf Mariko, 2021).

Bacterial culture remains the reference test to confirm the presence of the live bacteria sought in a sample contaminated by other bacteria. This is the conventional method for the isolation and identification of Salmonella. Salmonella are mesophilic bacteria, not very demanding from a nutritional point of view. They can be grown on ordinary medium containing meat extracts. They are facultative aero-anaerobic (Jakdalli Morad et Chettouh Saad, 2018). The biological confirmation of typhoid fever is based, for direct diagnosis on blood culture and stool culture and for indirect diagnosis, on the serodiagnosis of Widal and Félix. Blood culture and stool culture make it possible to demonstrate the etiological agent, salmonella. These two diagnostic techniques are indicated, one for the first week of the disease and the other for the second week. As for the serodiagnosis of Widal and Félix, it targets antibodies directed against the bacteria and is indicated when blood culture and stool culture are no longer possible (Dorothée M. Houngnon, 2020). Salmonella are more specifically adapted to humans, mammals (rodents), birds (poultry) and cold-blooded animals (reptiles). The main mode of contamination in humans is ingestion from water (mainly Salmonella typhi), food (e.g. dairy products, eggs, meat) or from carrying pets (turtles). Typhoid and paratyphoid fevers are caused by four Salmonella serovars, strictly human, antigenically distinct but with similar pathogenicity: Salmonella typhi, Salmonella paratyphi A, Salmonella paratyphi B and Salmonella paratyphi C. These salmonellas are called major because of the severity of the pathology they cause (En Fuli Wu, 2016). Like all enterobacteria, Salmonella potentially possess three types of antigens of diagnostic interest: somatic O antigens, flagellar H antigens and surface or virulence antigens (Sylla Hawa, 2019). In a liquid medium, enterobacteria cause a homogeneous cloudiness of the broth (Douhan Hassana, 2021). The serodiagnosis of the Widal reaction becomes positive after approximately one week of development. The serum is tested against O and H suspensions of Salmonella typhi and Salmonella paratyphi A, B and C. The O antibodies appear first but disappear shortly after recovery. H antibodies appear a few days later, reach hgher levels and can persist for several months after illness (En, 2019).

MATERIAL AND METHODS

Study environment and framework: this study was carried out in the Urban Commune of Dalaba. It is one of three prefectures falling within the administrative region of Mamou. The Dalaba prefectural hospital served as the setting for our study.

Work materials: Materials and reagents for Widal and Félix : 5cc syringe, EDTA-free tubes, white background plate, disinfectant, racks, Widal and Félix box (TO ; TH).

Equipment and reagents for Coproculture: incubator 37°, Bain Marie 50-60°, Hood, Vortex, Stirring hot plate, Petri dishes, Bunsen burner, Autoclave, 1ml graduated pipettes, Platinum handle, Binocular microscope, Centrifuge, EPT: Peptone water buffer, Rappaport Vasiliadis, MacConkey Agar, Gram box, Distilled water etc.

Materials and reagents for the Antibiogram: densitometer or turbidity standards (Mac Farland scale), Petri dishes (square at 120 mm or round at 90 mm), Screw tubes containing 10 ml of physiological water, Müeller Hinton agar , Antibiotic discs etc.

Working methods: this is a comparative, prospective and descriptive study of a transversal type which was carried out over a period of three months, going from April 2 to July 2, 2023. All patients received in consultation at the Dalaba prefectural hospital during our survey period constituted our study population. Sampling was simple random and the sample size (n=230) was using the Schwartz formula. The patients were received at the laboratory of the Dalaba prefectural hospital with the following information: First and last names, age, sex, residence, professions, contacts and identification numbers which were systematically noted on the samples.

Bio-material: It consists of blood and stools of patients.

Variables subject to study

Biological variables: Widal/Félix, Stool culture and Antibiogram.

Epidemiological variables: Ages, Sex, Professions and Residence.

Data collection method: Information is collected from:

- Pre-established survey sheets;
- Laboratory records
- Consultation notebooks.

Ethical considerations: before carrying out the study, we obtained the agreement and consent of each patient, confidentiality was respected throughout the data collection procedure and the results were used for purposes strictly therapeutic and scientific purposes. Our study complied with the Helsinki Declaration on Ethical Principles Applicable to Medical Research Involving Human Beings of 1975 as amended in 2008.

Computer analysis of the data: the information collected was analyzed manually, entered using Microsoft Word and Excel software under Windows 2016 and the analyzes were carried out using Epi Data software. For the analysis, we used SPSS® version 21 software.

Limitations and difficulties: The relative weakness of the sample size, the non-use of blood culture and PCR in the diagnosis of salmonellosis.

RESULTS AND DISCUSSION

The application of the research methodology led to the following results in the form of tables interpreted, commented and discussed according to the available literature data.

Table 1. Diagnosis of salmonellosis in patients according to the type of agglutination of the Widal/Felix test

N°	Agglutination	Number	Percentage
1	TO+/TH+	5	2.17
2	TO+ /TH-	9	3.91
3	TO- /TH+	30	13.04
4	TO- /TH-	186	80.87
	Total	230	100

This table indicates that of the 230 patients received at the laboratory, samples from 5 patients revealed antigen reactions (TO+/TH+), i.e. a prevalence of 2.17%, 9 samples reacted only to the TO antigen (TO+/TH+). TH-), i.e. 3.91% and 30 patients reacted to the TH antigen (TO-/TH+), i.e. 13.04%. On the other hand, the majority of blood samples did not react to any of the antigens (TO-/TH-), i.e. 80.87%. The high prevalence of TO+/TH+ cases could be explained by the fact that patients were consulted at an advanced stage of the disease (chronic). Patients in the TO+/TH- category would be received at the primary infection stage (acute phase). The immunological reaction with flagellar antigens (TH) only could be explained by the presence of immunological scars (presence of flagella in the blood) which persist even after healing. Those from TO-/TH- have not been in contact with salmonella.

Table 2. Distribution of comparison results between the Widal/Felix method and coproculture

Methods	Results				
	Effective	Positives		Négatives	
		Number	Percentage	Number	Percentage
Widal/Félix	230	14	6	216	93.91
Coproculture	230	04	1.74	226	98.26

The comparison results of this table showed that out of 230 blood and stool samples examined, the diagnosis by the Widal/Félix method is more sensitive with 14 positive cases, i.e. 6% compared to that of stool culture with 4 cases. positive, or 1.74%.

However, this higher sensitivity could be explained by the fact that the serodiagnosis of Widal/Félix is based on antibodies directed against Salmonella (somatic "O" and flagellar "H" antigen), on the one hand and the use of antibiotics by certain patients making stool culture negative. Coproculture made it possible not only to make a correct biological diagnosis of salmonellosis but also to identify some subspecies and types of Salmonella: (Salmonella enterica typhimerium and Salmonella enterica spp arizonae).

Table 3. Determination of antibiotic susceptibility of Salmonella typhi

Antibiotiques	S N (%)	I N (%)	R N (%)	ND N (%)	Total
Salmonella typhi					
Ciprofloxacine	4(29)	1(7)	7(50)	2(14)	14(100)
Ceftriaxone	2(14)	5(36)	5(36)	2(14)	14(100)
Erythromycine	3(21)	2(14)	5(56)	4(9)	14(100)
Kanamycine	3(21)	2(14)	7(50)	2(15)	14(100)
Amoxicilline	7(50)	0(00)	7(50)	0(00)	14(100)

The results of this table showed that of the 14 positive stool and blood samples (blood serum and stool culture), the Salmonella showed sensitivity to Amoxicillin in 7 cases, i.e. 50% and resistance with 50%, followed by of Ciprofloxacin with 4 cases of sensitivity, i.e. 29%, 7% intermediate sensitivity, 50% resistance and 14% undetermined sensitivity. This antibiotic is followed by Erythromycin and Kanamycin with 21% sensitivity, 56 and 50% resistance respectively. Ceftriaxone recorded the lowest sensitivity with 2 cases or 14%, but on the other hand it presented 56% resistance for 5 cases. This resistance of Salmonella to these antibiotics would be due to the self-medication of patients, taking antibiotics before sampling, on the one hand and by the acquisition of resistance by chromosomal and plasmid mechanisms, on the other hand.

Table 4. Distribution of patients with typhoid fever according to socio-demographic parameters

Parameters	Effective	Percentage
Sexes		
Female	9	64,28
Male	5	35,71
Socio-professional categories		
Mason	1	7,14
Drivers	3	21,43
Students	4	28,57
Workers	3	21,43
Housewives	2	14,28
Butcher	1	7,14
Marital status		
Marrieds	6	42,86
Singles	8	57,14
Age groups		
0-10 years	-	-
11-20 years	4	28,57
21-30 years	3	21,43
31-40 years	4	28,57
41-50 years	2	14,28
51-60 years	1	7,14
61 years and over	-	-
Residence		
Urban community	9	64,28
Rural municipalities	5	35,71
Total	14	100

In this table, we see that of the 14 patients suffering from typhoid fever, the female sex was the most represented with 9 cases, i.e. 64.28% compared to 5 cases for the male sex, i.e. 35.71% for a gender. ratio of 1.8 in favor of the female sex. This high prevalence among females is random because both sexes are exposed to contamination in the same way. It would be due to the high attendance of women at the Dalaba prefectural hospital during the period of our investigation. Compared to socio-professional categories, Students are the most represented in this series with 4 cases, i.e. 28.57%, followed by Drivers and Workers with 3 cases each, i.e. 21.43% and

Housewives with 2 cases. , or 14.28%. Butchers and Masons are the least represented with 1 case for each, or 7.14%. The high prevalence among students reflects the insufficient food hygiene in Dalaba schools. In relation to marital status, Singles are the most represented with 8 cases, i.e. a prevalence of 57% compared to 6 cases among Married people with 42.86%. Compared to age groups, patients whose age groups are between 11-20 years and 31-40 years are the most affected by typhoid fever with 4 cases each, or 28.57% followed by those of 41-50 years old and 51-60 years old with 14.28% and 7.14% respectively. Compared to the residence, patients from the urban municipality are the most represented with 9 cases, or 64%. Patients from the Dalaba sub-prefectures represent only 36%.

DISCUSSION

Mawazo et al. in 2019, found good statistical agreement (kappa = 0.33) between stool culture and blood culture and poor agreement (kappa = 0.01) between Widal titration and stool culture (Mohamed Stitou, 2019). Similar findings were observed in a study conducted by Andualem et al. in 2014 (Andualem, 2014). Another study done in Ethiopia also found similar results. This indicates that the result of the Widal/Felix test in the diagnosis of typhoid fever is less likely to corroborate with that of stool culture. In our study, female patients were the most represented with 64.28% compared to 35.71% for males for a sex ratio of 1.8 in favor of females (F/M) (Table 4). Our results are contrary to those reported by Godefroid et al. in 2023, in a study involving 63% of male subjects compared to 37% female subjects, i.e. a sex ratio (M/F) of 1.7 (Godefroid Ngeda Gombima, 2023). In our study, patients whose age groups are between 11-20 years and 31-40 years were the most affected by typhoid fever with 28.57% followed by those aged 41-50 years and 51-60 years. with 14.28% and 7.14% respectively (Table 4). Our results are contrary to those reported by Godefroid et al. in 2023, most of the respondents were aged between 0-10 years, 10 out of 30, or 33.3%, followed by those who were aged between 11-20 years, 07 respondents out of 30, or 23, 3% (Godefroid Ngeda Gombima, 2023). The comparison results of our study showed that out of 230 blood and stool samples examined, diagnosis by the Widal/Félix method was the most sensitive with 6% compared to stool culture which represented only 1.74% (Table 2). Our results are different from those reported by Godefroid et al. in 2023 who found that the prevalence of salmonellosis confirmed by the Widal/Felix test was 83% while with stool culture it was 17% (Godefroid Ngeda Gombima, 2023). A study carried out by Youssouf Mariko, in 2022, in Mali, showed that the sensitivity, specificity, negative predictive value and positive predictive value of the Widal/Félix test remain less than 80%. Its effectiveness in diagnosing typhoid fever without the need for another confirmatory test has no diagnostic value today. Therefore, the Widal/Félix test should not be used as a diagnostic tool to rule out a case of typhoid fever unless it is consistent with the clinical and blood or stool culture results (Youssouf Mariko, 2022).

CONCLUSION

This study showed that typhoid fever remains a major public health problem in the Republic of Guinea. Diagnosis by the Widal/Félix method is the most sensitive with 6% compared to that of coproculture which represented only 1.74%. This first method would contribute to increasing the number of positive cases of typhoid fever, thanks to its numerous false positive results which can lead to self-medication, economic losses and excessive antibiotic prescriptions within the population, while bacterial culture remains the best method in the diagnosis of salmonellosis. Of the 14 positive stool and blood samples (Widal/Félix and Coproculture), Salmonella showed sensitivity to Amoxicillin with 50% followed by Ciprofloxacin with 29%. This indicates a significant increase in resistance of Salmonella to antibiotics. Coproculture made it possible not only to make a correct biological diagnosis of salmonellosis but also to identify some subspecies of Salmonella: (Salmonella enterica typhimerium and Salmonella enterica spp arizonae).

Conflicts of interest: None.

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