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Susceptibility profile of *Salmonella typhi* isolated from typhoid patients attending the Federal Medical Centre, Gusau, Nigeria

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ABSTRACT: A total of 83 blood samples from patients suspected of typhoid fever were collected and examined for *S. typhi* infection by Widal and Blood culture methods and then antibiotics susceptibility testing was conducted on the *S. typhi* isolates. Thirty-five (42.2%) had *S. typhi* 'O' and 'H' antibody titre of 1:160 and 1: 80 respectively, 15 (18.1%) had *S typhi* 'O' and 'H' antibody titre of 1:160 and 1:40 respectively while 13 (15.7%) had 1:20 antibody titre in both 'O' and 'H' *S. typhi* antigens. The reciprocal titre of 1:320 and 1:80 for 'O' and 'H' antigens was shown in 15(18.3%) samples. Twenty-nine (34.9%) were blood culture positive. Ampiclox, ciprofloxin, chloramphenicol and erythromycin were the most sensitive, with susceptibility pattern of 14 (46.7%), 14 (46.7%), 10 (34.5%) and 7 (23.3%) respectively. The incidence of typhoid fever will greatly reduce if isolation of *S. typhi* from clinical specimen like blood is encouraged and the typhoid treatment based on susceptibility testing.

Keywords: Isolation, *Salmonella typhi*, Patients, Susceptibility, Antibiotics

Introduction

Typhoid fever is a bacterial illness caused by *Salmonella typhi*, a Gram-negative, rod-shaped bacterium found only in humans (David, 2005). Single Widal test on serum during acute illness together with compatible features has been the adopted option in most clinics/hospitals in an endemic area of typhoid/paratyphoid fevers in developing nations like Nigeria (Tanyigna *et al.*, 1999). This is the case in Gusau township where single Widal test is used in diagnosing typhoid fever. Similarly, the ease in the procurement of antibiotics and their indiscriminate use prior to coming to the clinic/hospital has made the treatment of typhoid fever particularly with chloramphenicol become less effective.

There appears to be no record of susceptibility pattern of *S. typhi* isolates to some antibiotics commonly obtainable at the market in most clinics/hospitals in Gusau. This informed the need for this study to be carried out so that proper antibiotics treatment is procured. Multiple drug resistance by *S. typhi* has been documented in many parts of the world including Nigeria. This may be probably due to widespread and indiscriminate use of anti-typhoid drugs in cases that were not bacteriologically proven. Thus the aim of this work was to isolate *S. typhi* from blood of patients suspected of typhoid fever and test the isolates against some commonly available antibiotics sold in the Market.

Materials and Methods

Blood was collected from eighty-three (83) febrile patients reporting to Federal Medical Center Gusau, Zamfara State, north-western Nigeria within the first (10) days of fever and had not started taking treatment and clinically diagnosed as having typhoid fever. Five millimeters (5ml) of blood was collected by venepunture. A quantity (2 ml) of the blood was allowed to clot at room temperature and centrifuged for 5 minutes at 3000 rpm to obtain the serum.

Widal test was conducted following the manufacturer's instructions (Antec Diagnostic Products, 2005). A quantity (3ml) was dispensed into McCartney bottles containing 10mls thioglycholate broth. These were inoculated into *Salmonella-Shigella* (SS) medium, and then incubated at 37°C for 24 hours. Samples without growth were re-incubated at the same temperature for 7 days, and sub-cultured again into SS medium before being discarded. Pale non-lactose fermenting colonies were picked from the SS plates and confirmatory biochemical tests, which include Hydrogen Sulphide Production, Lysine decarboxylation, glucose, lactose, motility, indole, and oxidase were performed according to Chesebrough (1984).

Isolates confirmed by biochemical test were tested for their susceptibility to ten (10) antimicrobial agents using MIC (Minimum Inhibitory Concentration) test based on the method described by Ericson and Sheriss (1971) at the following disc contents: Ampiclox (30ug), Ciprofloxin (10ug), Norfloxaxin (10ug), Gentamycin (10ug), Lincocin (30ug), Streptomycin (30ug), Rifampin (10ug), Floxapen (30ug), Erythromycin (30ug) and Chloramphenicol (20ug). Nutrient agar plates were used for the sensitivity test and incubated at 37°C for about 24 hours. The minimum inhibition concentration (MIC) was the least antimicrobial concentration that yielded no visible growth.

Results

Out of the 83 blood samples, sera from 35(42.2%) had reciprocal titre level of 1:160 and 1:80 for 'O' and 'H' antigens respectively and out of which 15 (45.8%) were confirmed by culture. From the 20(24.1%) sera having the titre of 1:160 and 1:40 of 'O' and 'H' antigen respectively, 8(53.3%) were confirmed by culture. Six (46.2%) salmonellae were isolated from 15(18.1%) that had 'O' and 'H' antigens levels of 1:320 and 1:80 respectively. None was discovered from the Widal negative samples (Table 1).

Table 1: Results of Widal test and blood culture

S. typhi antigens		No. (%) of patients	No. of samples from which S. typhi was isolated
O/ag	H/ag	13 (15.70)	0 (0.00)
1:20	1:20	20 (24.10)	8 (53.30)
1:160	1:40	35 (42.20)	15 (42.80)
1:160	1:80	15 (18.10)	6 (46.20)
1:320	1:80		

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Table 2 presents the concentrations and the susceptibility pattern of the S. typhi isolates to the antibiotics commonly available and sold at the market. The microbial susceptibility testing revealed that none of the isolates was susceptible to all the drugs. However, 14(46.7%) of the isolates were susceptible to ampiclox and ciprofloxin each. Ten (34.5) and 7(23.3) isolates were susceptible to chloramphenicol and erythromycin respectively. The number and percent of resistance to other antimicrobial agents were 28(96.6%) in each of gentamycin, lincocin, rifampin and floxapen while 27(93.1%) and 26(89.7%) were resistant for streptomycin and norfloxacin respectively.

Table 2: Results of susceptibility patterns of the S. typhi isolates to the antibiotics

Antibiotics/Conc. (µg)		Isolates sensitive (%)	Resistant isolates (%)
Ampiclox	30	14 (46.70)	15 (51.70)
Ciprofloxacin	10	14 (46.70)	15 (51.70)
Norfloxacin	10	3 (10.30)	26 (89.70)
Gentamycin	10	1 (3.40)	28 (96.60)
Lincocin	30	1 (3.40)	28 (96.60)
Streptomycin	30	2 (6.90)	27 (93.10)
Rifampin	10	1 (3.40)	28 (96.60)
Floxapen	30	1 (3.40)	28 (96.60)
Erythromycin	30	7 (23.30	22 (75.90)
Chloramphenicol	20	10 (34.50)	19 (65.50)

Discussion

Salmonellae are motile, Gram negative, rod-shaped bacteria that belong to the Enterobacteriaceae family. The specie is a close relative of *Escherichia coli* (Heran and Virginia, 1995). *S. typhi*, which is the causative agent of typhoid fever, has the somatic (O) and the flagellar (H) antigens that can be detected by Widal agglutination test (Chew *et al.*, 1992). However, the diagnostic value of this test is debatable, for it gives variable results in areas of the tropic and thus many found it difficult to interpret in endemic areas (Antec Diagnostic Products, 1995). Definitive diagnosis depends on isolating the aetiological agents from clinical specimens (Awotedu *et al.*, 1992). Typhoid organisms can usually be isolated from the blood stream in the first 7 to 10 days of the febrile period. However, highest percentage of positive cultures in the second week of the illness had been found by some authors (Akoh, 1991).

The reliability of isolation of the organism from clinical specimen (blood) has been confirmed by this study in which out of the 70 (84.3%) that were positive by Widal, only 29(34.9%) were confirmed by the blood culture. So much controversy surrounds preference for Widal test to culture tests and vice versa. However, the general agreement has been that Widal test has lots of pitfalls including false negatives and positives (Tanyigna *et al.*, 1991; 2001). The suggestion is that therefore, presumptive serology test (Widal test) should go together with culture. According to WHO (2005), efficacy, availability and cost are important criteria to be used in developing countries for the treatment of typhoid fever. Chloramphenicol is the drug of treatment in many countries. However, it is a very safe drug and aplastic anaemia, which may arise from its use, is particularly dreaded (Punjabi, 2000). The flouroquinolines (oxfloxacin, afrofloxacin, perfloxacin, fleroxacin, perfloxacin) are widely regarded as optimal for treatment of typhoid fever in adults (Awotedu *et al.*, 1992). They are relatively inexpensive, well tolerated and more rapidly and reliably effective than the former first line drugs viz. chloramphenicol, ampicillin and amoxacillin. Odugbemi *et al* (1994) commented that in all cases of positive cultures, antibiotics sensitivity results to chloramphenicol,

ampicillin, cotrimoxazole and norfloxacin (or ciprofloxin) should be considered, for these antimicrobials have been useful in the treatment of typhoid, the susceptibility pattern of the isolates in this study is disturbing, since many of isolates were resistant to those antibiotics commonly used in the medical practice (Table 2). Until the indiscriminate use of those antibiotics is controlled and the sensitivity testing be relied upon purely, the treatment of typhoid fever will become increasingly difficult.

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