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ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF CLINICAL ISOLATES OF SALMONELLA TYPHI ASSOCIATED WITH SEPTICEMIA AMONG CHILDREN AGED 0-5 YEARS IN KANO, NIGERIA

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ABSTRACT

Aim: Typhoid fever is a preventable and treatable enteric disease which can be effectively treated when adequately diagnosed and right antibiotic choice made. It is a major cause of morbidity and mortality in 10-18% of affected children aged 0-5 years. The aim of this work was to determine the antibiotic susceptibility pattern of clinical isolates of *Salmonella typhi* associated with septicaemia among children aged 0-5 years in Kano.

Methods: One hundred and eighty (180) of the isolates were randomly selected from four hospitals in Kano metropolis. The samples were analyzed for in vitro sensitivity pattern by disk diffusion technique.

Results: This study showed that cefriaxone and cefotaxime were the most sensitive (>90), ciprofloxacin was of intermediate sensitivity while more than 50% were resistant to chloramphenicol and trimethoprim/sulphamethoxazole. Eight (4.44%) multi drug resistant (MDR) *S. typhi* were detected and screened for Extended Spectrum β-Lactamase (ESBL) by double disk synergistic test using amoxicillin clavulanate, whereby 2 (25%) were positive while all the multidrug resistant (MDR) including the ESBL positive were sensitive to meropenem.

Conclusion: Blood culture is a useful method for effective diagnosis of *Salmonella typhi* infection.

Key Words: Susceptibility, Isolates, Salmonella typhi, Septicaemia

INTRODUCTION

The World Health Organisation (WHO, 2011) estimates 16-33 million cases of typhoid fever worldwide and 500,000-600,000 deaths annually (Archana and Russell 2014 and Wain et al., 2015). It is endemic in many developing areas of the world (Wain et al., 2015). Increasing numbers of septicaemia cases due to S. typhi are being reported in Africa especially in Kenya (Mills-Robertson et al., 2002), Ghana **(**Kariuki et al., 2010**)** and Nigeria (Ogunleye et al., 2005 and Obaro et al., 2011). It is termed as the leading cause of vaccine-preventable disease among international travellers in developed countries (Wain et al., 2015). Chloramphenicol, ampicillin and cotrimoxazole were defined as the first line drugs in the treatment of typhoid fever (WHO. 2003; Mathew et al., 2015 and Wain et al., 2015). S. enterica serovar Typhi strains resistant to all the first line drugs emerged in the 1970s, thus termed Multi Drug Resistant (MDR). Since then, these MDR strains have spread in an epidemic form and have rapidly emerged worldwide (Basudha et al., 2007 and Mathew et al., 2015). Consequently, the quinolones and fluoroquinolones (ciprofloxacin and ofloxacin), third generation cephalosporins (ceftriaxone and cifixime), and azithromycin came up as the 2nd line drugs for the treatment of multidrug resistant strains (Raveendran et al., 2010; Arora and Arora, 2012 and Mathew et al., 2015). Unfortunately, there is a changing trend in susceptibility pattern of *S.*

enterica serovar Typhi worldwide with emerging resistance to fluoroquinolones. This has greatly reduced the therapeutic options available (Sehra et al., 2013 and Srirangaraj et al., 2014). Currently, Fluoroquinolones such as Ciprofloxacin are recommended by the World Health Organization (WHO, 2011) because they are the most effective, orally active, inexpensive and well-tolerated drugs for the treatment of typhoid fever (Wain et al., 2015). Lately, multi-drug resistance in *S.* Typhi equally resistant to Cephalosporins or Fluoroquinolones has been reported and has become a global challenge (Basudha et al., 2007; Rahelah et al., 2014 and Wain et al., 2015). As a result, Aztreonam and imipenem are also potential third line drugs that have been used recently in serious infections (Mathew et al., 2015 and Wain et al., 2015) but surveillance data from many countries worldwide shows that ceftriaxone resistant to *S. enterica* serovar Typhi still remain low all over the world (Raveendran et al., 2010). The threat of invasive, multidrug-resistant organisms circulating in a region with little capacity for epidemiology and laboratory study show the need for international collaboration and standardized methods to identify and subtype these pathogens (Santos et al., 2001 and Wain et al**.,** 2015). This work was undertaken to determine the sensitivity pattern of some clinical isolates of *S. typhi* by dick diffusion technique and to determine the Extended Spectrum Beta Lactamases (ESBL) producing strains among the Multidrug Resistant isolates (MDR *S. typhi).*

MATERIALS AND METHODS

Study Area

Clinical isolates of *S. typhi* isolated from children aged 0-5 years with septiceamia were randomly selected from Aminu Kano Teaching Hospital (AKTH), Murtala Muhammad Specialist Hospital (MMSH), Hasiya Bayero Pediatric Hospital (HBPH) and Muhammad Abdullahi Wase Specialist Hospital (MAWSH) Kano.

Ethical Consideration

An ethical clearance was obtained from relevant authorities while an accent formed were completed by each consenting parent on behalf of the child.

Sample Size

One hundred and eighty (180) clinical isolates of *S. typhi* isolated from children aged 0-5 years with septiceamia were randomly selected from the study hospitals in Kano.

Sample Analysis

Culture: Automated blood culture BACTEC™ system was used. 1-3ml of patient blood was inoculated into the Paediatrics Plus BACTEC vial, which comprises of non-radioactive highly enriched culture media including a patented resin which aid neutralise the antibiotics in the blood sample. Incubation was carried out at 35-37^{0}C.

Identification: All the bacterial isolates that conform to the colonial morphology and are gram negative bacilli and also oxidase negative were subjected to the Analytical profile index (API) 20E (Bomeriex France, API) for the identification and differentiation of members of the family Enterobacteriacea based on the manufacturer's instructions.

Serological Test: Serologicat test were performed with polyvalent and monovalent antisera (Becton, Dickinson (BD) Difco™, 2010).

Antimicrobial Sensitivity: Following primary identification of the bacteria isolates, all confirmed S. Typhi were purified and subjected to antibiotic susceptibility testing using a modified form of the Kirby Bauer method (Bauer *et al.,* 1966 and Arora and Arora, 2012). Single disc antimicrobial agents (Oxoid, Basingstoke United Kingdom) of Ciprofloxacin (5µg), Cotrimozaxole (1.25/23.75µg), Amoxicillin/Clavulanic acid (30µg), Ceftriazone (30µg), Cefotaxime (30µg), and Chloramphenicol (30µg) were dispensed onto the surface of the agar plates. The plates were aerobically incubated at 37°c for 18 -24 hours. Results were recorded by measuring the diameter of the zone of inhibition around the antibiotic (CLSI, 2015).

Detection of ESBL: Isolates resistant to Amoxicillin/Clavulanic acid, Ceftriazone, Cefotaxime, only or resistant to Ciprofloxacin as well were further screened for Extended Spectrum β-lactamase (ESBL). A double disk synergistic method using Amoxicillin clavulanic

acid and two third generation Cephalosporins and Ceftazidime was adopted. A ≥5mm increase in zone diameter for either antimicrobial agent tested in combination with Clavulanic acid versus its zone when tested alone is interpreted as ESBL positive (CLSI, 2015).

Data Analysis

Descriptive statistics was performed for all analysis. Data were presented in tables and percentages.

RESULTS

There were a total of 180 clinical isolates of the *S. typhi* that were analysed and the distribution is presented in Table1. The results of the antibiotic sensitivity patterns against *S. typhi* that were studied using various antibiotics are shown in Table 2. Among the six antibiotics tested the study revealed that cefriaxone and cefotaxime showed the highest sensitivity with percentage sensitivity values of 92.2 % and 90.0 %, respectively, and ciprofloxacin was of intermediate sensitivity. Chloramphenicol and cotrimoxazole showed 66.1 and 35.0 % resistance among the obtained clinical isolates respectively. Eight (4.44%) multi drug resistant (MDR) *S. typhi* were detected and screened (Table 3) for Extended Spectrum β-Lactamase (ESBL) by double disk synergistic test using amoxicillin clavulanate, whereby 2 (25%) were found to be positive, and all the multidrug resistant (MDR) including the ESBL positive were sensitive to meropenem.

Table 1: Distribution of Clinical Isolates of *S. typhi* Based on Study Site.

Site	Frequency	%
AKTH	15	8.3
MMSH	53	29.5
HBPH	112	62.2
MAWSH	0	0.0
Total	180	100

Key: AKTH = Aminu Kano Teaching Hospital; HBPH = Hasiya Bayero Pediatric Hospital; MAWSH = Muhammad Abdullahi Wase Specialist Hospital; MMSH = Murtala Muhammad Specialist Hospital, Kano.

Table 2: Antibiotic Sensitivity Pattern of Clinical Isolates of S. typhi by Disk Diffusion Method.

Antibiotic	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
CRO	166(92.2)	10 (5.6)	4 (2.2)
CTX	162 (90.0)	14 (7.8)	4(2.2)
CIP	63 (35.0)	107 (59.4)	10 (5.6)
AMC	128 (71.1)	18 (10.0)	34 (18.9)
CHL	114(63.3)	3 (1.7)	63 (35.0)
SXT	54 (30.0)	6 (3.9)	119 (66.1)

Key: CRO=Ceftriazone (30µg); CTX= Cefotaxime (30µg); CIP= Ciprofloxacin (5µg); AMC= Amoxycillin/Clavulanic acid (30); CHL= Chloramphenicol (30µg); SXT= cotrimoxazole (1.25/23.75); MEM-Meropenem (10µg).

Table 3: Distribution of ESBL Producing *S. typhi* among the MDR *S.typhi* Isolates.

ESBL	MDR	%
Positive	2	25.0
Negative	6	75.0
Total	8	100

Key: ESBL=Extended Spectrum β-Lactamase; MDR=Multi Drug Resistance

DISCUSSION

The results of this study demonstrated a considerable resistance in *S. typhi* to chloramphenicol (35.0%) and cotrimoxazole (66.1%) and that agrees with other research conducted in Nigeria by Ogunleye et al., (2005) who reported 36.8% and 84.2% resistance of *S. typhi* to chloramphenicol and cotrimoxazole

respectively, similarly in parts of Asia as reported by Thiem et al., (2011) and by Wain et al., (2005) as a global challenge. But our finding disagree with that of Ishaleku (2015) who reported in his study a high susceptibility of 85.9% for *S. Typhi* strain to chloramphenicol in Nassarawa state of Nigeria. The emergence of MDR S. Typhi led to the use of ciprofloxacin as the mainstay in the treatment of enteric fever as it is orally effective and economical there by leading to selective pressure been exerted by the overuse of these drugs and subsequent selection of the resistant strains over the susceptible strains. The emergence of S. typhi highly resistant to ciprofloxacin is a cause for worry for both clinicians and microbiologists as well as for patients. Though fluoroquinolone resistance is chromosomally mediated. The finding of clinical isolates exhibiting intermediate sensitivity in present study is an indication that *S. Typhi* in the research area have the potential to develop resistance for routinely prescribed antimicrobial especially to Ciprofloxacin which is currently a first line drug for the treatment of typhoidal sepsis in all age groups drugs, and pose considerable health hazards to consumers, and these agree with the finding of Abakpa et al., (2015) and Wain et al., (2015). Increasing MIC and resistance to newer quinolones in this research strengthens the fear for of potential treatment failures and development of complications and necessitate the need for new, alternative antimicrobials as reported by Ishaleku, (2015). Coincidentally, resistance to third generation (broad spectrum) cephalosporins was also detected in this research and is similarly reported in other parts of Nigeria by Abakpa et al., (2015). This potentially renders treatment with Extended-spectrum cephalosporins unsuitable as is reported in different parts of the world especially in Asia by Wain et al., (2015) and in Africa by Mwueu and English, (2008) in Kenya. On the other hand the discovery of the low incidence of MDR *S. typhi* strain in this research is in accordance with the findings of Wong et al., (2016) which reported that the rapidly expanding resistant *S. typhi* clade (H58) previously associated with multiple antimicrobial resistances in Asia and in east, central and southern Africa, was not detected in a research that included isolates from Kano.

CONCLUSION

Septicaemia due to *S. typhi* is common among the children in the study area and the clinical isolates were found to be most sensitive to third generations cephalosporins but infection with MDR and ESBL may also occurs. Therefore, conducting blood culture as early as possible in suspected children will greatly reduce mortality and morbidity as well as aid in prompt detection of MDR strains.

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