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Antimicrobial Resistance Profile of *Salmonella enterica* Serovar *typhi* Isolated from Human Clinical Samples in Ebonyi State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The emergence of resistance to the frequent use of empirical treatment of uncomplicated enteric fever caused by *Salmonella enterica* serovar *Typhi* is on the increase. This study was designed to determine the antimicrobial Resistance profile of *Salmonella enterica* serovar *Typhi* isolated from human clinical samples in Ebonyi State. A non-duplicated stool culture of *Salmonella enterica* serovar *Typhi* of patients diagnosed with typhoid fever at General Hospital Onicha Igboeze were collected from the hospital ward namely: A & E (n = 4), MS (n = 3), FS (n = 3), PD (n = 7), LW (n = 4), ORT (n = 1), LAB (n = 17), THE (n = 9), GOPD (n = 4), MM (n = 3). Antimicrobial studies of *Salmonella enterica* serovar *Typhi* were determined using the Kirby–Bauer disk diffusion method. The proportion of resistance ranges from 33 %-100% against colistin, cefepime, nalidixic acid, cefoxitin, amikacin, cefuroxime, and piperacillin-tazobactam but isolates were only susceptible to meropenem 100%. The use of antimicrobial agents for the treatment of *Salmonella enterica* serovar *typhi* infection should be guided with antimicrobial susceptibility testing, Nonetheless, the diversity of the *Salmonella* isolates as a result of the dissemination of these resistant genes is a call for concern and emphasizes a need for an extensive investigation for the presence of these genes in Ebonyi State as well as the implementation of strict antimicrobial policies in a bid to restrict the spread of these resistance genes and prevent the emergence of new resistant strains.

Keywords: Salmonella typhi; antimicrobial resistance; human clinical; typhoid fever.

1. INTRODUCTION

Salmonella enterica serovar *Typhimurium* (*S. typhi*) is a Gram-negative, rod-shaped, catalase positive flagellated bacterium [1,2]. Moreover, the three *Salmonella* (*S. paratyphi*) types such as A, B and C are responsible for causing paratyphoid fever [1]. Typhoid fever being a systemic infection caused by *Salmonella enterica* serotype *Typhi* and is a highly adapted human specific pathogen which possesses remarkable mechanism for persistence in host [3].

Earlier studies have shown that majority of enteric fevers are prevalent in most of the developing countries like India, Pakistan, Bangladesh, and several African nations [4,5]. It is estimated to have approximately 5.4 million cases worldwide per year [3]. It can be transmitted via contaminated food, water and is mainly present in area with poor hygiene or low socioeconomic status [6].

According to the World Health Organization (WHO), they estimated that typhoid fever accounts for 222,000 deaths and 21 million infections annually on a global scale [7]. Africa is classified under region with medium incidence rate per year (10-100/100,000 cases/year) centered on a 22 community-based incidence study with only three African countries included in the analysis [7]. As of 2013, the annual mortality rate was reported at 2.8 per 100,000 persons in Africa with 2.5 per 100,000 persons reported for Nigeria [3,8]. Appropriate treatment reduces the mortality rate as low as 0.5% [3]. Appropriate treatment reduces the mortality rate as low as 0.5% [3].

The emergence of resistance to Co-trimaxazole, chloramphenicol, fluoroquinolones and third-generation cephalosporins had led to the frequent use of azithromycin for empirical treatment of uncomplicated enteric fever. However, the recent emergence of cephalosporin resistant strains of *Salmonella enterica* serovar *Typhi* is a cause for concern in the management of enteric fever and has been noted in human patients as a serious problem in most healthcare settings. This incidence makes typhoid fever an infectious disease worth investigating for better treatment plan and management with the knowledge of local antimicrobial resistance profile of *Salmonella enterica* serovar *Typhi*

2. METHODS

2.1 Culture Processing

A non-duplicated stool culture of *Salmonella enterica* serovar *Typhi* of patients diagnosed with typhoid fever at General Hospital Onicha Igboeze located latitude 6^0 6' 34" N and longitude 7^0 49' 3" E were collected according to the hospital ward namely: A & E (n = 4), MS (n = 3), FS (n = 3), PD (n = 7), LW (n = 4), ORT (n = 1), LAB (n = 17), THE (n = 9), GOPD (n = 4), MM (n = 3).The isolated *S. typhi* strains were genotypically confirmed by 16S rRNA sequencing at Bioinformatics Service laboratory, Ibadan, Nigeria, with universal specific primers.

2.2 Antibiotic Susceptibility Studies

Antibiotic susceptibility was performed by employing the Kirby-Bauer disk diffusion method using sterilized Mueller-Hinton agar per the guidelines of the Clinical and Laboratory Standards Institute [9]. *S. typhi* suspension of the test isolates was prepared using 0.5 McFarland standards and seeded on solidified Mueller–Hinton agar. The plates were allowed to pre-diffuse for 15 min. Thereafter, the following antibiotics disc: Colistin (30 µg), Amoxicillin-clavulanic acid (30 µg), Piperacillin-Tazobactam (5 µg), Cefuroxime (20 µg), Cefepime (15 µg), Nalidixic Acid (20 µg), Meropenem (30 µg), Cefoxitin (30 µg), Ofloxacin (10 µg), Amikacin (5 µg) was impregnated on the inoculated Mueller-Hinton (MH) agar plates and incubated at 37° C for 24 h. After overnight incubation, the diameters of zones of inhibition were measured and the results were interpreted in accordance with the criteria of Clinical and Laboratory Standards Institute [9,10,11].

3. RESULTS AND DICUSSION

3.1 Results

3.1.1 Antimicrobial resistant pattern of *S. typhi*

S. typhi were resistance to Piperacillin-Tazobactam (33-100%), Nalidixic Acid (57-100%), Cefepime (67-100 %) Cefuroxime (33-100%), Ofloxacin (100 %) colistin (33-100%) as shown in Table 1. The resistant pattern were colistin 50.0 %-100 %, Amoxicillin-clavulanic acid 25 %-100 %, Cefoxitin 55-100%, Cefuroxime 65-100%. All *S. typhi* were susceptible to meropenem (Table 2). Susceptibility of the isolates to monobactams (piperacillin-tazobactam), cefamycin (cefoxitin) and carbapenem (meropenem) antibiotics ranged between 10 % and 90%. In addition, low susceptibility (11% to 47%) was also observed against the common beta lactam and non-beta-lactam antibiotics.

3.2 Discussion

Majority of our studied isolates demonstrate resistant to second and third-generation cephalosporins, while 50% of them were resistant to fourth-generation cephalosporin. The higher resistance of *Salmonella typhi* to some antibiotics may be due to the over use of these antibiotics in animal rearing. The fluroquinolone, resistance was considered to be expressed by plasmid mediated quinolone (PMQR) determinants. These findings are in agreement with some recent studies which reported MDR *Salmonella* species and Non-typhoidal *Salmonella* species producing ESBL that were isolated from cattle [12], poultry [13], pigs [14], environment, [15] and humans [16]. The present study further reported a low susceptibility of *Salmonella typhi* species towards other common non β-lactam antimicrobials such as aminoglycosides, fluoroquinolone, tetracycline, etc. This findings tandem with a Canadian survey, where 62.8% of *Salmonella* species isolates were exhibit MDR [17].

Table 1. Antimicrobial resistant pattern of *S. typhi* Isolated from fecal samples of patients at A&E, MS, FS, PD and LW

	A&E (n = 4)	MS (n = 3)	FS (n = 3)	PD (n = 7)	LW (n = 4)
Antibiotic (µg)	**R (%)**	**R (%)**	**R (%)**	**R (%)**	**R (%)**
Colistin (30)	3 (75)	3 (100)	1 (33)	5 (71)	2 (50)
Amoxicillin- clavulanic acid (30)	2 (50)	3 (100)	3 (100)	6 (86)	2 (50)
Piperacillin-Tazobactam (5)	4 (100)	1 (33)	3 (100)	5 (71)	4 (100)
Cefuroxime (20)	4 (100)	1 (33)	3 (100)	5 (71)	4 (100)
Cefepime (15)	3 (75)	3 (100)	3 (100)	7 (100)	4 (100)
Nalidixic Acid (20)	4 (100)	3 (100)	3 (100)	4 (57)	4 (100)
Meropenem (30)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Cefoxitin (30)	4 (100)	2 (67)	1 (33)	5 (71)	4 (100)
Ofloxacin (10)	4 (100)	2 (67)	3 (100)	7 (100)	4 (100)
Amikacin (5)	4 (100)	2 (67)	2 (67)	7 (100)	4 (100)

Key: R- Resistance, n=number of isolates, A&E = Accident and Emergency ward, MS = Male surgical ward, FS = Female surgical ward, PD = Paediatric ward, LW = Labour ward

Table 2. Antimicrobial resistant pattern of *S. typhi* Isolated from fecal samples of patients at ORT, LAB, THE, GOPD and MM

	ORT (n = 1)	LAB (n = 17)	THE (n = 9)	GOPD (n = 4)	MM (n = 3)
Antibiotic (µg)	**R (%)**	**R (%)**	**R (%)**	**R (%)**	**R (%)**
Colistin (30)	1 (100)	14 (82)	9 (100)	2 (50)	0 (0)
Amoxicillin-clavulanic acid (30)	1 (100)	10 (59)	4 (44)	1 (25)	3 (100)
Piperacillin-Tazobactam (5)	0 (0)	17 (100)	7 (77)	4 (100)	3 (100)
Cefuroxime (20)	0 (0)	11 (65)	7 (77)	4 (100)	3 (100)
Cefepime (15)	1 (100)	9 (53)	9 (100)	1 (25)	2 (67)
Nalidixic Acid (20)	1 (100)	12 (71)	9 (100)	1 (25)	2 (67)
Meropenem (30)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Cefoxitin (30)	1 (100)	15 (88)	5 (55)	4 (100)	3 (100)
Ofloxacin (10)	1 (100)	17 (100)	7 (77)	3 (75)	1 (33)
Amikacin (5)	1 (100)	17 (100)	9 (100)	3 (75)	1 (33)

Key: R- Resistance, n=number of isolates, ORT = Orthopaedic ward, LAB = Laboratory, THE = Theatre, GOPD = General outpatient department, MM = male medical ward

Research has shown that the resistance to beta-lactam antimicrobials, such as ceftriaxone, is linearly correlated with the lactamase level over a period and resistance to beta-lactams and can be achieved by increasing enzyme levels [10]. Hence, the prolong use or misapplication of cephalosporins may lead to resistance over time.

However, our study revealed that the isolates were 100 % susceptible to meropenem. Meanwhile for some of these isolates to be susceptible to a panel of antibiotics tested in the present study, the possibility of acquiring resistance due to horizontal gene transfer or mutation is highly possible. For the treatment of *S. typhi* infections that demonstrated resistant to both fluoroquinolones and cephalosporins, carbapenems (meropenem) may be the last drug of choice.

The high level of tetracycline, Amoxicillin-clavulanic acid resistance in the present study is not surprising as it is an over-the-counter medication used by most farmers in the study area to treat bacterial and tick-borne infections in livestock. Interestingly, antimicrobial agents such as piperacillin-tazobactam are not commonly used in animal health and production in Nigeria and this suggests the importance of other sources and mechanism of MDR *Salmonella* species such as: the horizontal transfers of mobile resistance genetic element, imported *Salmonella*-contaminated feed, antimicrobials used in human treatment, livestock and foodstuff, and possibly cross-resistance among the related antimicrobials, the environment, or co-selection of resistances carried on the same DNA element [3,11,18].

This study showed high rates of resistant to ofloxacin and nalidixic acid was 33% -100%. This agrees with several studies carried out where *Salmonella* isolates demonstrated 100% resistance to nalidixic acid [18,19,20]. These antibiotics belong to the quinolone/ fluoroquiniolone drug class. Studies have shown quinolone resistance in salmonellae to be as a result of mutations in the DNA gyrase (*gyrA* and *gyrB*) and topoisomerase IV encoding (*parC* and *parE*) genes [3,18,19]. Other studies have also reported the presence of plasmid mediated quinolones resistant (PMQR) genes carried by the ESBL-producing plasmid, which facilitates the selection of higher-level resistance to quinolone drugs [3,21,22, 23,24,25].

Other studies have also suggested that the *in vitro* resistance to ofloxacin and nalidixic acid could be used as a pointer to the actual level of *in vitro* resistance to other ciprofloxacin, enorfloxacin, Levofloxacin and norfloxacin etc., [26,27]. This implies that the resistance observed by the *Salmonella typhi* to nalidixic acid in this study is a pointer to the development of resistance to other members of the fluoroquinolone class of antimicrobial agents in

humans in the Onicha region with time. This is important information give that fluoroquinolones are regarded as the antimicrobial class of first choice for the treatment of severe infections caused by *S. typhi* as well as other pathogenic Enterobacteriaceae in humans. For such patients, this study recommends the use of meropenem for case management as evidence with the 0.0 % resistant proportion reported in this study.

4. CONCLUSION

This study profiled antibiotic resistance, however, the trend of resistant from this study may emerge from injudicious use of antimicrobial agent in both human population, food and veterinary medicine and it is believed that this could be the most probable reason for the selective pressure and consequent resistance to these drugs. Therefore, this study suggests strict selection and rotation of antimicrobial agents coupled with the continuous monitoring of susceptibility profiles of antimicrobial agents in order to manage the emergence and spread of antimicrobial resistance.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Prior to this research, ethical approval with reference No: SMOH/ERC/042/21 was obtained from the Research and Ethics Committee of Ebonyi State Ministry of Health, Abakaliki, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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