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Multidrug Resistant *Salmonella enterica* Strains in South Western Nigeria: Prevalence and Susceptibility to Ceftriaxone

P. A. Idowu[1], O. T. Jemiseye[1] and T. S. Agidigbi[1,2*]

[1]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan, Nigeria.
[2]Department of Medicine, Inha University, Incheon, South Korea.

Authors' contributions

This work was carried out in collaboration among all authors. Author PAI designed the study and drafted the protocol and supervised the work. Authors OTJ and TSA did the sample collection, isolation and antibiotics susceptibility testing. Authors OTJ and TSA did the literature searches and analyzed the data. Authors TSA and OTJ wrote the first draft and final draft of the manuscript. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: To investigate the prevalence and susceptibility pattern *Salmonella enterica* strains to ceftriaxone in southwestern Nigeria.
Place and Duration of Study: Faculty of Pharmacy, University of Ibadan, Nigeria from November 2012-May 2013.
Methodology: Isolates of *Salmonella enterica* were characterized by established standard cultural and biochemical tests and was screened *in-vitro* for their sensitivity to different antibiotics (ampicillin, amoxicillin, chloramphenicol, cotrimoxazole and ceftriaxone) using the agar well diffusion method and their MICs determined.

**Corresponding author: E-mail: samuelgeneration@yahoo.com;*

Results: The susceptibility pattern of these strains to ceftriaxone and other antibiotics was examined to determine their prevalence among patients in South West Nigeria. 21 clinical isolates were screened *in-vitro* against five antibiotics. A higher number of the isolates showed MDR (76.19%) even at higher concentration of the antibiotics, while 61.9% were sensitive to ceftriaxone. Among the isolates, 71.43% resistance was recorded against ampicillin, 66.67% against amoxicillin, 38.1% resistance against ceftriaxone, 80.95% resistance against chloramphenicol and 57.14% resistance against cotrimoxazole. However, 3 isolates (14.29%) were completely sensitive to all of the antibiotics. The MICs obtained were higher (ranging from 30µg/ml to >100µg/ml), compared to the CSLI breakpoint standard. The result obtained showed an increased in incidence of MDR *S. enteric*a strain in southwestern Nigeria, and that ceftriaxone is still remain the drug of choice against *Salmonella enterica* strains, even though the number of isolates producing resistance against the antibiotics is on the increase.
Conclusion: The results above proces that the rate and prevalence of MDR *Salmonella enterica* strains are of major concern mostly in developing countries, therefore clinicians and public health practitioners should reduce the rate of antibiotic prescriptions and encourage public health and personal hygiene to prevent *S. enterica* infection.

Keywords: Salmonella enterica; multidrug resistance; antibiotic resistance pattern; ceftriaxone.

1. INTRODUCTION

Typhoid fever simply referred to as typhoid is a global bacterial infectious disease caused by ingestion of *Salmonella enterica* serotype Typhi. Infections due to multidrug-resistant *Salmonella enterica* serovar Typhi is of public health concern, with an estimated 30 million cases and 600,000 (2%) deaths annually [1,2], presently the incidence of death rate have declined to (1%) 217,000 out of 21,700,000 cases [3,4]. Since the beginning of the 1990s, there has been an increasing prevalence of multidrug resistance in *S. enterica* to first-choice antibiotics like chloramphenicol, ampicillin, and co-trimoxazole, thereby refocusing the treatment of typhoid to fluoroquinolones and ceftriaxone [5,6] Since the mid 1980s to early 1990s, strains of *Salmonella enterica* resistant to chloramphenicol, ampicillin, and trimethoprim (multidrug-resistant strains) have been responsible for a large number of outbreaks in Bangladesh, South America, India, African, Pakistan, Southeast and Asia.

Increasing in the rate of *S. enteric*a resistance to ciprofloxacin, most especially in Indian and South-east Asia is of major concern as possible transmission of resistance gene(s) cannot be undermined. As a result of the antimicrobial treatment option has been refocused on ceftriaxone, carbapenems and is even better to use of azithromycin [7-9].

However, Li-Hui et al. [10] has shown that a strain of *Salmonella enterica* serovar Anatum isolated from a 70 years old patient in Chang Gung Memorial Hospital, Taoyuan, Taiwan produces a resistance to ceftriaxone. This study is aimed to validate the susceptibility pattern of *Salmonella enterica* strains to ceftriaxone, a third-generation cephalosphorin and to determine the incidence of their multidrug resistance.

2. MATERIALS AND METHODS

2.1 Study Area and Period

The study area of this research involves collection of sample from different hospitals across south west Nigeria between November 2012 and May 2013.

2.2 Sample Collection

A total of 21 clinical isolates of *Salmonella enterica* strains were collected from different hospitals across South West Nigeria using Bismult Sulfite Agar and identified accordingly using the numerous biochemical tests described for *Salmonella* such as urease test, appearance on selective media, sugar utilization pattern, Methyl red and Voges-Proskauer test (MRVP) test, indole test, Gram reactions. All the isolates were collected from faeces and blood samples of the patients. The isolates were further screened on Salmonella-Shigella agar. *Salmonella enterica* serovar Typhimurium ATCC 14028 was used as a reference standard for all of the tested strains.

2.3 Identification of *Salmonella enterica*

Isolates	Names
SE2	*Salmonella enteric*
SE3	*Salmonella enteric*
SE4	*Salmonella enteric*
SE5	*Salmonella enteric*
SE6	*Salmonella enteric*
SE7	*Salmonella enteric*
SE8	*Salmonella enteric*
SE9	*Salmonella enteric*
SE10	*Salmonella* Paratyphi A
SE11	*Salmonella enteric*
SE12	*Salmonella enteric*
SE13	*Salmonella enteric*
SE16	*Salmonella enterica* serovar Typhi
SE17	*Salmonella enteric*
SE18	*Salmonella enteric*
SE19	*Salmonella enterica* serovar Typhimurium ATCC 14028
SE20	*Salmonella* Paratyphi A
SE21	*Salmonella enteric*
SE22	*Salmonella enteric*
SE23	*Salmonella enterica* serovar Typhi
SE24	*Salmonella enteric*

2.4 Media Used

Bismult sulfite agar (Oxoid Laboratories, England), Salmonella-Shigella agar (LAB M, United Kingdom), Mueller-Hinton agar (Oxoid Ltd, England) and Nutrient broth (LAB M, United Kingdom) were the media used for the research work.

Bismult sulfite agar was prepared by weighing the required amount (in grams) into the corresponding volume of sterile distilled water and boiled to dissolve, sterilized by autoclaving at 121℃ for 15 minutes, and then allowed to cool down before pouring into plates. Collection plates were stored at 2-8℃ unless used.

The same process was used for the preparation of Mueller-Hinton agar.

Salmonella-Shigella agar was prepared by weighing the stipulated amount (in grams) in required volume of sterile distilled water, and then heated till boiled as described by the manufacturer.

2.5 Antibiotics Used

The antibiotics used for the research work were ampicillin (Greenfield Pharma Ltd, China), amoxicillin (Beeecham Pharma, England), cotrimoxazole (SKG Pharma Ltd, Nigeria), chloramphenicol (Ciron Drugs & Pharma Ltd, India) and ceftriaxone (Furen Pharma Group Company Ltd, China). All the antibiotics were in vials with the exception of cotrimoxazole, which is in tablets. 30 µg/ml, 50 µg/ml, 75 µg/ml and 100 µg/ml concentrations, each of the antibiotics were made by dissolving appropriate powder in DMSO and make up with required volume of sterile distilled water. The prepared solutions were used immediately.

2.6 Standardization of Inoculums and Antimicrobial Susceptibility Testing

"Agar well diffusion method, as described by Perez et al. [11]" was used. Mueller-Hinton agar (20 ml) was prepared and allowed to cool to a temperature of about 20-30℃. Cells from the overnight cultures were suspended in nutrient broth until it produces turbidity equal to the 0.5 McFarland standard No. 1 from which 0.1 ml was introduced into the cooled molten Mueller-Hinton agar where it is uniformly mixed and poured into a sterile Petri dish and allowed to set. A sterile cork borer (diameter 8 mm) was used to make equidistance uniform wells on each of the set and dried agar. Each well was filled with the different concentration (30 µg/ml, 50 µg/ml, 75 µg/ml and 100 µg/ml) of the antibiotics and allowed to diffuse for about 40 minutes. The plates were then incubated in the incubator at 37℃ for 18-24 hrs. The process was repeated in triplicates. The zones of inhibitions produced were measured and the interpretive criteria for susceptibility testing of all the antibiotics used were based on the performance standard of antimicrobial susceptibility screening, Clinical Laboratory Standard Institute (CLSI) [12].

2.7 MIC Determination

The broth dilution method as described by [13] was used. The antibiotics were prepared in standard graduated decreasing concentrations of 100 µg/ml, 75 µg/ml, 50 µg/ml, 30 µg/ml, 15 µg/ml, 7.5 µg/ml, 3.75 µg/ml, 1.875 µg/ml and 0.9375 µg/ml. Cells from the overnight cultures were suspended in nutrient broth until it showed turbidity equal to a 0.5 McFarland standard No. 1 of which 0.2 ml was introduced into tubes containing the antibiotics and nutrient broth, which were later incubated at 37℃ for 18-24 hrs. A control experiment was used.

Table 1. Antibiotic resistance pattern of *S. enterica* showing the of zone of inhibition in (mm)

	AMP (µg/ml)				AMX (µg/ml)				CEF (µg/ml)				CHL (µg/ml)				COT (µg/ml)			
	30	**50**	**75**	**100**	**30**	**50**	**75**	**100**	**30**	**50**	**75**	**100**	**30**	**50**	**75**	**100**	**30**	**50**	**75**	**100**
SE2	22	24	25	26	23	23	25	27	34	36	36	36	18	20	21	23	28	29	30	33
SE3	R	R	R	R	21	22	24	25	30	32	33	35	R	R	R	R	27	29	29	30
SE4	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
SE5	R	R	R	R	R	R	R	R	40	41	41	43	R	R	R	R	R	R	R	R
SE6	R	R	R	R	R	R	R	R	37	39	39	40	R	R	R	R	R	R	R	R
SE7	R	R	R	R	R	R	R	R	34	36	37	39	R	R	R	R	R	R	R	R
SE8	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
SE9	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
SE10	10	12	14	15	31	33	34	36	30	32	33	35	14	15	17	19	33	34	34	36
SE11	29	30	33	35	23	25	27	30	R	R	R	R	R	R	R	R	R	R	R	R
SE12	32	33	33	35	34	35	37	40	20	23	25	27	R	R	R	R	30	30	31	34
SE13	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
SE16	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
SE17	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
SE18	18	20	21	23	22	24	25	26	40	40	42	44	24	25	27	29	19	21	23	34
SE19	R	R	R	R	R	R	R	R	26	28	29	30	R	R	R	R	R	R	R	R
SE20	R	R	R	R	25	26	26	28	27	29	30	32	R	R	R	R	22	23	25	25
SE21	12	14	14	15	R	R	R	R	39	39	40	41	11	13	13	15	30	32	34	35
SE22	R	R	R	R	R	R	R	R	40	40	42	43	R	R	R	R	40	41	43	44
SE23	R	R	R	R	R	R	R	R	34	35	36	39	R	R	R	R	40	42	42	43
SE24	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R

KEYS: R- Resistant, AMP- ampicillin, AMX- amoxcillin, CEF- ceftriaxone, COT- cotrimoxazole, CHL- chloramphenicol, SE_2 – SE_{24}: isolates

3. RESULTS

The results of the susceptibility pattern to various antibiotics of all 21 isolates were summarized in Table 1. Among the 21 isolates tested against the 5 antibiotics, 16 isolates (76.19%) showed multidrug resistance, i.e. resistance to two or more antibiotics while 80.95% resistance was observed against chloramphenicol. 14 isolates (66.67%) were resistant to amoxicillin, while 12 isolates (57.14%) produced resistance against cotrimoxazole, with 15 isolates (71.43%) exhibited resistant to ampicillin. 13 isolates (61.9%) were however found to be sensitive to ceftriaxone making it the only antibiotics that had the highest antimicrobial effect against the organisms (Table 1). The MICs of the antibiotics ranged from 30 µg/ml to >100 µg/ml (Table 2).

4. DISCUSSION AND CONCLUSION

It is no news that the rate of multidrug resistance (MDR) of *Salmonella enterica* strains to first line of antibiotics (ampicillin, cotrimoxazole, amoxicillin and chloramphenicol) is on the increase. This can be seen in the result of the susceptibility screening of the aforementioned antibiotics. The results obtained from this study buttress the previously concluded discussions that prevalence and incidence of multidrug *Salmonella enterica* strains is associated with Africa and now precisely within the south-west region of Nigeria. MDR *Salmonella enterica* strains are now endemic in many developing countries and has also been isolated from people returning from the developed countries. As resistance had emerged against these antimicrobial agents, the use of chloramphenicol, ampicillin and trimethoprim were no longer visible. This has made ciprofloxacin being used as the first choice of drug for the treatment of *Salmonella enterica* related infections. However, cases of Resistance to Ciprofloxacin have also been observed in Bangladesh [14], where 10 strains of *S. enterica* serovar Typhi produce a high degree of resistance to Ciprofloxacin but were however sensitive to ceftriaxone.

Generally, fluoroquinolones have been the preferable choice of antibiotics for the treatment of typhoid fever. Although resistance changes over a period of time, still fluoroquinolones and its new derivative remain potent in the treatment of typhoid fever. Alternatively, a third-generation cephalosporin such as ceftriaxone or cefotaxime becomes the drugs of choice for first line of treatment [15].

Table 2. Minimum inhibitory concentrations of the antibiotics

	CEF (µg/ml)	AMP (µg/ml)	AMX (µg/ml)	COT (µg/ml)	CHL(µg/ml)
SE2	30	30	30	30	50
SE3	50	>100	50	30	>100
SE4	>100	>100	>100	>100	>100
SE5	50	>100	>100	>100	>100
SE6	50	>100	>100	>100	>100
SE7	50	>100	>100	>100	>100
SE8	>100	>100	>100	>100	>100
SE9	>100	>100	>100	>100	>100
SE10	50	100	50	30	75
SE11	>100	50	50	>100	>100
SE12	50	50	50	30	>100
SE13	>100	>100	>100	>100	>100
SE16	>100	>100	>100	>100	>100
SE17	>100	>100	>100	>100	>100
SE18	30	75	50	50	50
SE19	50	>100	>100	>100	>100
SE20	50	>100	50	50	>100
SE21	30	100	>100	100	100
SE22	30	>100	>100	30	>100
SE23	30	>100	>100	30	>100
SE24	>100	>100	>100	>100	>100

KEYS: CEF- ceftriaxone, AMP- ampicillin, AMX- amoxicillin, COT-cotrimoxazole, CHL- chloramphenicol, SE_2-SE_{24}: isolates

The indiscriminate drug administration without prescription, antibiotics mis-used and rampant abuse of cephalosphorins, co existence of other infectious diseases [16] in South Western Nigeria might be the factors contributing to the high prevalence of reduced susceptibility (61.9%) thereby giving an increased in emergence of very high level or complete resistance of isolates of *Salmonella enterica* strains to cephalosphorins. The prevalence of multidrug resistance is also on the increase (76.19%), and may result in total antibiotic treatment failure in clinical practice.

Most of the antibiotics used on the farm are not ultimately used in treating sick birds, as observed by [17]. These antibiotics are used by the farmers to enable for rapid growth of the birds. Thinking the feeds are meant to fight off infections, however, researchers have now found out that the use of antibiotics on farms has led to an increase in antibiotic resistant cases of food poisoning caused by *Salmonella* bacteria in human.

It is a general believe that the abuse of antibiotics by people and individuals has greatly contributed to the inability of drugs to cure infections, moreover, the addition of antibiotics as low level diet in the feeds of poultry birds may also cause the development of resistant strains of bacteria, which can end up in people ingesting these resistant strains of *Salmonella* through handling or eating of such contaminated meats. Additionally, residual antibiotics have been detected in drinking water and water used in the industries [18,19], yet this is no law in Nigeria regulating the use of antibiotics in water treatment and industries. Also, circulation of counterfeit drugs and indiscriminate outflow of antibiotics might have well being a factor contributing to antibiotic resistance [20].

This study has shown that ceftriaxone is still the drug of choice against *Salmonella enterica* strains, even though the number of isolates producing resistance against the antibiotics is on the increase. This proves that the rate and prevalence of MDR *Salmonella enterica* strains are of a major concern in developing countries, therefore clinicians and public health practitioners may face an extensive challenge handling untreatable infectious diseases caused by MDR *S. enterica* strains in the nearest future. Public health and personal hygiene remain the major way of preventing infections due to *S. enterica.*

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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