

Original Research Articles

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

Abstract

Salmonella enterica strains have shown resistance to chloramphenicol, ampicillin, amoxycillin and cotrimoxazole (multidrug-resistant [MDR]) since 1989 and have been responsible for numerous outbreaks in many countries including Africa, Southeast Asia and Indian subcontinent. 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 (ampicillin, amoxycillin, chloramphenicol, cotrimoxazole and ceftriaxone) using the agar well diffusion method and their MICs determined. A higher number of the isolates showed MDR (76.19%) even at higher concentration of the antibiotics, while 61.9% was sensitive to ceftriaxone. Among the isolates, 71.43% resistance was recorded against ampicillin, 66.67% against amoxycillin, 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 study has shown that the prevalence of MDR *Salmonella enterica* strains is on the increase and that ceftriaxone is still the most potent among the antibiotics. It is regrettable that resistance to Ceftriazone has now emerged in MDR *Salmonella enterica* strains and it is of paramount importance to limit the unnecessary use of this vital drug so that its efficacy should not be further jeopardized.

Keywords: *Salmonella enterica*, multidrug resistance, antibiotics susceptibility, ceftriaxone

Introduction

Typhoid fever due to multidrug-resistant *Salmonella enterica* serovar Typhi is a global public health problem, with an estimated 30 million cases and 600,000 deaths annually (WHO, 1998). Since the beginning of the 1990s, there has been an increasing prevalence of multidrug resistance to the first-line antimicrobials, such as chloramphenicol, ampicillin, and co-trimoxazole, shifting the drug of choice for the treatment of typhoid fever to fluoroquinolones (Rahman *et al.*, 2005 and Renuka *et al.*, 2005). Since 1989, strains of *Salmonella enterica* resistant to chloramphenicol, ampicillin, and trimethoprim (i.e., multidrug-resistant [MDR] strains) have been responsible for numerous outbreaks in countries in the Indian subcontinent, Southeast Asia, and Africa (Rowe *et al.*, 1997).

Ciprofloxacin resistance is an increasing problem, especially in the Indian subcontinent and Southeast Asia. Many centres are therefore moving away from using ciprofloxacin as the first line for treating suspected typhoid originating in South America, India, Pakistan, Bangladesh, Thailand, or Vietnam. For these patients, the recommended first line treatment is ceftriaxone. (Effa *et al.*, 2011).

However, Lin-Hui *et al.*, 2003 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 cephalosporins and to determine the incidence of their multidrug resistance.

Materials and methods

Salmonella enterica strains

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, 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.

Microorganisms

ISOLATES	NAMES
S2	<i>Salmonella enterica</i>
S3	<i>Salmonella enterica</i>
S4	<i>Salmonella enterica</i>
S5	<i>Salmonella enterica</i>
S6	<i>Salmonella enterica</i>
S7	<i>Salmonella enterica</i>
S8	<i>Salmonella enterica</i>

S9	<i>Salmonella enterica</i>
S10	<i>Salmonella</i> Paratyphi A
S11	<i>Salmonella enterica</i>
S12	<i>Salmonella enterica</i>
S13	<i>Salmonella enterica</i>
S16	<i>Salmonella enterica</i> serovar Typhi
S17	<i>Salmonella enterica</i>
S18	<i>Salmonella enterica</i>
S19	<i>Salmonella enterica</i> serovar Typhimurium ATCC 14028
S20	<i>Salmonella</i> Paratyphi A
S21	<i>Salmonella enterica</i>
S22	<i>Salmonella enterica</i>
S23	<i>Salmonella enterica</i> serovar Typhi
S24	<i>Salmonella enterica</i>

62

63 **Media used**

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

67 Bismult sulfite agar was prepared by weighing the required amount (in grams) into the
68 corresponding volume of sterile distilled water and boiled to dissolve, sterilized by autoclaving at
69 121°C for 15 minutes, and then allowed to cool down before pouring into plates. Collection
70 plates were stored at 2-8°C unless used.

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

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

74 **Antibiotics used.**

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

82 **Broth culture**

83 Nutrient broth was prepared according to the manufacturer's description. The required quantity
84 (in grams) of the media was dissolved in appropriate volume of sterile distilled water in test
85 tubes and sterilized by autoclaving at 121⁰C for 15minutes and then allowed to cool . The test
86 tubes were then inoculated with the organism and incubated at 37⁰C for 16 to 18hrs until it
87 achieved the turbidity of the McFarland 0.5 standard to get an overnight culture.

88

89 **Antimicrobial susceptibility testing**

90 The agar well diffusion method, as described by Perez, *et al.*, 1990 was used. Mueller-Hinton
91 agar (20ml) was prepared and allowed to cool to a temperature of about 20- 30⁰C. Cells from the
92 overnight cultures were suspended in nutrient broth until it produces turbidity equal to the 0.5
93 McFarland standard No. 1 from which 0.1ml was introduced into the cooled molten Mueller-
94 Hinton agar where it is uniformly mixed and poured into a sterile Petri dish and allowed to set. A
95 sterile cork borer (diameter 8mm) was used to make equidistance uniform wells on each of the
96 set and dried agar. Each well was filled with the different concentrations (30µg/ml, 50µg/ml,
97 75µg/ml and 100µg/ml) of the antibiotics and allowed to diffuse for about 40 minutes. The plates
98 were then incubated in the incubator at 37⁰C for 18-24hrs. The process was repeated in
99 triplicates. The zones of inhibitions produced were measured and the interpretive criteria for
100 susceptibility testing of all the antibiotics used were based on the standard of Clinical Laboratory
101 Standard Institute (CLSI, 2010).

102 **MIC determination**

103 The broth dilution method as described by (Rollins and Joseph, 2000) was used. The antibiotics
104 were prepared in standard graduated decreasing concentrations of 100µg/ml, 75µg/ml, 50µg/ml,
105 30µg/ml, 15µg/ml, 7.5µg/ml, 3.75µg/ml, 1.875µg/ml and 0.9375µg/ml. Cells from the overnight
106 cultures were suspended in nutrient broth until it produces turbidity equal to a 0.5 McFarland
107 standard No. 1 from which 0.2ml was introduced into tubes containing the antibiotics and
108 nutrient broth, which were later incubated at 37⁰C for 18-24hrs. A control experiment was used.

109 **Results**

110 The results of the susceptibility pattern to various antibiotics of all 21 isolates were summarized
111 in Table 1. Among the 21 isolates tested against the 5 antibiotics, 16 isolates (76.19%) showed
112 multidrug resistance, i.e resistance to two or more antibiotics while 80.95% resistance was
113 observed against chloramphenicol. 14 isolates (66.67%) were resistant to amoxycillin, while 12
114 isolates (57.14%) produced resistance against cotrimoxazole, with 15 isolates (71.43%) found
115 resistant to ampicillin. However, lesser percentage (38.1%) resistance was found against
116 ceftriaxone with only 3 isolates (14.29%) found to be completely sensitive to all of the
117 antibiotics used. 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 30µg/ml to >100µg/ml. (Table 2).

120

TABLE 1 Antibiotic susceptibility testing showing the diameter of zone of inhibition (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
S2	22	24	25	26	23	23	25	27	34	36	36	36	18	20	21	23	28	29	30	33
S3	R	R	R	R	21	22	24	25	30	32	33	35	R	R	R	R	27	29	29	30
S4	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
S5	R	R	R	R	R	R	R	R	40	41	41	43	R	R	R	R	R	R	R	R
S6	R	R	R	R	R	R	R	R	37	39	39	40	R	R	R	R	R	R	R	R
S7	R	R	R	R	R	R	R	R	34	36	37	39	R	R	R	R	R	R	R	R
S8	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
S9	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
S10	10	12	14	15	31	33	34	36	30	32	33	35	14	15	17	19	33	34	34	36
S11	29	30	33	35	23	25	27	30	R	R	R	R	R	R	R	R	R	R	R	R
S12	32	33	33	35	34	35	37	40	20	23	25	27	R	R	R	R	30	30	31	34
S13	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
S16	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
S17	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
S18	18	20	21	23	22	24	25	26	40	40	42	44	24	25	27	29	19	21	23	34
S19	R	R	R	R	R	R	R	R	26	28	29	30	R	R	R	R	R	R	R	R
S20	R	R	R	R	25	26	26	28	27	29	30	32	R	R	R	R	22	23	25	25
S21	12	14	14	15	R	R	R	R	39	39	40	41	11	13	13	15	30	32	34	35
S22	R	R	R	R	R	R	R	R	40	40	42	43	R	R	R	R	40	41	43	44
S23	R	R	R	R	R	R	R	R	34	35	36	39	R	R	R	R	40	42	42	43
S24	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- amoxycillin, CEF- ceftriaxone,

COT- cotrimoxazole, CHL- chloramphenicol, S₂ – S₂₄: isolates

125

126 **TABLE 2 Minimum Inhibitory Concentrations of the antibiotics**

	CEF (µg/ml)	AMP (µg/ml)	AMX (µg/ml)	COT (µg/ml)	CHL(µg/ml)
S2	30	30	30	30	50
S3	50	>100	50	30	>100
S4	>100	>100	>100	>100	>100
S5	50	>100	>100	>100	>100
S6	50	>100	>100	>100	>100
S7	50	>100	>100	>100	>100
S8	>100	>100	>100	>100	>100
S9	>100	>100	>100	>100	>100
S10	50	100	50	30	75
S11	>100	50	50	>100	>100
S12	50	50	50	30	>100
S13	>100	>100	>100	>100	>100
S16	>100	>100	>100	>100	>100
S17	>100	>100	>100	>100	>100
S18	30	75	50	50	50
S19	50	>100	>100	>100	>100
S20	50	>100	50	50	>100
S21	30	100	>100	100	100
S22	30	>100	>100	30	>100
S23	30	>100	>100	30	>100
S24	>100	>100	>100	>100	>100

127 **KEYS:** CEF- ceftriaxone, AMP- ampicillin, AMX- amoxycillin, COT-cotrimoxazole,

128 **CHL- chloramphenicol, S₂-S₂₄: isolates**

129

130 **DISCUSSION AND CONCLUSION**

131 It is no news that the rate of multidrug resistance (MDR) of *Salmonella enterica* strains to first
 132 line of antibiotics (ampicillin, cotrimoxazole, amoxycillin and chloramphenicol) is on the
 133 increase. This can be seen in the result of the susceptibility screening of the aforementioned
 134 antibiotics. The results obtained from this study buttress the previously concluded discussions
 135 that prevalence and incidence of multidrug *Salmonella enterica* strains is associated with Africa
 136 and now precisely within the South-West region of Nigeria. MDR *Salmonella enterica* strains is
 137 now endemic in many developing countries but also has been isolated from returning travelers in
 138 developed countries. As a result of the proliferation of such strains, the use of chloramphenicol

has been compromised, and that of ampicillin and trimethoprim similarly impaired. This has made ciprofloxacin being used as the first choice of drug for the treatment of *Salmonella enterica* related infections. However, in 1992, the isolation in the United Kingdom of an MDR strain of *Salmonella enterica* serovar Typhi Vi phage type El with plasmid-encoded resistance to chloramphenicol, ampicillin, and trimethoprim (and chromosomal resistance to ciprofloxacin at a concentration of 0.30 mg/L) was reported (Rowe *et al.*, 1992). The patient was a 1-year-old child who had been infected in India and did not respond to treatment with ciprofloxacin (Umasankar *et al.*, 1992). Resistance to Ciprofloxacin have also been recorded in Bangladesh by Dilruba *et al.*, 2006, where 10 strains of *Salmonella enterica* serovar Typhi produce a high level resistance to Ciprofloxacin but were however sensitive to ceftriaxone.

Where resistance is uncommon, the treatment of choice is a fluoroquinolone such as ciprofloxacin (Parry and Beeching, 2009). Otherwise, a third-generation cephalosporin such as ceftriaxone or cefotaxime is the first choice (Soe and Overturf, 1987).

The injudicious administration and rampant use of cephalosporins in South Western Nigeria probably contributed to the high prevalence of reduced susceptibility (61.9%) and the emergence of very high level or complete resistance of isolates of *Salmonella enterica* strains to cephalosporins. The prevalence of multidrug resistance (resistance to two or more drugs) is also on the increase (76.19%).

Most of the antibiotics used on the farm are not ultimately used in treating sick birds, as observed by Kaufman, (2000). 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 contribute 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.

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 is of a major concern in developing countries, and we therefore face the imminent prospect of encountering untreatable infections in the nearest future.

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