Original Article

Multidrug resistant *Salmonella* Concord is a major cause of salmonellosis in children in Ethiopia

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Abstract

Introduction: *S.* Concord in Ethiopia. The objective of this study was to determine the aetiology of febrile and diarrhoeic illness in Ethiopian children focussing on *Salmonella*.

Methodology: Paediatric patients (n = 1,225) presenting with diarrhoea or fever from the paediatric outpatient department of Tikur Anbessa University Hospital, Addis Ababa (n = 825), and Jimma University Hospital, South West Ethiopia (n = 400), were investigated for pathogens from January to August 2006.

Results: Parasites were detected in 337 cases, *Salmonella* in 65, and *Shigella* in 61. Serotyping of *Salmonella* (including 48 stored isolates) demonstrated the dominance of *S.* Concord: *S.* Concord (85), *S.* Typhimurium (7), *S.* Paratyphi B (2), *S.* Haifa (1), *S.* Typhi (2), *S.* Enteritidis (4), *S.* Butantan (2), *S.* Infantis (1), *S.* Pomona (1), *Salmonella* group M (28:y:-) (1), and *S.* Oskarshamn (1). Six isolates in serogroups B and D were untypeable. Of 81 *S.* Concord isolates, 30% were invasive, most (86.5%) were positive for ESBL production by E-test and 70% were multiply resistant to trimethoprim-sulphamethaxole, ceftriaxone, chloramphenicol and gentamicin, of which over one quarter (27%) also showed reduced susceptibility to ciprofloxacin.

Conclusion: Multi-drug resistant *S.* Concord was the major cause of salmonellosis in two regions of Ethiopia. The strain isolated was highly invasive, highly antibiotic-resistant, and represents a threat to heath care globally.

Key words: Salmonellosis; *Salmonella* Concord; multidrug resistance; Ethiopia

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Introduction

The global nature of infectious diseases is well-established, and the spread of antibiotic resistance is of major concern for both developed and developing countries. If, as a scientific community, we are to address these problems, we need to understand the origins of strains which become resistant and combat them at source. An example of the global threat of antibiotic resistance is the multidrug-resistant (MDR) serogroup C *Salmonella enterica* serovar Concord (*S.* Concord) [1,2]. Isolates have been widely reported in Europe and America from travellers and from children adopted in Ethiopia. Isolates are usually resistant to ampicillin, aztreonam, cefazolin, cefepime, cefpodoxime, ceftazidime, ceftiofur, cefuroxime, cephalothin, chloramphenicol, streptomycin, sulfamethoxazole, trimethoprim, and ceftriaxone [1]. Resistance is encoded on plasmids and a chromosomal island and includes two extended spectrum β-lactamase (ESBL) genes: CTXM-15 and SHV-12 [2]. The connection between *S.* Concord and Ethiopia has been made by the investigation of babies in Europe and America who were adopted from Ethiopia [2]. There has been no report in the international literature about the actual source of *S.* Concord in Ethiopia. Previous studies in Ethiopia on isolates from humans, animals and food products indicate the presence of a number of different *Salmonella enterica* serogroups circulating; however, the only serotype fully described is *Salmonella* Typhi. These studies are reviewed in the *Journal of Infection in Developing Countries* December 2008 issue [3] and show that group C *Salmonella* predominate among non-typhoidal *Salmonella* (NTS) infections [4-14]. One study, with 216 isolates from Addis Ababa from 1974-81, reported serotypes of *Salmonella* isolates [6]: among 216 isolates, there were 26 different serovars of which *S.* Concord

(12.5%) was the most common NTS. Furthermore, an increase in the resistance of *Salmonella* to commonly used antimicrobials has also been noted in both public health and veterinary sectors in Ethiopia [6-10,14,15].

To establish if MDR *S.* Concord was circulating in the population or being selected in orphanages by the overuse of antibiotics, and to inform local health systems responsible for controlling *Salmonella* infection in Ethiopia, a hospital-based survey of children with diarrhoeal or febrile illness was conducted at two centres, one rural site (Jimma) and one urban site (Addis Ababa). Full serotyping, subtyping and antibiotic resistance typing was performed for all *Salmonella* isolated.

Methodology

Study design and period

A hospital-based, prospective, cross-sectional study was conducted in Tikur Anbessa and Jimma Specialized University Hospital to determine the common pathogens in children (aged 6 months to 15 years) with febrile and diarrhoeal illness from January to August 2006. Diarrhoea was defined as the presence of at least three loose stools or one watery stool per day [16]. Fever was defined as a child with an axillary temperature higher than 37.5^0C.

Study subjects

A total of 1,225 consecutive children (6 months to 15 years of age) with diarrhoeal illness and/or febrile illness from the Paediatrics Department of Tikur Anbessa (n = 825) and Jimma University (n = 400) hospitals were investigated.

Children below six months and above 15 years of age and those whose parents did not agree to allow sampling were excluded from this study. Histories were taken from each child and informed consent was obtained from the parents or guardians before sample collection was attempted by the attending paediatrician. All the relevant demographic, clinical and laboratory data were recorded and transferred to the questionnaire prepared for this study. In addition to the study, 48 *Salmonella* strains from stock cultures were also analysed. These strains were collected between January 2004 and December 2005 from children of a similar age to the study group from the same hospital, Tikur Anbessa Hospital.

Identification of pathogens

All stool and blood specimens were collected by the laboratory technician.

Stool: Either a freshly passed stool or a rectal swab was collected, placed immediately in Cary Blair transport medium (Oxoid Ltd, Basingstoke, UK) and transported to the laboratory within six hours of collection. Stool and rectal swab specimens were placed in Selenite F enrichment broth (Oxoid) and incubated at 37°C for 24 hours, then subcultured onto deoxycholate agar (DCA) and xylose lysine deoxycholate agar (XLD) (Oxoid) agar at 37°C for 18-24 hours. The growth of *Salmonella* and *Shigella* species was detected by their characteristic appearance on XLD agar (*Shigella*: red colonies, *Salmonella* red with a black centre) and DCA (pale colonies). API 20E identification kits (API systems S.A., Montalieu-Vercieu, France) were also used to confirm identification. *Shigella flexneri* (NBISC 530) and *Salmonella* Typhimurium (NBISC-11) were used for quality control throughout the study. Microscopic examination of stool specimens for ova and parasites was performed using saline preparations stained with iodine.

Blood: About 2 ml of venous blood was drawn aseptically from each patient by cleaning the skin using tincture of iodine, and placed into Brain Heart Infusion (BHI) broth (Oxoid) containing 0.05% sodium polyanetholesulfonate (Oxoid). A minimum blood-to-broth ratio of 1 in 10 was maintained [17]. Blood culture broths were incubated at 37°C and checked for signs of bacterial growth daily for up to seven days. Bottles which showed signs of growth were subcultured onto DCA and XLD. Blood culture broth with no bacterial growth after seven days were subcultured before being reported as a negative result [18].

Antimicrobial susceptible testing

Disk diffusion testing: Antimicrobial susceptibility testing was performed for all *S.* Concord isolates using the disc diffusion method. Results were interpreted using international criteria [19]. The drugs for disk diffusion testing were obtained from bioMerieux, Lyon, France, in the following concentrations: ampicillin (AM) (10μg), ceftriaxone (CRO) (30μg), chloramphenicol (C) (30μg), ciprofloxacin (CIP) (5μg), gentamycin (GM) (10μg), nalidixic acid (NA) (30μg), ofloxacin (OFX) (5μg), tetracycline (TE) (30μg) and trimethoprim-sulfamethoxazole (SXT) (1.25 + 23.75μg). The criteria used to select the antimicrobial agents to be

tested were based on local clinical need and global use for treating salmonellosis [personal communication with local clinicians].

E-test: MIC by E-test was performed for *Salmonella* Concord against ciprofloxacin using E-test strips (AB Biodisk, Solna, Sweden). The isolates were classified as susceptible, reduced susceptibility, intermediate susceptibility or resistant according to the E-test application sheet (EAS-013) supplied by the manufacturer. A standard reference strain of *Escherichia coli* (ATCC 25922), susceptible to all antimicrobial drugs tested, was used as a quality control for both the disk diffusion and the E-test.

Detection of extended spectrum β-lactamase

Salmonella Concord isolates showing zones of inhibition by disc diffusion method for ampicillin and ceftriaxone ≤ 11 and ≤ 13 mm respectively were tested for ESBL production by E-test. Each suspicious isolate was subcultured and processed under the same conditions as described under E-test. The isolates were tested for susceptibility to ceftazidime (TZ, MIC graded scale 0.5-32 μg/ml) and cefotaxime (CT, MIC graded scale 0.25-16 μg/ml) individually, and in combination with clavulanic acid (L) (4μg/ml): TZL (4-0.064 μg/ml) and CTL (1- 0.016 μg/ml) respectively (AB Biodisk, Solna, Sweden).

ESBL-negative control strain *E. coli* ATCC 35219, and ESBL-positive control strain *Klebsiella pneumoniae* ATCC 700603 demonstrated the expected zone patterns (NCCLS, 2003). The result was interpreted as ESBL-positive if the MIC ratio for TZ/TZL was ≥ 8 or CT/CTL was ≥8. The result would be non-determinable if the TZ MIC was > 32 μg/ml and TZL > 4 μg/ml, and if the CT MIC was > 16 μg/ml and CTL >1 μg/ml [20].

Phenotyping characterization

Serogrouping: *Salmonella* strains were serogrouped by slide agglutination tests using poly O and single O-groups antisera (Remel Europe Ltd, Dartford, UK). These strains were further tested against poly H antisera. Those strains identified biochemically as *Salmonella* Typhi were also tested against Vi antisera. *Shigella* isolates were serogrouped by slide agglutination tests using *Shigella* polyvalent and group antisera (*Shigella* group A, B, C and D antisera) from Difco laboratories Inc., Detroit, USA.

Serotyping: Serotyping of *Salmonella* species isolates was performed after serogrouping, on the basis of phase 1 and phase 2 flagellar antigens by tube agglutination tests with known antisera (Remel Europe Ltd), according to the Kaufmann–White scheme [21]. Flagellar phase change was conducted using bridge plates when the test organisms occurred in one of the two phases only. For negative control purposes, a drop of saline was placed on another slide/tube and bacterial cultures were emulsified without antiserum. *Salmonella* Typhimurium (NBISC-11) was used as a control.

Subtyping by pulsed field gel electrophoresis (PFGE) of Xba*I digested chromosomal DNA*

Chromosomal DNA from NTS isolates was prepared in agarose plugs as described previously [22]. DNA in agarose plugs was digested using 20 units each of *Xba*I (Promega, Madison, USA). PFGE of agarose plug inserts was then performed on a CHEF-DR III system (Bio-Rad Laboratories, Hercules, USA) on a horizontal 1% agarose gel for 24 hours at 6 V/cm, with a pulse time of 2.2 seconds to 68 seconds at 10°C. Digested DNA from *S.* Braenderup H9812 was loaded every five lanes as the molecular marker, as recommended by Pulse Net [23]. The gel was stained with ethidium bromide and photographed on an ultraviolet trans-illuminator (UVP Inc., San Gabriel, USA). The restriction endonuclease digest patterns were compared visually and isolates with the same number and molecular weight band were considered as the same strain.

Statistical analysis

All demographic, clinical and laboratory data obtained from this study were analysed and interpreted using the statistical package for social sciences (SPSS, Applied Maths, Belgium). Chi-square was used to test the difference between proportions, and P-values less than 0.05 were considered statistically significant.

Ethical clearance

Ethical approval for the study was obtained from the Medical Faculty at Addis Ababa University, the Armauer Hansen Research Institute at Jimma University, and the National Ethical Review Committee of the Ethiopian Science and Technology Commission. Written informed consent was obtained from the parents/guardians of the children participating in the study.

Table 1. Pathogens in 1,225 children who presented to hospital with diarrhoea or fever

Enteropathogens **n = number of samples collected**	**Addis Ababa** **No. (%)** **n = 825**	**Jimma** **No. (%)** **n = 400**	**Total** **No. (%)** **n = 1,225**
***Salmonella* (most common serovar)** Serogroup B (Typhimurium)	7 (0.8)	1 (0.3)	8 (0.7)
Serogroup C (Corcord)	43 (5.2)	9 (2.3)	52 (4.2)
Serogroup D (Enteritidis)	5 (0.6)	0	5 (0.4)
Total	**55 (6.7)**	**10 (2.5)**	**65(5.3)**
Shigella			
Sergroup B *(flexineri)*	20 (2.4)	22 (5.5)	42 (3.4)
Serogroup C *(boydii)*	4 (0.5)	2 (0.5)	6 (0.5)
Serogroup D *(sonnei)*	2 (0.2)	11 (2.8)	13 (1.1)
Total	**26 (3.2)**	**35 (8.8)**	**61 (5)**
Parasites			
Entamoeba histolytica	63 (7.6)	8 (2)	71 (5.8)
Giardia lamblia	59 (7.2)	49 (12.3)	108 (8.8)
Ascaris lumbricoides	16 (1.9)	25 (6.3)	41 (3.5)
Hymenolepis spp.	29 (3.5)	8 (2)	37 (3.0)
Trichuris trichuria	12 (1.5)	26 (6.5)	38 (3.1)
Schistosoma mansoni	0	4 (1)	4 (0.3)
Hookworm	0	1 (0.3)	1 (0.1)
Strolongyloides stercoralis	1 (0.1)	**0**	1 (0.1)
Total	**180 (21.8)**	**121 (30.3)**	**301 (24.6)**

Results

Study subjects

A total of 1,225 children visiting the outpatient paediatric departments with fever alone (222 children; 18.1%), fever and diarrhoea (244 children; 9.9%) and diarrhoea alone (759 children; 62%) were investigated for enteropathogens. The ages ranged from six months to 15 years with a mean age of 4.8 (SD ± 3.93) years. The majority of the patients (61.7%) were between the ages of six months and five years. The study consisted of 654 (53.4%) males and 571 (46.6%) females, resulting in an overall female to male ratio of 1:1.5. Of these 1,225 patients, 400 (32.7%) were from Jimma and 825 (67.3%) were from Addis Ababa.

Pathogen identification and subtyping

Results for all pathogens are summarised in Table 1.

Salmonella species: A total of 65 isolates of *Salmonella* were cultured from febrile and/or diarrhoeic children. The typing data for these 65 *Salmonella* isolates are presented in Figure 1. In addition, 48 *Salmonella* strains (collected between 2004 and 2005) from stock cultures were also analysed.

The antimicrobial susceptibility testing results of all 113 *Salmonella* isolates are shown in Table 2 and those for *S.* Concord are summarised in Table 3. The resistance patterns varied from four to eight drugs, and in general, *S.* Concord showed high-level resistance to the drugs used commonly for treatment of invasive salmonellosis, including third generation cephalosporins (ceftriaxone), ampicillin, trimethoprim-sulphamethaxole, chloramphenicol, and gentamicin. Susceptibility to fluoroquinolones (ciprofloxacin and ofloxacin) was tested by E-test. Of 82 *S.* Concord isolates tested, 62 were susceptible (MIC < 0.125 μg/ml), 18 showed reduced susceptibility (0.125 to < 1) and two were resistant (MIC ≥ 1 μg/ml). Nalidixic acid has been used as a marker of reduced susceptibility to fluoroquinolones in *Salmonella*, but this relationship does not seem to hold true for *S.* Concord. Of eight isolates resistant to nalidixic acid, five gave reduced susceptibility to ciprofloxacin (CIP^{RS}), two were susceptible, and one was resistant. Of 11 isolates with intermediate resistance to nalidixic acid, one was resistant, nine showed reduced susceptibility, and one was susceptible to ciprofloxacin. Of the 63 nalidixic acid susceptible isolates, 59 were susceptible and four gave reduced susceptibility to ciprofloxacin. Taken

together, the ciprofloxacin and nalidixic acid data (Figure 2) show that isolates with zone sizes around a nalidixic acid disc of 10-20 mm have notably variable MICs of ciprofloxacin, suggesting that more than one mechanism for fluoroquinolone resistance is present amongst the *S.* Concord isolates in Ethiopia, including target site mutations (Nal^R Cip^{RS}) and possibly *qnr* mediated resistance (Nal^S CIP^{RS}). The presence of extended spectrum beta-lactamase producing *S.* Concord was shown by phenotypic testing of 81 *S.* concord isolates of which 71 (86.5%) were ESBL-positive (MIC ratio for TZ/TZL was ≥ 8 or CT/CTL was ≥ 8) and 10 were unable to be determined (ND) (MIC of TZ was > 32 μg/ml and TZL > 4 μg/ml. CT was > 16 μg/ml and CTL was > 1 μg/ml).

Most of the *Salmonella* isolates were from stool (68%), but a very high proportion was from blood (32%); the most invasive serovar was *S.* Concord (Table 4), which suggests that the *S.* Concord circulating in Ethiopia is not just resistant to most antibiotics, but may also be highly invasive. It is certainly more invasive than *S.* Typhimurium in the same environment.

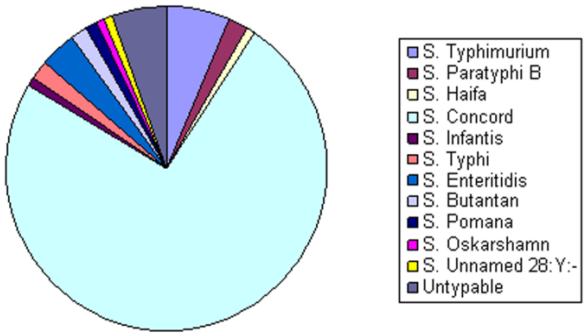
Sub-typing *S.* Concord by PFGE was not very informative. Isolates produced 9 to 13 fragments which ranged in size from 1135 kb to 50 kb. Overall, 16 different PFGE types/ profiles were seen among all isolates. There were 13 PFGE profiles seen among 24 *S.* Concord isolates. Six profiles were observed among the ten blood isolates. There was no association between the PFGE profiles of strains that were either isolated from different locations or from different specimens (blood/stool). *S.* Pomona (group M), *S.* Haifa (group B), and *S.* Butantan (Group E) showed different profiles from *S.* Concord.

Table 2. Resistance pattern by serovar for 113 *Salmonella enterica* isolates from two locations in Ethiopia.

SG	Serotypes	No.	Number of strains (%) resistant to									
			AM	AMX	SXT	CRO	C	TE	GM	NA	OFX	CIP
B(13)	*S.* Typhimurium	7	2 (28.6)	3 (42.9)	2 (28.6)	1 (14.3)	3 (42.9)	3 (42.9)	1 (14.3)	1 (14.3)	0	1 (14.3)
	S. Paratyphi B	2	0	0	0	0	0	0	0	0	0	0
	S. Haifa	1	0	0	0	0	0	0	0	0	0	0
	Untypeable	3	0	1 (33.3)	1 (33.3)	0	0	2 (66.6)	0	0	0	0
C_1(86)	*S.* Concord	85	84 (98.8)	83 (97.6)	84 (98.8)	83 (97.6)	83 (97.6.)	37 (43.5)	80 (94.1)	8 (9.4)	1 (1.2)	0
	S. Infantis	1	0	0	0	0	0	1 (100)	0	0	0	0
D(9)	*S.* Typhi	2	0	0	0	0	0	0	0	0	0	0
	S. Enteritidis	4	3 (75)	3 (75)	2 (50)	2 (50)	2 (50)	0	0	0	0	0
	Untypable	3	1 (33.3)	1 (33.3	1 (33.3	1 (33.3	1 (33.3	1 (33.3	0	0	0	0
E[52]	*S.* Butantan	2	0	0	0	0	0	0	0	0	0	0
M(3)	*S.* Pomana	1	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)			
	S. Oskarshamn	1	1 (100)	1 (100)	0	1 (100)	1 (100)	0 0	1 (100)			
	S. Unnamed 28:y:-	1	1 (100)	1 (100)	0	0	1 (100)	0	1 (100)			
All		**113**	**93 (82.3)**	**94 (83.2)**	**91 (80.5)**	**89 (78.8)**	**92 (81.4)**	**45 (39.8)**	**84 (74.3)**	**9 (8.0)**	**1 (0.9)**	**1 (0.9)**

AM: Ampicillin; AMX: Amoxicillin; SXT: Trimethoprim-sulphamethaxole; CRO: Ceftriaxone; C: Chloramphenicol; TE: Tetracycline; GM: Gentamycin; NA: Nalidixic acid; OFX: Ofloxacin; CIP: Ciprofloxacin

Figure 1. Proportion of serotypes for 65 *Salmonella* isolates from 1,225 diarrhoeal or febrile children in Ethiopia.



Pathogens other than Salmonella

The parasites isolated were *Giardia lamblia* (108; 8.8%), *Entamoeaba histolytica* (71; 5.8%), *Ascaris lumbricoides* (41; 3.5%), *Trichuris trichuria* (38; 3.1%), *Schistosoma mansoni* (4; 0.3%), hookworm and *Strongyloids stercoralis* (1; 0.1% each) (Table 1). Among the total 301 identified parasites, *G. lamblia* was the most frequently identified parasite in both study sites with an isolation rate of 32%.

Shigella *species*

A total of 61 *Shigella* species were isolated from stool specimens. The serogroup distribution of the 61 *Shigella* isolates is presented in Table 1. Serogroup B (*S. flexneri)* was the most frequently isolated species (68.9%) followed by group D (*S. sonnei*; 21.3%) and group C (*S. boydii*; 9.8%). *Shigella* species were more prevalent among children under five years of age.

Table 3. Resistance patterns in *S.* Concord

Resistance antibiogram	**No.**
C, TE	1
AM, SXT, TE	1
AM, SXT, CRO, C, GM	1
AM, SXT, CRO, GM	1
AM, SXT, CRO, C	1
AM, SXT, CRO, C, OFX	1
AM, SXT, CRO, C, GM	41
AM, SXT, CRO, TE, C	1
AM, SXT, CRO, C, GM, NA	3
AM, SXT, CRO, TE, C, GM	29
AM, SXT, CRO, TE, C, GM, NA	5
Total	**85**

Note: AM: Ampicillin; SXT: Trimethoprim-sulphamethaxole; CRO:Ceftriaxone; C: Chloramphenicol; TE: Tetracycline; GM: Gentamycin; NA: Nalidixic acid; OFX: Ofloxacin; CIP: Ciprofloxacin

Figure 2. Scatter plot showing the relationship between nalidixic acid resistance (disc diffusion zone diameter) and ciprofloxacin resistance (E-Test MIC).

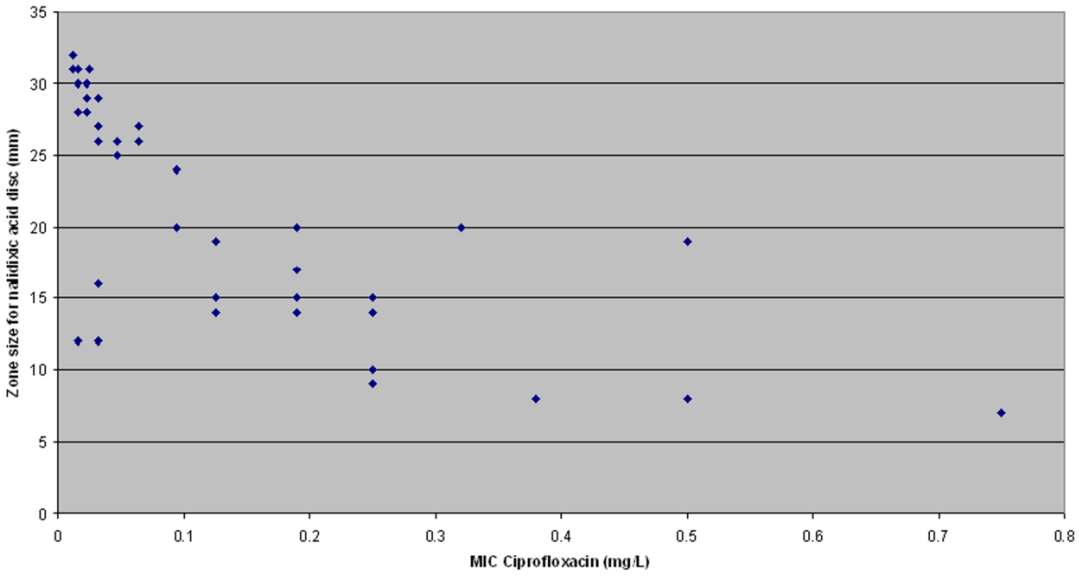


Table 4. Invasiveness of serovars of *Salmonella enterica* in Ethiopian children. *Invasive index = (blood isolates/total number of isolates for serotype) *100. As numbers for individual servars in some cases is low, the average for the serogroup is presented in the row labelled Total.

Serogroup	Serotypes	No	Invasive index *
B	*S*. Typhimurium	7	14%
	S. Paratyphi B	2	Insufficient data
	S. Haifa	1	Insufficient data
	nontypeable	3	Insufficient data
	Total	**13**	**7.7%**
C1	*S*. Concord	85	30.6%
	S. Infantis	1	Insufficient data
	Total	**86**	**30.2%**
D	*S*. Typhi	2	Insufficient data
	S. Enteritidis	4	Insufficient data
	nontypable	3	Insufficient data
	Total	**9**	**77.8%**
E	*S*. Butantan	2	Insufficient data
M	*S*. Pomana	1	Insufficient data
	S. Oskarshamn	1	Insufficient data
	S. Unnamed 28:Y:0	1	Insufficient data
	Total	**3**	**66.7%**
Total		**113**	**31.9%**

Discussion

In this study of febrile and diarrhoeic children, *Salmonella* was shown to be a common pathogen, present in 65/1,225 (> 5%) of cases. Detailed analysis of isolates from this study and stored isolates (n = 113) showed that NTS was far more common than *S.* Typhi (2/113). *S.* Concord (Serogroup C) predominated, which is similar to previous findings reported from Addis Ababa [8,15]. This observation suggests that *S.* Concord has been a major cause of human salmonellosis or food-borne disease in Ethiopia for at least two decades. Indeed, *S.* Concord was reported in Ethiopia for the first time more than two decades ago from a bone-processing factory in Addis Ababa [24].

The high rate of isolation of *S.* Concord in Ethiopia in the previous and present studies is unusual when compared to that of other countries. A few published reports mention *S.* Concord in Turkey [25], the Netherlands [26], and Saudi Arabia [27] but isolation is more common in Zaire [28] and Rwanda [29]. In many countries *S.* Typhimurium and *S.* Enteritidis are the predominant isolates reported although this may change over time. In Kenya, surveillance from 1994 to 1997 shows that *S.* Typhimurium predominated (prevalence of 75%) among cases of NTS bacteraemia; however, after 1997, the proportion of *S.* Enteritidis rose steadily and, by 2003, both were equally common [30]. In other parts of Africa (Cameroon, Mali, Morocco, Senegal and Tunisia), *S.* Enteritidis and *S.* Typhimurium are also reported in equal proportion [31]. The difference in the pattern of serotypes may be due to ecological (animal reservoirs) or to geographical differences in the many and varied sub-regions of the African continent, as well as variation over time [31]. A World Health Organization survey of all age groups on the global distribution of *Salmonella* between 2000 and 2002 showed that among human isolates, *S.* Enteritidis was the most common serovar, accounting for 65% of all isolates, followed by *S.* Typhimurium at 12% and *S.* Newport at 4% [31].

Even within Ethiopia, studies conducted in Gondar, Jimma, and Addis Ababa show that serogroup B isolates were the most common

[14,32,33], and, for some hospital-based studies [5,6], *S.* Typhi was predominant. However, what seems clear is that in many countries of Africa, NTS accounts for a steadily increasing proportion of human infections, including severe conditions such as septicaemia [34]. This is especially true in association with HIV infection [35-37].

In the study reported here, *S.* Concord was not only common, but was also variable by PFGE, suggesting the strains have had time to diverge. Worryingly, *S.* Concord was also the most invasive NTS: only 14% of *S.* Typhimurium was isolated from blood compared to 30% of *S.* Concord. The importance of NTS is also illustrated by data from Malawi: NTS bacteraemia was diagnosed in 299 children during a two-year period with a case fatality rate of 24% [38]. In the current study, a higher proportion of children under five years of age (as compared with children over five years) presenting with NTS infection developed bacteraemia; this finding may be attributed to lower immune status in the younger children. Although the HIV status of the subjects in this study was not investigated, the overall prevalence of HIV infection in all age groups of the Ethiopian population is about 3.5% [39]; therefore, it seems unlikely that HIV co-infection can explain the invasiveness of *S.* Concord. Dissemination of NTS might also be enhanced by intestinal inflammation resulting from chronic diarrhoeal disease, parasitic infection, or suboptimal nutrition [40]; all these factors are likely to be present in the Ethiopian children in this study as they were in Kenyan children [41]. Another possible explanation is the emergence of strains of *S. enterica* with an increased level of virulence. In Kenya and Malawi, using sequenced based typing, a newly emerged strain of *S.* Typhimurium has been identified [42]. It is vital that technologies capable of strain typing for local epidemiology and the tracking of newly emergent strains are translated onto platforms suitable for use in front-line laboratories in the developing world. If these powerful new technologies remain in the preserve of the research institutions, then an opportunity to gain information for public health action will be lost [43].

A worrying aspect of the *S.* Concord isolates from Ethiopian adoptees is the levels of antibiotic resistance. A study of Ethiopian adoptees in Denmark and the United States showed that out of 43 *S.* Concord isolates, 81% were multidrug-resistant (≥ 3 agents). In line with this study, the multidrug-resistant isolates reported previously were resistant to a third-generation cephalosporin and 14% had decreased susceptibility to ciprofloxacin [2].

Published data describing ESBL-producing *Salmonella* serovars in Africa is scarce. One study from South Africa showed that 5.6% of NTS produced ESBLs [44]. These ESBL positive isolates were *S.* Typhimurium, *S.* Isangi and *S.* Muechen. In the current study, 78/85 isolates of *S.* Concord from community-acquired salmonellosis harboured ESBLs encoding resistance to the third-generation cephalosporin ceftriaxone and at least four other resistance genes encoding resistance to the first-line drugs amoxicillin, trimethiprim-sulphamethoxazole, gentamicin, and chloramphenicol. This pattern of resistance has been observed for at least two decades [6], and resistance in *S.* Concord is not the result of selection in orphanages but represents an established strain capable of transmission in the population. Furthermore, a single isolate of *S.* Concord from an Ethiopian migrant in Ireland was also resistant to ampicillin, chloramphenicol, streptomycin, sulphonamide, tetracycline, trimethoprim, and gentamicin [45], further demonstrating the potential for international spread. Fluoroquinolones are also clinically compromised. The overall ciprofloxacin resistance rate in Concord (1.2%) seems low; however, the percentage of reduced susceptibility for ciprofloxacin (26.8%) indicated that the development of resistance to this drug is clinically relevant [46]. Thus, the emerging resistance and reduced susceptibilities to fluoroquinolones in our *Salmonella* isolates is of great concern for Ethiopia. This emphasises the need for local as well as national surveillance for emerging fluoroquinolone resistance.

The selection for resistance almost certainly comes from the availability of cheaper generic drugs for the treatment of this invasive bacterial infection in Ethiopia. A study on the practice of self-medication in Jimma town showed that at least 27.6% of 152 sick people self medicated [47]. The relatively low cost of generic medicine (35.7%) was the major reason for using self-medication. As unregulated suppliers are the only sources from which parents can obtain antibiotics for their sick children, this situation will not resolve until medical systems in Ethiopia improve, most likely in line with economic development.

Currently, the control of *S.* Concord infection in Ethiopia is stalled by the lack of data from acceptable epidemiological studies able to inform local control activities. Over two decades ago in 1985, Gebre Yohannes [7] commented that "the high isolation of

S. Concord in Ethiopia needs further study to clarify the animal or food source associated with its epidemiology." Nothing has been implemented; however, as there is now a concern over the global spread of antibiotic-resistant *S.* Concord, [2,3] perhaps funding can be improved. Epidemiological investigations of salmonellosis in developing countries such as Ethiopia are difficult to conduct because of the limited scope of strain typing available for the studies and a lack of coordinated surveillance systems. However, despite these difficulties, the overall isolation rate of *Salmonella* in this study, 5.3%, is comparable with other studies conducted in Ethiopia at different times (4.5% reported by Ashenaffi and Gedebou [15]; 6.4% reported by Mache *et al*. [8]; 4.5% reported by Asrat *et al*. [14]; 3.8% reported by Aseffa *et al.* [32]; 15% reported by Mache [33]; and 8.1% reported by Awol [48]) showing that NTS is a major problem in Ethiopia. It is essential for global control that countries such as Ethiopia are able to document the occurrence and trends of *Salmonella* serovars to detect local, regional, national, and even international outbreaks. This will enable early warning about potentially virulent strains and should facilitate the elimination of the source by suggesting preventive actions. To enable control measures, detection of the reservoir host of *S.* Concord is necessary, and this must involve studies in Ethiopia.

In conclusion, this study has shown that salmonellosis, in two study sites in Ethiopia, rural and urban, is mainly due to highly drug-resistant *S.* Concord. These results may not be consistent with results obtained in other countries in the East African region, so data from one African country cannot be used to represent an entire continent. It seems highly likely that the *S.* Concord infections seen in Ethiopian adoptees in Europe and America are of a highly virulent strain that is circulating in the Ethiopian population. Prevention of further international spread may depend on addressing the problem in Ethiopia.

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