

Full Length Research Paper

Serotypes and antibiotypes of *Salmonella* isolated at the University Teaching Hospital of Yopougon, Abidjan, Cote d'Ivoire from 2005 to 2009

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***Salmonella* are responsive of gastroenteritis or bacteremia through fecal-oral transmission. *Salmonella* are also incriminated in meningitis particularly in patients with immune deficiency. The emergence of new serotypes and antibiotic resistance in *Salmonella* is a public health concern in developing countries. The aim of this study is to determine *Salmonella* serotypes circulated in the country and describe antibiotic resistance phenotypes. A retrospective study was conducted from January 2005 to December 2009. The strains were identified according to standard bacteriological methods. Serotypes were determined by slide agglutination using sera according to Kauffmann-White scheme. Antimicrobial susceptibility was performed using Kirby- Bauer method and interpretation was made according to the Comité de l'Antibiogramme–Société Française de Microbiologi (CA-SFM). A total of 62 *Salmonella* strains were isolated from 2005 to 2009. These strains were identified in 53.2% of cases in blood cultures 16.1 % in stool and 3.2% in CSF. Typing was possible for 76.1% of strains. Among serotypes, non-typhoid *Salmonella* were prevalent, 76.1 versus 23.9% for *Salmonella typhi*. *S. typhimurium* was recovered in 37% and *Salmonella enteritidis* 16%. The resistance rates ranged from 66.6 to 100% for amoxicillin, 50 to 75% for cotrimoxazole and 41.7 to 75% for chloramphenicol. Multidrug resistance (to three or more antibiotics) rate was 34.1% for non-typhoid and 33.3% for *S. typhi*. Resistance to nalidixic acid was 38% and reduced susceptibility to ciprofloxacin 14%. This study highlight emergence of multidrug-resistant *Salmonella* to antibiotics in pediatric. *Salmonella* surveillance must be put in place in Côte d'Ivoire to improve epidemiological analysis of strains diversity.**

Key words: *Salmonella*, Pediatrics, serotype, antibiotic resistance.

INTRODUCTION

Salmonella infections, remains a public health concern in developing countries. Among these infections according to serotypes *Salmonella* infections, gastrointestinal and extradiagnostic infections are noted. The *Salmonella*

serotypes called typhoid are responsive for gastroenteritis, also for bacteremia (Benacer, 2010). Other serotypes may be responsive of extra intestinal infections such as urinary tract infections, deep suppuration and meningitis in immuno-deficient individuals (WHO, 1996). About 80% of deaths due to *Salmonella* are found in Africa (Onyango, 2009). Mortality rate is estimated to reach 5% in Côte d'Ivoire. This country is located in West Africa, (Bakayoko, 2000,

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University of Abidjan, Côte d'Ivoire, Personal communication). Before 1990, 25% of blood cultures were positive to *S. Typhi*. An increase in the number of *S. enteritidis* in blood culture was noticed beginning HIV infection in Côte d'Ivoire (Dosso, 1998).

Salmonella typhi bacteremias (*S. Typhi*) are common in children less than three years of age in Kenya (Doris, 2010). Moreover, in countries where salmonellosis is endemic especially in Kenya and India, the emergence multiple drugs resistant *Salmonella* with resistance ampicillin, cotrimoxazole and chloramphenicol was observed making treatment of salmonellosis becoming difficult (Ben Hassen, 1993). Currently, treatment of multi-resistant *Salmonella* uses fluoroquinolones (Parry 2002 and Mills-Roberson 2008). In 2005, emergence of multi-resistant *S. Typhi*, resistant to three antibiotics earlier mentioned and resistant to nalidixic acid was observed in Cameroon (Nkemngu, 2005). In Côte d'Ivoire few data are available regarding *Salmonella* serotypes. Moreover, of multidrug-resistant strains prevalence is unknown. This study aims to determine *Salmonella* serovars circulation and describe antibiotic resistance phenotypes of identified strains.

METHODS

Study design: From January 2005 to December 2009, a retrospective study was conducted at the Laboratory of Bacteriology-Virology, University Teaching Hospital of Yopougon (Côte d'Ivoire). A total of 62 strains of *Salmonella* were isolated from various specimen collected in children and adults hospitalized in pediatric ward and adult in patient services of the University Teaching Hospital Center of Yopougon, Abidjan, Côte d'Ivoire. The strains were collected from thirty-three peripheral blood, ten stools, two Cerebral Spinal Fluid (CSF) six urines and eleven pus. A survey form supplying socio-demographic information such as age and sex was to be completed for each microbiological record. Thus, to avoid the duplicate strain, we made so that each patient has a single identifier.

Bacteriological analysis: Specimens were collected and transported to laboratory for analysis. Specimens were carried out according to conventional microbiological methods. *Salmonella* were identified based on biochemical characteristics (Marnonnier, 1987). Antibiotics susceptibility was performed and strains *Salmonella* were inoculated in deep agar and send at 37°C to the reference laboratory for serotyping.

Serotyping: *Salmonella* serotypes were determined using agglutinating antisera (Biorad, Hercules, Californie, USA) based on Kauffmann and White scheme.

Antibiotic susceptibility testing: The Kirby-Bauer disc-diffusion test which conform to the recommendations of Antibiotic susceptibility Committee of the French Society of Microbiology (CA-SFM, 2009). Antibiotics discs with the following drugs content: Amoxicillin (A) 25 µg, amoxicillin / Clavulanic acid (AMC) 20/10 µg, Cefazidime 30µg (CTZ), Cefotaxime 30µg (CTX), Ceftriaxone 30 µg (CRO), Chloramphenicol 30 µg (C), Tetracycline 30 IU (T), Trimethoprim-sulfamethoxazole 1.25 / 23 , 75 µg (STX), Nalidixic acid 30 µg (NA), Ciprofloxacin 5 µg (CIP) (Antibiotic Biorad Hercules, Californie, USA), were placed at least 15 mm apart and

from edge of the plates to prevent the overlapping of the inhibition zones. Plates were incubated at 37°C for 24 h, and the diameters of the inhibition zones were compared with recorded diameters of the control organism *Escherichia coli* ATCC 25922 to determine the susceptibility or resistance of isolates to various drugs.

Statistical analysis: Data were analyzed using Epi Info 2000 software from the CDC in Atlanta

RESULTS

Epidemiological and serotype distribution

A total of 62 *Salmonella* strains were isolated from 2005 to 2009. Most of them came from children under 15 years (53.2%). 51.6% of specimens were identified in pediatric service followed by General Medicine service 35.5%. Less than 2% of the strains were from the Departments of Surgery and Obstetrics and Gynecology.

Salmonella strains were identified in 53.2% of cases from blood cultures, 27.4% of cases in various suppuration and 16.1% from stool samples. *Salmonella* were rarely isolated from urines 9.7% and from cerebrospinal fluid (CSF) 3.2% (Table 1). Of the 62 *Salmonella* strains isolated, 46 could be typed which give typing rate of 74.2%. Among these strains, *S. Typhi* accounted for 23.9% against 76.1% of non-typhoid *Salmonella* strains with *S. typhimurium* 50%, *S. enteritidis* 18.9% and *Salmonella dublin* 4.3%. Most of *S. Typhi* was isolated in patients under 15 years. 11.3 and 19.4% of strains of *S. enteritidis* and *S. typhimurium* respectively were isolated from children whose ages were less than 5 years (Table 1).

Antimicrobial drug resistance

The determination of resistance patterns showed 74.2% of strains resistant to amoxicillin and 58.1% to amoxicillin-clavulanic acid. 5 or 8.1% of strains were resistant to cefotaxime. Most of the strains were resistant to chloramphenicol, tetracycline and cotrimoxazole with over 50%. The rate of strains simultaneously resistant to amoxicillin, cotrimoxazole and chloramphenicol called multidrug-resistant bacteria or MDR was estimated to 30%. Concerning the quinolones, the resistance to nalidixic acid was 38% and the rate of strains with reduced susceptibility to ciprofloxacin 14%. The strains of *S. Typhi* showed resistance rates of 66.6% for amoxicillin and cotrimoxazole and 50% to chloramphenicol. For *S. typhimurium* resistance rates to these antibiotics were higher than those observed with *S. Typhi*. The distribution of MDR strains were 33.3% for *S. Typhi* and 34.1% for non-typhoid *Salmonella* with 28.6% of *S. typhimurium*. *S. enteritidis* 30.8% (Figure 1). *S. dublin* had the highest rate of multidrug-resistant (MDR) among non-typhoid *Salmonella* strains with 33.3%. *Salmonella* strains MDR to nalidixic acid (NA-MDR) were found in 16.7% of *S.*

Table 1. Distribution of *Salmonella* serotype according of age, site isolation and service.

Age	S. Typhi {N=11 (100%)}	S. non Typhi {N= 51(100%)}	Total {N=62(100%)}
Child [0 –15 years]	8 (72. 7)	25 (49)	33(53. 2)
Adults > 15	2(18. 2)	19 (37. 3)	21(33. 9)
NR*	1(9. 1)	7(13. 7)	8 (12. 9)
Specimen			
Blood culture	9 (81. 2)	24(47)	33 (53.2)
Stool cultures	1(9.1)	9(17. 6)	10(16.1)
Pus	1(9.1)	10 (19. 6)	17 (27.4)
ECBU	0	6(11. 7)	6(9. 7)
CSF	0	2 (3. 9)	2(3. 2)
Service			
Pediatric	7(63.7)	25 (49)	32(51.6)
Medicine	2(18.2)	20(39. 2)	22(35.5)
Surgery	0	1(1. 9)	1(1.6)
Obstetric-Gynaeco	0	1(1. 9)	1(1.6)
External	1(9.1)	7(13. 7)	8(12.9)

NR: No Report

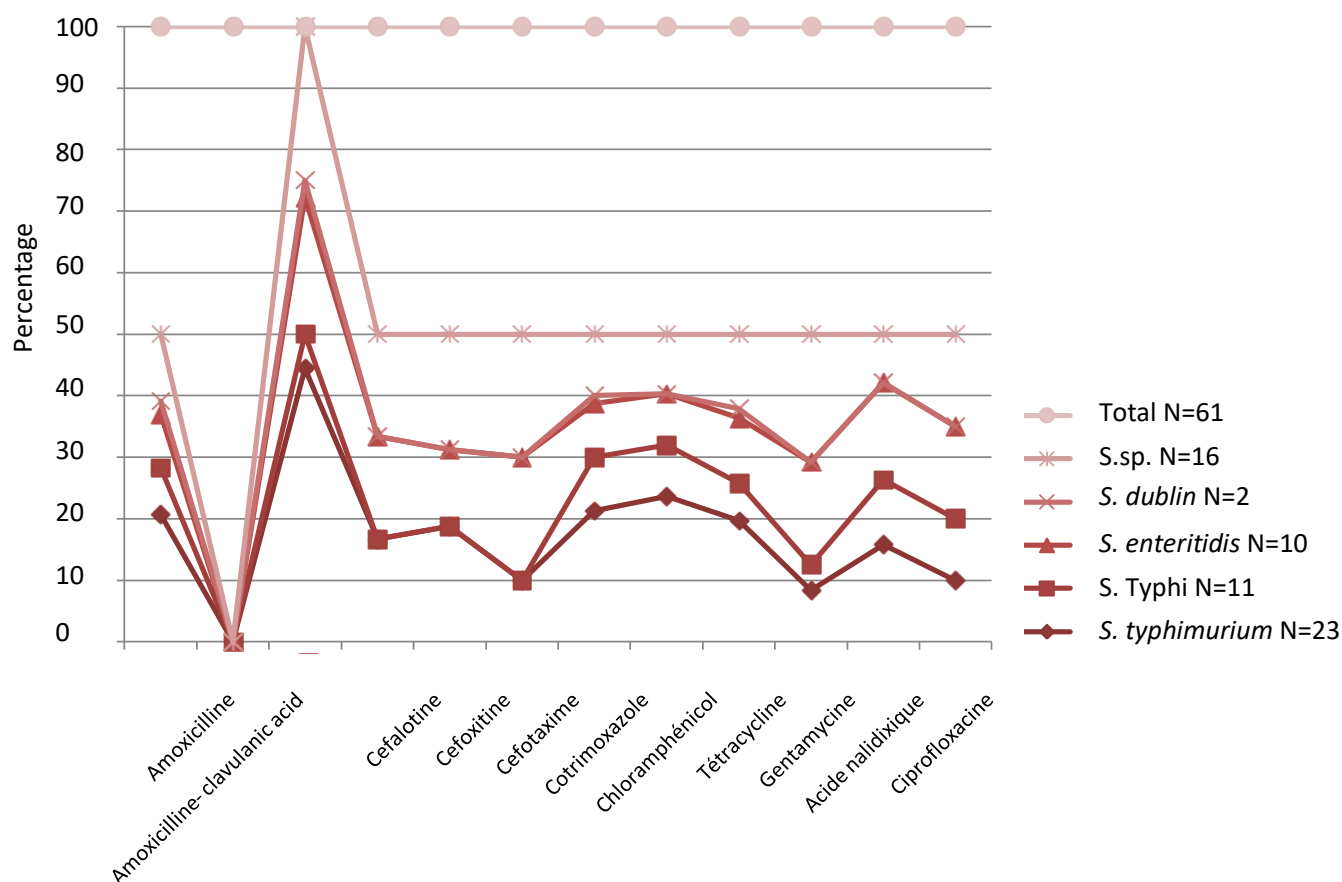


Figure 1. Number of *Salmonella* serotype resistant to antibiotics.

Table 2. *Salmonella* strains antibiotics resistant phenotype.

Variable	<i>S. Typhi</i>	<i>S. typhimurium</i>	<i>S. enteritidis</i>	<i>S. dublin</i>
	{N=6 (%)}	{N=28 (%)}	{N=13 (%)}	{N=3 (%)}
A	6(100)	15 (53.6)	8(61.5)	2(66.7)
Sxt	5(83.3)	13(46.4)	7(53.8)	2(66.7)
C	3(50)	13(46.4)	6(46.1)	1(33.3)
Ctx	0	1(3.57)	2(15.4)	0
T	3(50)	9(32.1)	5(38.5)	2(66.7)
Na	4(66.7)	6(21.4)	6(46.1)	0
Cip	1(16.7)	2(7.14)	4(30.8)	0
Asxt	5(83.3)	13(46.4)	5(38.5)	2(66.7)
ASxtC (MDR)	2(33.3)	8(28.6)	4(30.8)	1(33.3)
ASxtCNa (MDR-NA)	1(16.7)	3(10.7)	3(23)	0

A: Amoxicillin; SXT: Trimethoprim-sulphamethaxole; Ctx: Cefotaxim; C: Chloramphenicol; T: Tetracycline; NA: Nalidixic acid; CIP: Ciprofloxacin, MDR: Multi drugs resistance

Typhi, 10.7% of *S. typhimurium* and 23% of *S. enteritidis* (Table 2).

In this study no strain produces an extended spectrum beta lactamase (ESBL).

DISCUSSION

The emergence of new serotypes responsible for community acquired infections make a supervision of *Salmonella* strains in developing countries mandatory. Furthermore the emergence of multidrug-resistant *Salmonella* (MDR) to common antibiotics such as amoxicillin, cotrimoxazole and chloramphenicol is a problem in the therapeutic treatment of *Salmonella* infections especially in children. This study has enabled us to determine the prevalence of the major *Salmonella* serotypes circulating in Yopougon University Teaching Hospital and to identify antibiotic resistance phenotypes of isolates.

Epidemiologic and serotype distribution

Regarding the age distribution of patients according to the serovars isolated, there is a recruitment bias due to the fact that 50% of patients come from the pediatric ward. However extraintestinal infections caused by *Salmonella* species are a common cause of severe morbidity and mortality among young children from tropical Africa (Hill et al., 2007). Blood cultures are the main organic product from where *Salmonella* strains were isolated. This can be attributed to the fact that the blood culture is the systematic analysis test and the essential means of diagnosis of salmonellosis.

In our study, *S. Typhi* accounted for less than 10% of the isolated strains. *S. Typhi* is rarely isolated in industrialized countries but remains responsible for

bacteraemia and gastroenteritis among children in developing countries (Green, 1993). Non-typhoidal *Salmonella* (NTS) is one of the most important enteric pathogens causing bacteraemia in young children in many parts of the world, including Africa (Usman, 2007). NTS were the majority of strain isolated in our study. The high prevalence of NTS poses a challenge for empirical antibiotic treatment guidelines (Graham 2009 and Nadjm, 2010). In sub-Saharan African countries these *Salmonella* are becoming more isolated in bacteraemia in children under five (Bahwere, 2001). Studies carried out in Europe and America reported *S. enteritidis* as the first causative agent of bacteremia and gastroenteritis (Araque, 2009 and Betancor 2009). *S. typhimurium* was predominant in some Africa Country (Karuiki 2006, 2008; Fashea 2010). Recently published studies again highlight that the vast majority of invasive NTS disease in African adults occurs in association with HIV infection and in those with advanced immunosuppression (Gordon, 2008 and Reddy, 2010).

Antimicrobial drug resistance

The phenotypes of resistance to antibiotics used in the treatment of *Salmonella* bacteremia is not well known in Côte d'Ivoire. Treatment with an appropriate antibiotic is essential for salmonellosis and because of the nature of the disease it should commence as soon as the clinical diagnosis is made rather than after the results of antimicrobial susceptibility tests are available (Rowe, 1997). To follow the generic WHO advice, in Côte d'Ivoire, salmonellosis first line therapy is chloramphenicol. This scheme is change to ceftriaxone when MDR NTS suddenly emerged. The emergence of *Salmonella* resistant to third generation cephalosporins and fluoroquinolones is due to the overuse of these drugs in human medicine and animal husbandry (Coulbaly,

2010). It cannot be assumed that treatment with alternative antibiotics such as cephalosporins or fluoroquinolones will lead to a reduction in mortality because invasive NTS disease is commonly associated with co-morbidities which themselves carry a substantial risk of mortality (Graham, 2009). Typhoid and NTS are more resistant to Ampicillin, cotrimoxazole and chloramphenicol in this study. Recent studies from a range of settings in Africa report that *in-vitro* resistance to the same antibiotics is now very common (Morpeth, 2009 and Vadenberg, 2010). When non-typhoidal salmonellae can be identified, fluoroquinolones (eg, ciprofloxacin) or cephalosporins (eg, ceftriaxone) are increasingly relied on for treatment (Gordon, 2008) and non-typhoidal salmonellae seem to be susceptible to these antibiotics.(Enwere, 2006 and Graham 2009).

Conclusion

These epidemiological data show the distribution of *Salmonella* and the emergence of MDR. In this study the resistance of *Salmonella* strains to quinolones highlights the need for the establishment of a network of continuous monitoring of antibiotic resistance. Surveillance of circulation and emergence of new serotypes of *Salmonella* is necessary to adapt the therapy scheme in Côte d'Ivoire.

REFERENCES

- Araque M (2009). Nontyphoid *Salmonella* gastroenteritis in pediatric patients from urban areas in the city of Mérida. Venezuela J Infect Dev. Ctries., 3(1): 28-34.
- Bahwere P, Levy J, Hennart P (2001). Community-acquired bacteremia among hospitalized children in rural central Africa. Int. J. Infect. Dis., 5: 180–188.
- Bakayoko S (2000). Interest of serodiagnosis Widal and Felix in the diagnosis of salmonella Intérêt du sérodiagnostic de Widal et Félix dans le diagnostic des salmonelles. Med. Thesis. Abidjan., 2470 :110.
- Ben Hassen A, Bejaoui M, Lakhoua MR, Ben Redjeb S (1993). Profil épidémiologique de 153 souches de *Salmonella* (S. Typhi exclues) isolées en milieu pédiatrique tunisien de 1985 à 1990. Path. Biol., 41(8): 706-712.
- Benacer D , Kwai lin T, Haruo W, Savithri DP (2010). Characterization of drug-resistant *Salmonella enteric* serotype *Typhimurium* by antibiograms, plasmids, integrons, resistance genes, and PFGE. J. Microbiol. Biotechnol., 20(6): 1042-1052.
- Betancor L, Yim L, Fookes M et al. (2009). Genomic and phenotypic variation in epidemic-spanning *Salmonella enterica* serovar Enteritidis isolates. BMC Microbiol., 9: 237.
- Comité de l'Antibiogramme de la Société Française de Microbiologie (2009) : CA-SFM.
- Coulibaly E, Bakayoko S, Karou T (2010). Serotyping and antibiotic resistance of *Salmonella* strains isolated from the livers of chickens sold in the markets of Yopougon (Abidjan, Côte d'Ivoire) in 2005 .RASPA Vol.8 N°S.
- Dosso M, Coulibaly M and Kadio A. (1998). The role of bacterial diarrhea in developing countries. Bull Soc Pathol Exot.; 91(5): 1-2, 402-5.
- Doris M. Kariuki S, Muigai A, Revathi G (2010). Trends in *Salmonella enteric* serovar Typhi in Nairobi, Kenya from 2004 to 2006. J. infect. Dev. Ctries., 4(6): 393-396.
- Enwere G, Biney E, Cheung YB (2006). Epidemiological and clinical characteristics of community-acquired invasive bacterial infections in children aged 2–29 months in The Gambia. Pediatr. Infect. Dis. J., 25: 700–05.
- Fashae K, Ogunsola F, Aarestrup F (2010). Antimicrobial susceptibility and serovars of *Salmonella* from chickens and humans in Ibadan, Nigeria. J. Infect. Dev. Ctries., 4(8):484-494.
- Gordon MA, Graham SM, Walsh AL (2008). Epidemics of invasive *Salmonella enterica* serovar Enteritidis and *Salmonella enterica* serovar Typhimurium infection associated with multidrug resistance among adults and children in Malawi. Clin. Infect. Dis., 46: 963–969.
- Graham SM (2009). English M. Non typhoidal Salmonellae: a management challenge for children with community acquired invasive disease in tropical African countries. Lancet; 372: 267–269.
- Green SD, Cheesbrough JS (1993). *Salmonella* bacteraemia among young children at a rural hospital in western Zaire. Ann. Trop. Paediatr., 13: 45.
- Hill PC, Onyeama CO, Ikumapayi UNA, Secka O (2007). Bacteraemia in patients admitted to an urban hospital in West Africa. BMC, Infect. Dis., 7: 2.
- Kariuki S (2008). Typhoid fever in sub-Saharan Africa: Challenges of diagnosis and management of infections . J Infect Dev. Ctries., 2(6): 443-447.
- Kariuki S, Revathi G, Kariuki N (2006). Characterization of community acquired non-typhoidal *Salmonella* from bacteraemia and diarrhoeal infection in children admitted to hospital in Nairobi, Kenya BMC Microbiol; 6: 101.
- Marmonier A (1987). Techniques de diffusion en gélose méthodes des disques 137-248 In Bactériologie Médicale Techniques Usuelles : Carbonnelle B, Denis F, Marmonier AA, Pinon G, Vagues R. Paris ; France 3eme tirage. p. 323.
- Mills-Robertson F, Addy ME, Mensah P, Crupper S (2002). Molecular characterization of antibiotic resistance in clinical *Salmonella typhi* isolated in Ghana. FEMS, Microbiol. Lett., 215: 249–253.
- Morpeth SC, Ramadhani HO, Crump JA (2009). Invasive non-Typhi *Salmonella* disease in Africa. Clin. Infect. Dis., 49:606–611.
- Nadjm B, Amos B, Mtove G (2010). WHO guidelines for antimicrobial treatment in children admitted to hospital in an area of intense *Plasmodium falciparum* transmission: prospective study. BMJ; 340: c1350. doi:10.1136/ bmj.c1350
- Nkemngu NJ, Asonganyi ED, Njunda AL (2005). Treatment failure in a typhoid patient infected with nalidixic susceptibility to ciprofloxacin: a case report from Cameroon. BMC Infect. Dis., 5: 49.
- Onyango D, Ghebremedhin B, Waindi NE (2009). Phenotypic and genotypic analysis of clinical isolates *Salmonella* serovar Typhimurium in western Kenya. J. Infect. Dev. Ctries., 3(9): 685-694.
- Parry C, Wain J, Chinh NT, Vinh H, Farrar JJ (1998). Quinolonerésistant *Salmonella typhi* in Vietnam. Lancet, 351:1289.
- Reddy EA, Shaw AV, Crump JA (2010). Community-acquired bloodstream infections in Africa: a systematic review and meta-analysis. Lancet. Infect. Dis., 10: 417–432.
- Rowe B, Linda Ward R, John Threlfall E (1997). Multidrug-Resistant *Salmonella typhi*: A Worldwide Epidemic. CID; p. 24.
- Usman N, Martin A, Sonne-Hansen J (2007) .Molecular epidemiology of community-acquired invasive non-typhoidal *Salmonella* among children aged 2–29 months in rural Gambia and discovery of a new serovar, *Salmonella enterica* Dingiri. Journal of Medical Microbiology .56, 1479–1484
- Vandenbergh O, Nyarukweba DZ, Ndeba PM (2010). Microbiologic and clinical features of *Salmonella* species isolated from bacteremic children in eastern Democratic Republic of Congo. Pediatr. Infect. Dis. J., 29: 504–510.
- World Health Organisation WHO. (1996). The World Health Report, report of the director general WHO World Health Organisation: Geneva
- World Health Organisation WHO (2005). Pocketbook of hospital care for children: guidelines for the management of common illnesses with limited resources. Geneva: World Health Organization.