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Antibiotic Susceptibility Patterns of *Salmonella* Typhi in Jakarta and Surrounding Areas

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1. Introduction

Typhoid fever, also known as enteric fever, is a potentially fatal multi systemic illness caused primarily by *Salmonella* enterica serotype Typhi (*S*. Typhi). The classic presentation of the disease includes fever, malaise, diffuse abdominal pain, and constipation. Untreated, typhoid fever may progress to severe condition like delirium, intestinal hemorrhage, bowel perforation, and death. The disease remains a critical public health problem in developing countries. In 2000, it was estimated that over 2,16 million of typhoid occurrences worldwide, resulting in 216,000 deaths, and that more than 90% of this morbidity and mortality occurred in Asia [1]. A report from World Health Organization in 2008 on typhoid fever in five Asian countries showed the annual typhoid incidence (per 100,000 person years) among 5-15 years age group varied from 24.2 and 29.3 in Vietnam and China, to 180.3 in Indonesia; and to 412.9 and 493.5 in Pakistan and India, respectively; multidrug resistant *S.* Typhi were 23% (96/413) [2]. Further, unlike *S*. Typhi originated from Pakistan, Vietnam and India, those from Indonesia collected in North Jakarta, were all susceptible to antibiotic tested, i.e. Chloramphenicol, Ampicillin, Trimethoprim-Sulfamethoxazole; none of multidrug resistance were found. Nalidixic acid resistance was rather high in Pakistan, India and Vietnam, but none was found in Indonesia [2].

In Indonesia the prevalence of typhoid fever was 358-810 per 100,000 populations in 2007, with 64% of the disease was found in people aged 3-19 years. Mortality rate varies from 3.1-10.4% among hospitalized patients. Hatta and Ratnawati, 2008 reported a rise of resistance of *S*. Typhi to 6.8% to all three of first line drugs (Chloramphenicol, Ampicillin, Co-trimoxazole) in South Sulawesi (East of Indonesia) [3]. Antibiotics Fluoroquinolone and 3rd generation of Cephalosporin are frequently used for therapy of patients suspected typhoid fever in the past decade in many places especially in endemic countries including Indonesia due to resistance issues against conventional antibiotics [4, 5, 6, 7]. This study aimed to overview antibiotic susceptibility of *S*. Typhi originated from Jakarta and surrounding areas in particular to those

recommended by Performance Standard for Antimicrobial Susceptibility Testing for Clinical and Laboratory Standard Institute within 9 years period up to 2010.

2. Materials and methods

2.1 Specimens

Specimens used in the study were blood received in our laboratory i.e. Laboratory of Clinical Microbiology Faculty of Medicine University of Indonesia (CML-FMUI) Jakarta between 2002-2007. Our laboratory accepted specimens from hospitals, mainly the National Hospital Cipto Mangunkusumo (a tertiary general public hospital), primary health cares, private practices, and individuals. As in CML-FMUI, blood specimens from in and outpatients as well as other sources examined in Siloam Hospital Kebon Jeruk and St. Carolus Private Hospital in 2008-2010 were also included.

2.2 Culture and antibiotic susceptibility tests

Culture and antibiotic susceptibility tests were established in each of the above-mentioned institutions. Microbiology tests were performed according to microbiology standard practices and Performance Standards for Antimicrobial Susceptibility Testing for the Clinical and Laboratory Standards Institute (CLSI) [8]. Cultures were performed using Bac-T Alert™ (Enseval)/Bactec™ 9050 (Becton Dickinson), and sub cultured were on Salmonella-Shigella and MacConkey agar. Microorganism identification was determined using conventional biochemical reactions i.e. acid production from glucose, lactose, maltose, mannitol and saccharose, IMViC tests (Indole, Methyl Red, Voges Proskauer and Citrate) and H2S production in TSI agar. In recent years, API20E biochemical identification system (BioMerieux, Paris, France) was used instead. Susceptibility of microorganisms to antibiotics was assessed using the disc diffusion method. Antimicrobial susceptibility results were categorized in to three groups: Sensitive (S), Intermediate (I) and Resistant (R) according to CLSI guidelines. The antibiotics susceptibility data was then entered into the WHO-Net 5.4 program.

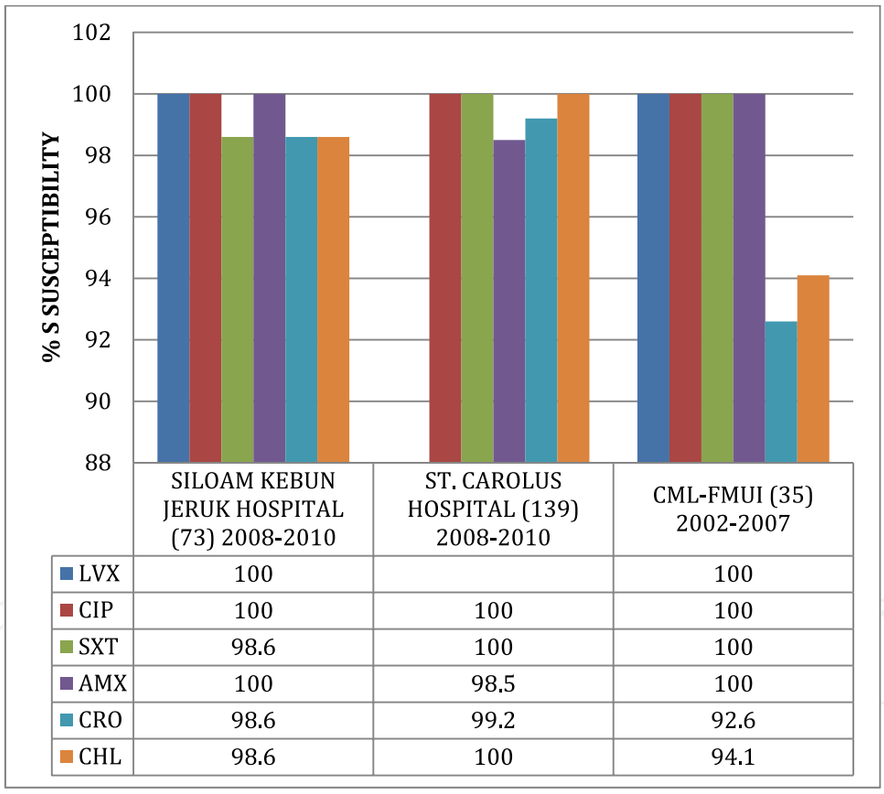
2.3 Antibiotics

Standard disc diffusion method was employed. The following antibiotic discs Chloramphenicol (CHL) 30µg, Amoxycillin (AMX) 25µg, Trimethoprim-Sulfamethoxazole (SXT) 1.25/23.75µg, Ceftriaxone (CRO) 30µg, Ciprofloxacin (CIP) 5µg, and Levofloxacin (LVX) 5µg were included in the study. These antibiotics are frequently used to treat typhoid fever in Jakarta, Indonesia. Susceptibility of *S.* Typhi to antibiotics was tabulated, and good activity in-vitro was defined by antimicrobial susceptibility of 80% or greater. Minimal inhibitory concentration was not examined.

3. Results

During nine years period from 2002-2010, 247 isolates of *S.* Typhi were collected, in which 35 isolates were from CML-FMUI, 73 and 139 isolates came from Siloam Kebon Jeruk and St. Carolus Hopitals respectively. In 2002-2007, all *S.* Typhi isolated in CML-FMUI was susceptible to antibiotics Levofloxacin, Ciprofloxacin, Trimethoprim-Sulfamethoxazole and

Amoxycillin (Figure 1). In 2008-2010, all of *S*. Typhi isolated in Siloam Hospital Kebon Jeruk was susceptible to Levofloxacin and Ciprofloxacin as also found in St. Carolus Hospital except antibiotic Levofloxacin was not tested on isolates from St. Carolus Hospital (see Figure 1). Susceptibility of the microorganism to Trimethoprim-Sulfamethoxazole showed almost similar pattern to those of CML-FMUI in the earlier years ranging from 98.6% to 100%, and so Amoxycillin that was 98.5% to 100%. Susceptibility of these microorganisms to Ceftriaxone seemed to increase from 92.6% in 2002-2007 to 98.6% or greater in 2008-2010. Lastly, although antibiotic Chloramphenicol was scarcely used in the treatment of typhoid fever compared to Fluoroquinolones, this antibiotic was still effective. The susceptibility of *S*. Typhi isolates to Chloramphenicol was 94.1% in 2002-2007, and was apparently increase to 98.6% or greater in 2008-2010 (Figure 1). Overall, during 9 years period up to 2010, antibiotic Chloramphenicol, Amoxycillin, Trimethoprim-Sulfamethoxazole, Ceftriaxone, Ciprofloxacin and Levofloxacin showed good activity in-vitro against *S*. Typhi originated from Jakarta and adjacent areas.



	SILOAM KEBUN JERUK HOSPITAL (73) 2008-2010	ST. CAROLUS HOSPITAL (139) 2008-2010	CML-FMUI (35) 2002-2007
LVX	100		100
CIP	100	100	100
SXT	98.6	100	100
AMX	100	98.5	100
CRO	98.6	99.2	92.6
CHL	98.6	100	94.1

Fig. 1. Susceptibility of *S*.Typhi to Antibiotics in Jakarta and Surrounding Areas

4. Discussions

Jakarta, as a capital city, is the biggest urban area in Indonesia. The city connects to five other satellites cities i.e. Bogor, Depok, Tangerang, Bekasi, Karawang, and it is densely populated where some districts are still impoverished. Health care system in the country does not support extensive program that covers laboratory examinations. This reflected in

the limited samples received in the laboratories. Clinical Microbiology Laboratory of Faculty of Medicine University of Indonesia is located in the center of Jakarta and known as the referral Microbiology Laboratory as it is part of the Department of Microbiology FMUI. The laboratory receives specimens from other laboratories, hospitals, primary health cares and also individuals. Furthermore, Siloam Kebon Jeruk in West Jakarta and St. Carolus in Central Jakarta are private hospitals; their laboratories serve for the hospitalized, outpatients and also other sources. In this study, 247 isolates of *S.* Typhi examined during 2002-2010 were susceptible to antibiotics Chloramphenicol, Amoxycillin, Trimethoprim-Sulfamethoxazole, Ceftriaxone, Ciprofloxacin, and Levofloxacin. In addition, *S.* Typhi was in fact still susceptible to antibiotic Tetracycline (Data not shown).

Some reports on antibiogram of *S.* Typhi from other institutions within the country were similar i.e. Central Laboratory of Cipto Mangunkusumo Hospital reported all S. Typhi isolated from hospitalized typhoid fever cases in 2009 were all susceptible to antibiotics (Chloramphenicol, Cotrimoxazole, Ceftriaxone, Cefuroxime, Ampicillin-Sulbactam, Amoxycillin-Clavulanic acid, Ciprofloxacin, Levofloxacin) [9]. In 2003-2005, ninety isolates of *S.* Typhi were collected from some districts in East Jakarta. All of these isolates were susceptible to first line drugs (Chloramphenicol, Trimethoprim-Sulfamethoxazole, Ampicillin) and Tetracycline except for only 1 isolate which was resistant to Chloramphenicol [10].

Data obtained from outside Jakarta such from Pakanbaru in Sumatra Island in 2009-2010 also showed very similar susceptibility patterns. Those *S.* Typhi isolates were all susceptible to Chloramphenicol, Tetracycline, Trimetoprim-Sulfamethoxazole, Amoxycillin-Clavulanic Acid, Cefotaxim, Cefepime, Ceftazidime, Cefazolin, Ceftriaxone and Ciprofloxacin [11]. *S.* Typhi isolated from South Sulawesi, however, showed an increase resistant against Chloramphenicol and Ciprofloxacin between 2001-2007, which was 1.04% to 7.84% and 0.11% to 6.83% respectively [3]. In 2003, a collaborated study on enteric bacteria in patients with diarrhea had been carried out in United States Naval Medical Research Unit, Jakarta that involved many health institutions from many cities in Indonesia including Medan, Padang, Batam, Jakarta, Pontianak, Denpasar and Makassar. A total of 111 *S.* Typhi had been isolated from feces, and all were susceptible to antibiotic tested i.e. Ampicillin, Trimethoprim-Sulfamethoxazole, Chloramphenicol, Tetracyclin, Cephalotin, Ceftriaxone, Norfloxacin and Ciprofloxacin. Nalidixic acid resistance was not found [12].

Resistance to Chloramphenicol was reported to emerge in only two years after its introduction in 1948, and was not until 1972 that typhoid fever caused by Chloramphenicol-resistant *S.* Typhi became a major problem; outbreaks occurred in Mexico, India, Vietnam, Thailand, Korea, and Peru (cited from Parry et al, 2002 [13]). Toward the end of the 1980s and the 1990s, *S.* Typhi developed resistance simultaneously to all the drugs that were then used as first line treatment (Chloramphenicol, Trimethoprim, Sulfamethoxazole, and Ampicillin). Despite multidrug-resistant *S.* Typhi are still common in many areas of Asia, strains that are fully susceptible to all first line antibiotics have reemerged in some areas [13]. Chau et al, 2007 reported that of eight endemic countries from Indian continent to China, multidrug resistance (MDR) *S.* Typhi varied from 16 to 37% and Nalidixic acid resistance were 5 to 51% [7]. In some places Nalidixic Resistant *S.* Typhi (NRST) was reported to cause more complication and poorer outcome of the disease; the presence of NRST is critical and influenced the successful rate of therapy with Fluoroquinolone [14].

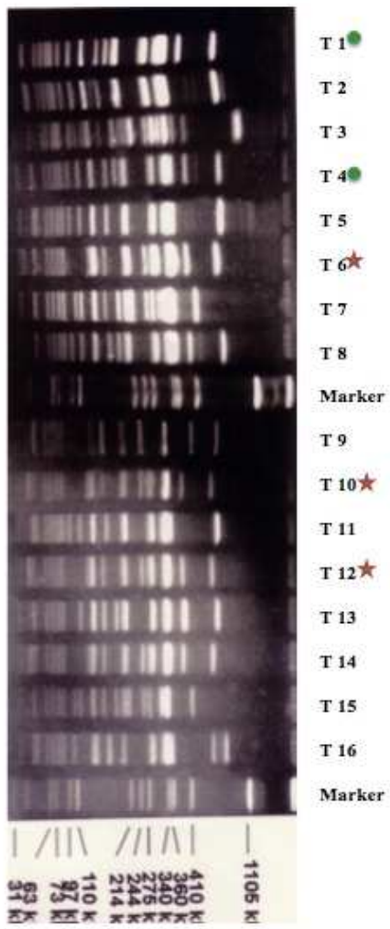
Despite an increase resistance elsewhere, certain areas especially in Northern India, reported that Chloramphenicol resistance has reduced from a high of 18% to only 2% [15].

Differences in the antibiotic susceptibility profiles as well as clinical appearance of typhoid fever cause by *S.* Typhi rely on many factors. Conditions such as disease control programs, inadequate policy of using antibiotics, local conditions that include personal hygiene, availability of clean drinking water, food handling and sanitation contribute to the complexity and outcome of the disease. Nevertheless, one important element of the diverse clinical manifestation of typhoid fever is the presence of genome plasticity of *S.* Typhi. Many studies had been conducted in the late nineties on genome profile of *S.* Typhi and showed the diversity of the genome. Some of strains originated from a certain region or country share some degree of similarity to other strains from different places. Our earlier study on genetic relationship using pulsed-field gel electrophoresis (PFGE) found that *S.* Typhi originated from five cities i.e. Medan, Jakarta, Pontianak, Makassar and Jayapura in Indonesia expanding from west to east part of the country, had clusters of endemic strains in certain geographic areas [16]. The presence of specific strains in localized area might have been the reason of varied symptoms of the disease and, possibly their susceptibility to antibiotics. Despite heterogeneity and different clonality of the 33 isolates of *S.* Typhi used in the study, these endemic strains were in fact all susceptible to Chloramphenicol, Ampicillin and Cotrimoxazole [16].

Some investigators reported a correlation of certain genome profiles of *S.* Typhi strains and their ability to cause a fatal typhoid fever [17]. Others reported specific flagellar types were associated with severe outcome of the disease [18]. A study carried out in our laboratory by Tjita in 2000 showed that 3 *S.* Typhi strains had identical genome profiles deduced from PFGE [19]. Each of the strains showed different susceptibility against several antibiotics i.e. one strain was resistant to Tetracycline; another two were multi resistant to Chloramphenicol/Tetracycline, and Ampicillin/Chloramphenicol/ Tetracycline respectively (see Figure 2). In addition to the findings, two other *S.* Typhi strains which resistant to Ampicillin and Tetracycline were found to be an identical strains [19]. The mentioned conditions could have been the reasons that conventional drugs such as Chloramphenicol, Amoxycillin, Trimethoprim- Sulfamethoxazole and Tetracyclin still have good activity in-vitro against *S.* Typhi strains in Jakarta and surrounding areas. In recent years, antibiotic Fluoroquinolone has been widely used in the treatment of typhoid fever in Indonesia, and it showed superiority in term of efficacy and safety [20]. Previous reports by Ochiai et al, 2008 [2] and Tjaniadi et al, 2003 [12] showed Nalidixic acid resistant *S.* Typhi was not found thus far in Indonesia. Cautious is adviced, however, since quinolone resistant *S.* Typhi strains have been an important issue in regional and global [6, 21, 22, 23].

In conclusion, despite resistance issue of *S.* Typhi from other countries, this study showed that most of all *S.* Typhi isolated in certain places in Jakarta and neighboring areas were susceptible to antibiotic tested (Chloramphenicol, Amoxycillin, Trimethoprim-Sulfamethoxazole, Ceftriaxone, Ciprofloxacin, Levofloxacin). This information is important since antimicrobial therapy plays a key role in management of typhoid fever disease. The susceptibility profiles, however, were only derived from certain strains, which may not represent all strains, which present in Indonesia. Therefore it is necessary to perform cultures and antibiotic sensitivity tests on patients with suspected typhoid fever, and so the

patients can be treated with definitive antibiotic therapy. Needless to say, adequate antibiotic therapy will prevent the spread of antibiotic-resistant *S*. Typhi strains. Lastly, promotion of public health such as personal hygiene, sanitations, clean drinking water, food handlings and also vaccination are equally important as prevention of the disease.



Sixteen *S*. Typhi isolates were originated from patients with typhoid fever in Jakarta. The genome was digested *Xba*I restriction enzyme. T6, T10 and T12 [★] were identical strains, which showed different susceptibility patterns i.e. T6 resistant to Tetracycline, T10 resistant to Chloramphenicol/Tetracycline, T12 resistant to Ampicillin/Chloramphenicol/Tetracycline. T1 and T4 [●] had identical genome profiles but each showed different susceptibility patterns i.e. T1 resistant to Ampicilin, and T4 resistant to Tetracycline. Modified from Tjita, 2000 [19].

Fig. 2. Genome profile of *S*. Typhi isolates from Jakarta using Pulsed-Field Gel Electrophoresis

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6. References

[1] Crump JA, Luby SP, Mintz ED. The global burden of typhoid fever. Bull World Health Organ 2004; 82: 346-53.

[2] Ochiai RL, Acosta CJ, Danovaro-Holliday MC, Baiqing D, Bhattacharya SK, Agtini MD, Bhutta ZA, Canh DG, Ali M, Shin S et al. A study of typhoid fever in five Asian countries: disease burden and implications for controls. Bull World Health Organization 2008; 86: 260–268.

[3] Hatta M and Ratnawati. Enteric Fever in endemic areas of Indonesia: an increasing problem of resistance. J Infect Developing Countries 2008; 2(4): 279-282.

[4] Hasan R, Zafar A, Abbas Z, Mahraj V, Malik F and Zaidi A. Antibiotic resistance among *Salmonella enterica* serovars Typhi and Paratyphi A in Pakistan (2001-2006). J Infect Developing Countries 2008; 2(4): 289-294.

[5] Kumar S, Rizvi M and Berry N. Rising prevalence of enteric fever due to multidrug-resistant Salmonella: an epidemiological study. Journal of Medical Microbiology 2008; 57: 1247-1250.

[6] Jin Yang. Enteric Fever in South China: Guangxi Province. J Infect Developing Countries 2008; 2(4): 283-288.

[7] Chau TT, Campbell JI, Galindo CM, Hoang NVM, Diep TS, Thi Nga TT, Chau NVV, Tuan PQ, Page AL, Ochiai RL et al. Antimicrobial Drug Resistance of *Salmonella enterica* Serovar Typhi in Asia and Molecular Mechanism of Reduced Susceptibility to the Fluoroquinolones. Antimicrobial Agents and Chemotherapy 2007; 51 (12): 4315-4323.

[8] Performance Standards for Antimicrobial Susceptibility Testing. Clinical and Laboratory Standard Institute (CLSI); year up to date.

[9] Loho T. Microorganism mapping and their Susceptibility to antibiotics in National Hospital Cipto Mangunkusumo Jakarta in 2009. (Unpublished).

[10] Ali S. Antibiotic Susceptibility of *Salmonella* Typhi in Jakarta in 2001-2003. (Unpublished).

[11] Anggraini D. Susceptibility of *Salmonella* Typhi in Private Hospital in Pakanbaru Sumatra in 2010. (Unpublished).

[12] Tjaniadi P, Lesmana M, Subekti D, Machpud N, Komalarini S, Santoso W, Simanjuntak CH, Punjabi N, Campbell JR, Alexander WK, Beecham III HJ, Corwin AL and Oyofo BA. Antimicrobial Resistance of Bacterial Pathogens Associated with Diarrheal Patients in Indonesia. Am J Trop Med Hyg 2003; 68 (6): 666-670.

[13] Parry CM, Hien TT, Dougan G, White NJ and Farrar JJ. Typhoid Fever. Medical progress. N Engl J Med 2002; 347: 1770-1782.

[14] Kadhiravan T, Wig N, Kapil A, Kabra SK, Renuka K and Misra A. Clinical outcomes in typhoid fever: adverse impact of infection with nalidixic acid-resistant *Salmonella* Typhi. BMC Infectious Diseases 2005; 5:37. http://www.biomedcentral.com/1471-2334/5/37

[15] Shrikala Baliga. Drug Resistance in *Salmonella typhi:* Tip of the Iceberg. *Online J Health Allied Scs.* 2004; 4: 1.

[16] Moehario LH. The molecular epidemiology of *Salmonella typhi* across Indonesia reveals bacterial migration. J Infect Dev Ctries 2009; 3(8): 579-584.

[17] Thong KL, Passey M, Clegg A, Comb BG, Yassin RM, and Pang T. Molecular analysis of isolates of *Salmonella* Typhi obtained from patients with fatal and non fatal typhoid fever. J Clin Microbiol 1996; 34: 1029-1033.

[18] Franco A, Gonzales C, Levine OS, Lagos R, Hall RH, Hoffman SL, Moechtar MA, Gotuzzo E, Levine M and Hone DM. Further consideration of the clonal nature of *Salmonella typhi*: Evaluation of molecular and clinical characteristics of strains from Indonesia and Peru. J Clin Microbiol 1992; 30(8): 2187-2190.

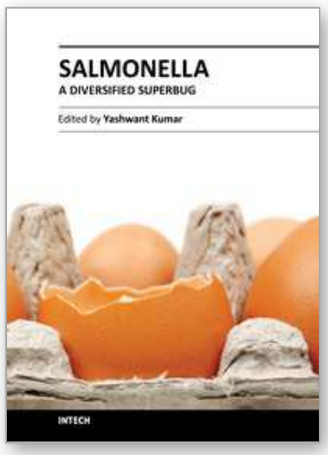
[19] Tjita PJ. Analysis of genetic diversity of *Salmonella typhi* resistant against first line drugs using Pulsed-Field Gel Electrophoresis. (Magister Thesis in Biomedical Science, 2000. Unpublished).

[20] Nelwan RH, Chen K, Nafrialdi and Paramita D. Open study on efficacy of Levofloxacin in treatment of uncomplicated typhoid fever. Southeast Asian J Trop Med Public Health 2006; 37(1): 126-130.

[21] Accou-Demartin M, Gaborieau V, Song Y, Roumagnac P, Marchou B, Achtman M, and Weill FX. *Salmonella enterica* Serotype Typhi with Non-classical Quinolone Resistance Phenotype Emerging Infectious Diseases 2011; 17(6): 1091-1094.

[22] Keddy KH, Smith AM, Sooka A, Ismail H, and Oliver S. Fluoroquinolone-Resistant Typhoid, South Africa. Emerging Infectious Diseases 2010; 16(5): p. 880.

[23] Joshi S and Amarnath SK. Fluoroquinolone resistance in *Salmonella typhi* and *S. paratyphi* A in Bangalore, India. Transactions of the Royal Society of Tropical Medicine and Hygiene 2007; 101(3): 308-310.



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Salmonella is an extremely diversified genus, infecting a range of hosts, and comprised of two species: enterica and bongori. This group is made up of 2579 serovars, making it versatile and fascinating for researchers drawing their attention towards different properties of this microorganism. Salmonella related diseases are a major problem in developed and developing countries resulting in economic losses, as well as problems of zoonoses and food borne illness. Moreover, the emergence of an ever increasing problem of antimicrobial resistance in salmonella makes it prudent to unveil different mechanisms involved. This book is the outcome of a collaboration between various researchers from all over the world. The recent advancements in the field of salmonella research are compiled and presented.

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