ORIGINAL ARTICLE

Isolation and Antibiotic Susceptibility Profile of *Salmonella* spp. from Patients in a Tertiary Care Hospital in Thailand

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Objective: The impact of COVID-19 on the number and antibiogram profile of *Salmonella* was studied between January 2018 and December 2021. The present time period included years before the COVID-19 pandemic, which are 2018 and 2019, and during the pandemic, which are 2020 and 2021.

Materials and Methods: *Salmonella* infections were classified into eight distinct serogroups using slide agglutination with specific antisera (A, B, C, D, E, F, G, and I). The susceptibility to antimicrobial agents were evaluated by the standard disk diffusion method.

Results: Four hundred fifty-one isolates were detected (139 in 2018, 119 in 2019, 102 in 2021, and 91 in 2021). *Salmonella* infection decreased by 25.2% from 258 isolates in 2018 and 2019 to 193 in 2020 and 2021. When comparing *Salmonella* infections in different age groups (0 to 10, 11 to 20, 21 to 30, 31 to 40, 41 to 50, 51 to 60, 61 to 70, and older than 70 years), before and during COVID-19, statistical significance was noted only in patients aged 11 to 20 (p=0.016). For clinical specimens (stool, blood, urine, pus, etc.), statistical significance was found only in blood specimens (p=0.036). The four most predominant *Salmonella* serogroups were B (31.1%), C (30.6%), E (15.7%), and D (11.4%). *S.* Typhi was present in 2.1% (4/193) of *Salmonella* isolates during COVID-19. The findings of a susceptibility test using the disk diffusion method for four commonly used drugs in treatment of severe salmonellosis as ampicillin, cefotaxime, ciprofloxacin, trimethoprim/sulfamethoxazole, before and during COVID-19 demonstrated statistical significance only in *Salmonella* serogroup D (p=0.028). Overall, drug susceptibility of *Salmonella* serogroup B, C, D, and E was ampicillin (range 15.1% to 55.9%), cefotaxime (range 66.7% to 100%), ciprofloxacin (range 18.8% to 59.1%), and trimethoprim/sulfamethoxazole (range 70.0% to 93.8%).

Conclusion: The present study results suggested the importance of monitoring the prevalence of *Salmonella* at a hospital in Bangkok. The antibiogram of susceptibility helps provide guidelines for clinician to consider empirical treatment.

Keywords: *Salmonella*; COVID-19 pandemic; Thailand

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Salmonella is responsible for causing two diseases known as salmonellosis, which is enteric fever (typhoid) resulting from bacterial invasion of the bloodstream, and acute gastroenteritis, resulting from foodborne infection. The Centers for Disease Control and Prevention (CDC) estimates *Salmonella* causes about 1.35 million infections, 26,500 hospitalizations, and 420 deaths in USA every year[1]. Drug-resistant *Salmonella* have been reported in many countries[2,3].

The COVID-19 pandemic is a new, severe respiratory disease and a major worldwide public health burden. The disease is caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which originated in Wuhan, Hubei Province, China in December 2019 and rapidly spread throughout China[4]. In February 2020, the World Health Organization (WHO) announced that the disease caused by a novel coronavirus would be named coronavirus disease 2019 (COVID-19). In March 2020, the WHO declared COVID-19 a global health emergency, leading to an enormous impact on the healthcare system[5].

The authors related the *Salmonella* infection

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with the COVID-19 pandemic in Taksin Hospital because *Salmonella* was well known as a predominant pathogen causing intestinal infection globally. *Salmonella* is an important issue because it is a true pathogen that causes communicable disease. The authors expected the COVID-19 pandemic led to a decrease in human salmonellosis cases, mostly as a result of reduced exposure to *Salmonella* due to restrictions on international travels and gatherings (including house parties, receptions, etc.), closure of dine-in restaurants, catering, and the hospitality sectors at large and possibly changes in healthcare-seeking and behavioral diagnosis, as society and the healthcare system have been put under pressure by the pandemic.

The aim of the present study was to determine whether there was a change in *Salmonella* prevalence before and during the emergence of COVID-19, and drug resistance among various *Salmonella* serogroups.

Materials and Methods

Bacterial isolates and serogrouping

Ethical approval for the present study was obtained from the Human Research Committee at Siam University, reference code SIAMPY-IRB 2022/004. Four hundred fifty-one *Salmonella* isolates were obtained from different patients at Taksin Hospital, Bangkok over a four-year period between January 1, 2019 and December 31, 2021. *Salmonella* was isolated from stool, blood, urine, pus, and other clinical specimens according to standard microbiology methods[6]. The time period covered two years before COVID-19 (January 1, 2018 to December 31, 2019), and two years during the pandemic, which was January 1, 2020 to December 31, 2021. In brief, the collected stool specimen was processed for bacteriological analysis. The stool sample was inoculated into *Salmonella-Shigella* (SS) agar and Xylose Lysine Deoxycholate (XLD) agar (Oxoid, UK) by using sterile wire loop. The inoculum was incubated under aerobic condition at 35°C for 24 hours. After incubation, the plates were examined for colony characteristics of *Salmonella* spp. colorless colonies on MacConkey agar, and red colonies on XLD agar were further identified by species of pure isolates by biochemical tests. In addition, the stool sample was also inoculated into selenite broth for enrichment of *Salmonella* spp. and incubated under aerobic condition at 35°C overnight. Then, it was cultured on SS, Modified Semi-solid Rappaport Vassiliadis Medium (Oxoid, UK) and incubated under aerobic condition at 35°C overnight. After incubation, the plates were examined for colony characteristics of *Salmonella* spp. and followed the same procedure. However, *Salmonella* spp. from other clinical specimen than stool was cultured by using blood agar and MacConkey agar. For serogrouping, pure colonies of *Salmonella* were reacted with 11 specific *Salmonella* antisera (Serosystem, Clinag, UK) such as *Salmonella* polyvalent serogroup A-I, monovalent serogroups A, B, C, D, E, F, G, H, I, and Vi. Positive results were observed via agglutination on slides with the naked eye.

Drug susceptibility testing

Standard disk diffusion method and the interpretive criteria as described by the Clinical and Laboratory Standards Institute Guidelines were used[7]. Precisely, pure identified colonies from the overnight culture were suspended in 1% peptone broth and incubated for four hours at 37°C. Turbidity of broth culture was checked against 0.5 McFarland standards. By using sterile swab, the organism in broth was uniformly inoculated into Muller Hinton agar. The antibiotic disks were applied on the surface of the inoculated agar. The latter was incubated at 35°C in an ambient air incubator for 16 to 18 hours using disks (Oxoid, UK), with each containing ampicillin (10 µg), cefotaxime (30 µg), ciprofloxacin (5 µg), and trimethoprim/sulfamethoxazole (1.25/23.75 µg). *Escherichia coli* ATCC 25922 was used as a control strain. It was obtained from the Department of Medical Sciences, Ministry of Public Health because Taksin Hospital is a member of the National Antimicrobial Resistant. Surveillance Center, Thailand (NARST).

Data analysis

Data were entered and analyzed using IBM SPSS Statistics, version 20.0 (IBM Corp., Armonk, NY, USA) for descriptive analysis. Categorical variables such as age of patients, source of clinical specimens and *Salmonella* serogroups were expressed as frequency and percentages. The ages of patients were also expressed as range and median (interquartile range, IQR). Chi-square test and Fisher's exact test were used to compare the difference of categorical data between groups, before and during COVID-19. A p-value of less than 0.05 was considered statistically significant.

Results

There were 139, 119, 102, and 91 isolates in

Table 1. Comparison of *Salmonella* prevalence according to patient age groups, clinical specimens and serogroups before (2018-2019) and during COVID-19 (2020-2021)

	Total; n (%)	2018-2019; n (%)	2020-2021; n (%)	p-value#
No. of *Salmonella*	n=451	n=258	n=193	
Male:female	214:237 (1:1.1)	117:141 (1:1.2)	97:96 (1:1)	0.301
Age group (years)				0.016*
0 to 10	89 (19.7)	59 (22.9)	30 (15.5)	
11 to 20	21 (4.7)	19 (7.4)	2 (1.1)	
21 to 30	49 (10.9)	25 (9.7)	24 (12.4)	
31 to 40	36 (8.0)	18 (7.0)	18 (9.3)	
41 to 50	56 (12.4)	33 (12.8)	23 (11.9)	
51 to 60	59 (13.1)	30 (11.6)	29 (15.0)	
61 to 70	47 (10.4)	22 (8.4)	25 (13.0)	
>70	94 (20.8)	52 (20.2)	42 (21.8)	
Specimens				
Stool	344 (76.3)	204 (79.1)	140 (72.5)	0.107
Blood	81 (18.0)	36 (14.0)	45 (23.3)	0.010*
Urine	14 (3.1)	11 (4.2)	3 (1.6)	0.101
Pus[c]	7 (1.5)	3 (1.2)	4 (2.1)	0.468
Others[d]	5 (1.1)	4 (1.5)	1 (0.5)	0.398
Serogroups				
A	2 (0.4)	0 (0.0)	2 (1.0)	0.183
B	140 (31.1)	80 (30.0)	60 (31.1)	0.985
C	138 (30.6)	79 (30.6)	59 (30.6)	0.991
D	65 (14.4)	33 (12.8)	32 (16.6)	0.257
E	71 (15.7)	44 (17.0)	27 (14.0)	0.377
F	3 (0.7)	0 (0.0)	3 (1.5)	0.078
G	3 (0.7)	2 (0.8)	1 (0.5)	1.000
I	2 (0.4)	2 (0.8)	0 (0.0)	0.509
Salmonella Typhi	4 (0.9)	0 (0.0)	4 (2.1)	0.033*
Salmonella spp.[e]	23 (5.1)	18 (7.0)	5 (2.6)	0.036*

Chi-square test or Fisher's exact test, * Statistical significance at $p<0.05$

[c] Pus from ankle tissue, anus, bone marrow, buttock, hip, leg, and liver; [d] Ascitic fluid, pericardium fluid, peritoneal fluid, pleural fluid, and sputum; [e] *Salmonella* not serogroups A, B, C, D, E, F, G, H, I

2018, 2019, 2020, and 2021, respectively. Over the four-year study, *Salmonella* decreased by 25.2% from 258 isolates in 2018 and 2019, or before COVID-19 to 193 isolates in 2021 and 2022 or during COVID-19. For the patients, 47.5% (214/451) were male (male:female ratio of 214:237, 1:1.1). The ages ranged from three days to 99 years. The median age of patients was 45, with an IQR of 21 to 66. Before COVID-19, 45.3% (117/258 patients) were male (male:female ratio of 117:141, 1:1.2). The ages ranged from three days to 94 years and the median age was 42, with an IQR of 14 to 65. During COVID-19, 50.3% (97/193 patients) were male (male:female ratio of 97:96, 1:0.99). The age ranged from three days to 99 years, with a median 51, and an IQR of 28 to 68. The p-value was not statistically significant when comparing the male to female ratio (p=0.301) before and during COVID-19 (Table 1).

Comparison of different age groups (0 to 10, 11 to 20, 21 to 30, 31 to 40, 41 to 50, 51 to 60, 61 to 70, and older than 70 years), before and during COVID-19 revealed statistical significance only in patients aged 11 to 20 (p=0.016) (Table 1). A comparison of clinical specimens such as stool, blood, urine, pus, and others showed statistical significance only in the blood specimen (p=0.036). The three most common clinical specimens were stool (72.5% to 79.1%), blood (18% to 23.3%), and urine (1.6% to 4.2%). Overall, the four most predominant *Salmonella* serogroups were B (31.1%), C (30.6%), E (15.7%), and D (11.4%). However, the p-value was not statistically significant when comparing *Salmonella* serogroups before and

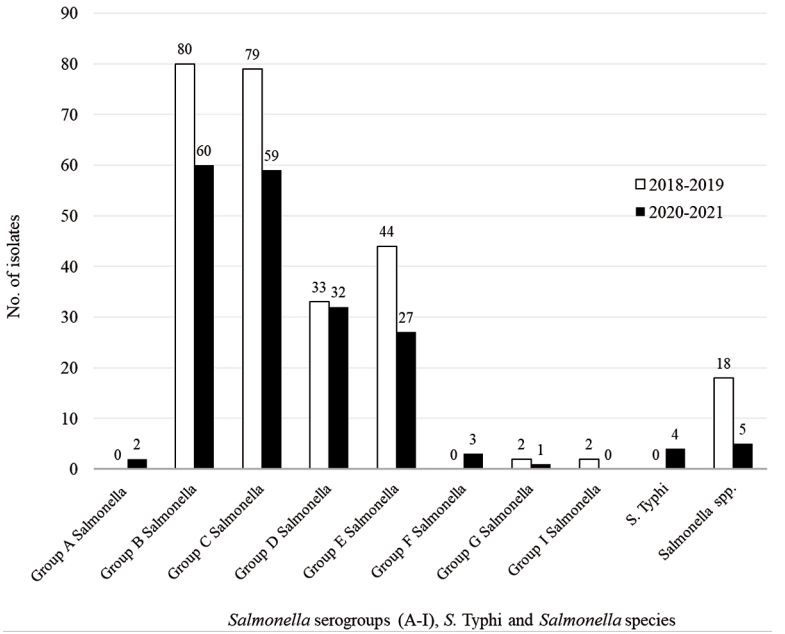

Figure 1. Comparison of *Salmonella* serogroups (A-I), S. Typhi and *Salmonella* species between 2018-2019 (□ before COVID-19) and 2020-2021 (■ during COVID-19).

during COVID-19 (p=0.172).

Figure 1 shows that COVID-19 led to a decrease in the number of *Salmonella* isolates. However, *S.* Typhi, which is uncommon in Bangkok and was not present before COVID-19 was found during the pandemic (4 isolates, 2.1%) from blood. All *Salmonella* Typhi patients were male (aged 11 months, 52, 69, and 73 years) and isolated from blood. Likewise, *Salmonella* serogroups A and F were also found only during COVID-19 (Table 1, Figure 1).

Table 2 shows four commonly used drugs to treat salmonellosis, which are ampicillin, cefotaxime, ciprofloxacin, and trimethoprim/sulfamethoxazole. The result revealed statistical significance only in *Salmonella* serogroup D (p=0.028) because they were more susceptible to ampicillin (40.6%) during COVID-19 (compared to 15.1% before COVID-19). Meanwhile, for other serogroups, drug susceptibility comparisons were not statistically significant. The overall drug susceptibility of *Salmonella* serogroup A, B, C, D, and E was ampicillin (range of 15.1% to 51.9%), cefotaxime (range of 70.0% to 93.8%), ciprofloxacin (range of 18.8% to 59.1%), and trimethoprim/sulfamethoxazole (range of 70.0% to 93.8%). In general, *Salmonella* serogroup D was more susceptible to all drugs tested compared to other *Salmonella* serogroups. For example, it was highly susceptible to cefotaxime (100%) and trimethoprim/sulfamethoxazole (93.8%). *S.* Typhi (n=4) were susceptible to ampicillin (1/4, 25%), 100% intermediate resistant to ciprofloxacin, 100% susceptible to cefotaxime and some to trimethoprim/sulfamethoxazole (data not shown). *Salmonella* serogroup A (n=2) were susceptible to ampicillin (50%), 100% susceptible to cefotaxime, ciprofloxacin, and trimethoprim/sulfamethoxazole (data not shown). *Salmonella* serogroup F (n=3) was 100% susceptible to ampicillin, cefotaxime, ciprofloxacin, and trimethoprim/sulfamethoxazole (data not shown). Due to the lack of clinical information in the present study, the authors could not tell that the isolated *Salmonella* spp. caused the infection or was just at the carrier stage. The authors compared the susceptibility patterns of *Salmonella* spp. from blood or pus or urine separately from the stool isolates, which may be pathogen or stool carrier as shown in Table 3.

Discussion

The Thai government implemented many measures to reduce COVID-19 transmission such as the closure of international borders, and national lockdowns. Public health prevention measures were also introduced such as mask wearing, frequent hand washing, avoiding sharing of meals, closure of dine-in restaurants, parties, festivals, pubs, bars, cafes, restaurants, stay at home orders, work from home, and online education. In the present study,

Table 2. Comparison of *Salmonella* drug susceptibility using the disk diffusion method before (2018-2019) and during COVID-19 (2020-2021)

Drugs	S/I/R	*Salmonella* serogroup B; n (%)		*Salmonella* serogroup C; n (%)		*Salmonella* serogroup D; n (%)		*Salmonella* serogroup E; n (%)	
		Before COVID-19 (2018-2019)	During COVID-19 (2020-2021)	Before COVID-19 (2018-2019)	During COVID-19 (2020-2021)	Before COVID-19 (2018-2019)	During COVID-19 (2020-2021)	Before COVID-19 (2018-2019)	During COVID-19 (2020-2021)
		n=80	n=60	n=79	n=59	n=33	n=32	n=44	n=27
Amp	S	29 (36.3)	21 (35)	36 (45.6)	33 (55.9)	5 (15.1)	13 (40.6)	18 (40.9)	14 (51.9)
	I	2 (2.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.3)	0 (0.0)
	R	49 (61.2)	39 (65)	43 (54.4)	26 (44.1)	28 (84.9)	19 (59.4)	25 (56.8)	13 (48.1)
	p-value#	0.566		0.302		0.028		0.669	
CTX	S	61 (76.3)	47 (78.3)	62 (78.5)	49 (83.0)	33 (100)	32 (100)	38 (86.4)	18 (66.7)
	I	0 (0.0)	1 (1.7)	1 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	R	19 (23.7)	12 (20.0)	16 (20.2)	10 (17.0)	0 (0.0)	0 (0.0)	6 (13.6)	9 (33.3)
	p-value#	0.529		0.808		N/A		0.072	
CIP	S	36 (45.0)	23 (38.3)	29 (36.7)	24 (40.7)	8 (24.2)	6 (18.8)	26 (59.1)	15 (55.6)
	I	41 (51.3)	32 (53.3)	31 (39.2)	28 (47.4)	25 (75.8)	25 (78.1)	12 (27.3)	10 (37.0)
	R	3 (3.7)	5 (8.3)	19 (24.1)	7 (11.9)	0 (0.0)	1 (3.1)	6 (13.6)	2 (7.4)
	p-value#	0.412		0.188		0.764		0.557	
SXT	S	63 (78.8)	42 (70.0)	60 (76.0)	44 (74.6)	27 (81.8)	30 (93.8)	38 (86.4)	23 (85.2)
	I	2 (2.5)	1 (1.7)	2 (2.5)	2 (3.4)	0 (0.0)	0 (0.0)	0 (0.0)	1 (3.7)
	R	15 (18.7)	17 (28.3)	17 (21.5)	13 (22.0)	6 (18.2)	2 (6.2)	6 (13.6)	3 (11.1)
	p-value#	0.424		1.000		0.258		0.669	

S=susceptible; I=intermediate; R=resistant; Amp=ampicillin; CTX=cefotaxime; CIP=ciprofloxacin; SXT=trimethoprim/sulfamethoxazole

Chi-square test, statistically significance at *Salmonella* serogroup D

Table 3. Comparison of drug susceptibility patterns of *Salmonella* according to types of clinical specimens using the disk diffusion method

Drugs	Stool (total n=344); n (%)			Blood (total n=81); n (%)			Urine (total n=14); n (%)			Pus (total n=7); n (%)		
	S	I	R	S	I	R	S	I	R	S	I	R
AMP	152 (44.19)	3 (0.87)	189 (54.94)	41 (50.62)	0 (0.00)	40 (49.38)	4 (28.57)	0 (0.00)	10 (71.43)	0 (0.00)	0 (0.00)	7 (100)
CTX	298 (86.63)	0 (0.00)	46 (13.37)	64 (79.01)	0 (0.00)	17 (20.99)	8 (57.14)	0 (0.00)	6 (42.86)	4 (57.14)	0 (0.00)	3 (42.86)
CIP	155 (45.06)	163 (47.38)	26 (7.56)	23 (28.40)	48 (59.25)	10 (12.35)	3 (21.43)	8 (57.14)	3 (21.43)	0 (0.00)	4 (57.14)	3 (42.86)
SXT	276 (80.23)	1 (0.30)	67 (19.47)	65 (80.25)	5 (6.17)	11 (13.58)	11 (78.57)	0 (0.00)	3 (21.43)	6 (85.71)	0 (0.00)	1 (14.29)

S=susceptible; I=intermediate; R=resistant; AMP=ampicillin; CTX=cefotaxime; CIP=ciprofloxacin; SXT=trimethoprim/sulfamethoxazol

Salmonella isolates decreased by 25.2% during the spread of COVID-19. This decline was lower than the previous reports from the Netherlands[8] and Israel[9] that found a wide range of decline in *Salmonella* at 37% to 57% and 33%, respectively. In the United States, data from 10 sites from the Foodborne Diseases Active Surveillance Network, showed 22% less *Salmonella* infection (95% confidence interval of 17% to 26%) were reported in 2020, compared to the average between 2017 and 2019[10]. In Australia, the impact of COVID-19 in 2020 led to 25% lower *Salmonella* infections than the median from the previous five years[11].

For serogroup study the authors found that *Salmonella* serogroup B (31.1%) was predominant, followed by serogroup C (30.6%), serogroup E (15.7%), and serogroup D (14.4%). In China, *Salmonella* serogroup B (67.5%) was predominant, followed by serogroup C (11.8%), serogroup D (11.6%), and serogroup E (4.2%)[12]. In addition, COVID-19 and *S.* Typhi co-epidemic was reported in Lahore, Pakistan between March and June 2020. Out of 220 patients, 48% were typhoid positive[13]. *S.* Typhi isolated in the present study is important because it causes typhoid fever. *S.* Typhi is solely a human pathogen with no known animal reservoir.

The authors focused on studying drug resistance to ampicillin, cefotaxime, ciprofloxacin, and trimethoprim/sulfamethoxazole because they are common drugs for severe salmonellosis treatment.

However, the present study indicated that serogroup B is the most resistant serogroup to ampicillin (65.0%). This resistance level was higher than a recent report[14] of *Salmonella* isolated in human cases in 2019 from 25 countries in Europe (EFSA), where the lowest resistance was in Greece (6.0%) and highest in Belgium (46.6%). For third-generation cephalosporin, or cefotaxime in the present study, the EFSA reported *Salmonella* isolates at a very low resistance level of 1.8%[14]. In the same study, *Salmonella* isolates were 2.8% and 29.0% resistant to ciprofloxacin and sulfonamide, respectively[14]. In addition, a previous study from CDC in USA, which was an average between 2015 and 2017, reported 3% and 7% resistance of *Salmonella* to ceftriaxone and ciprofloxacin, respectively[15], which is also significantly lower than the present study. *Salmonella* serogroup D was highly susceptible to cefotaxime (100%) and trimethoprim/sulfamethoxazole (93.8%) and was comparable with data (100% and 91%, respectively), reported by 11 chicken farmers in Thailand[16]. The limitation of the present study was that it was a single-center study. The present study is noticeably demonstrated that more *Salmonella* was in blood during the pandemic than in the previous studies.

Conclusion

The present study found 25.2% *Salmonella* prevalence reduction from 258 isolates in 2018 and 2019 to 193 isolates in 2020 and 2021. Four cases of *S.* Typhi were found during COVID-19 pandemic. The information will be helpful in epidemiology, prevention, and infection control.

What is already known on this topic?

Salmonella was reduced in many countries during COVID-19. The differences in the rate of decline could be due to social, behavioral, and geographical factors.

What this study adds?

Salmonella infections before and during COVID outbreak in Thailand were compared. It added new data including age, gender, specimen collection, *Salmonella* serotyping, and antibiotic susceptibility.

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Conflicts of interest

The authors declare no conflict of interest.

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