Hotspots sequences of $\text{gyrA}$, $\text{gyrB}$, $\text{parC}$, and $\text{parE}$ genes encoded for fluoroquinolones resistance from local $\text{Salmonella}$ Typhi strains in Jakarta

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Abstract

Background: Infection of $\text{Salmonella enterica}$ subsp. enterica serovar Typhi is the primary etiology of typhoid fever globally and is common in many developing countries, especially those with dense populations and poor environmental sanitation. Antibiotic fluoroquinolones were used for the treatment in the 1980s due to the resistance to the first-line antibiotics. However, many cases of treatment failure of fluoroquinolones in typhoidal patients have been reported from numerous countries in Asia, Europe, Africa, and America. Mutations in quinolone resistance determining regions (QRDR) genes, $\text{gyrA}$, $\text{gyrB}$, $\text{parC}$, and $\text{parE}$, are found in fluoroquinolone-resistant $\text{Salmonella}$ Typhi. Contrast reports came from the $\text{S.}$ Typhi isolates in Indonesia, mainly Jakarta and the surroundings, obtained from patients with typhoid fever, with good sensitivity to the fluoroquinolones, i.e., nalidixic acid, ciprofloxacin, moxifloxacin, and levofloxacin. The present study, therefore, aimed to identify the hotspot sequences of $\text{gyrA}$, $\text{gyrB}$, $\text{parC}$, and $\text{parE}$ genes of the local $\text{S.}$ Typhi strains based on their susceptibility to fluoroquinolones from patients with typhoid fever in Jakarta and its satellite cities.

Results: A total of 28 isolates were identified as $\text{S.}$ Typhi. All isolates were susceptible to nalidixic acid, levofloxacin, and moxifloxacin. Twenty-seven isolates (96.4%) were susceptible to ciprofloxacin, with one isolate (3.6%) being intermediate. The hotspot sequences of $\text{gyrA}$, $\text{gyrB}$, $\text{parC}$, and $\text{parE}$ genes from all isolates were identical to the fluoroquinolone-sensitive reference sequence $\text{Salmonella enterica}$ subsp. enterica serovar Typhi Ty2 (NCBI GenBank AE014613.1), including the isolate with intermediate susceptibility. The mutation was not found, and amino acid deduced from all hotspots in susceptible and intermediate isolates showed no replacement in all reported codons.

Conclusions: This study showed that the local $\text{S.}$ Typhi strains from Jakarta and surroundings were susceptible to fluoroquinolones (nalidixic acid, ciprofloxacin, levofloxacin, and moxifloxacin), and the hotspot sequences of the $\text{gyrA}$, $\text{gyrB}$, $\text{parC}$, and $\text{parE}$ genes were all identical to the reference sequence. Thus, the hotspot sequences of the $\text{gyrA}$, $\text{gyrB}$, $\text{parC}$, and $\text{parE}$ genes seemingly were conserved in Jakarta’s local $\text{S.}$ Typhi strains and could be considered wild type.

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Background

Typhoid fever is a public health problem, with high morbidity and mortality rates mostly in developing countries of Africa, South Asia, and Southeast Asia [1, 2]. Infection of Salmonella enterica subsp. enterica serovar Typhi is the leading cause of the disease. People living in poverty with poor sanitation system, low water quality, and improper food handling promote higher risk of S. Typhi infection. It is estimated that around 13% of the world’s population lives in extreme poverty, with a poverty rate of 60% in each country [3]. Data from the Global Burden of Disease (GBD) in 2017 showed the number of S. Typhi infections about 10 million cases, with the number of deaths of approximately 116,800. These numbers are much higher than paratyphoid fever cases, around 3 million and 19,100 deaths [4], with the case fatality rate of typhoid fever at 10–30% without treatment and falling to 1–4% after proper treatment [2]. GBD report also revealed the Years of Life Lost (YLL) and Years Lived with Disability (YLD) of typhoid fever were around 8,332,000 and 105,500. Although the trend of typhoid fever decreased each year from 1990 to 2010, the global case still reached 13–20 million cases, with mortality number of 145,000–202,000 cases. Areas with the highest issues were South Asia, East Asia, Southeast Asia, and Sub-Saharan Africa [4].

The only way to treat typhoid fever is through antibiotic therapy. The first-line antibiotics were ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole, where chloramphenicol became the earliest antibiotic introduced in 1948 on the Malay Peninsula [5]. Ampicillin and trimethoprim-sulfamethoxazole replaced chloramphenicol in the 1970s due to chloramphenicol resistance and severe side effects [5, 6]. The first report of S. Typhi strain resistant to chloramphenicol came from England in 1950. Later from 1962 to 1967, chloramphenicol resistance S. Typhi strains were also reported from India, West Africa, Greece, and Israel [7]. In 1972, MDR (multidrug resistance) S. Typhi was reported in Mexico [8]. Since the 1980s, the second-line antibiotic, fluoroquinolones, and third-generation cephalosporin have been recommended to treat MDR (multidrug resistance) S. Typhi. Alternatively, azithromycin can be applied to cure typhoid fever [9].

In recent years, multiple countries have reported the resistance and decrease-susceptibility of S. Typhi to fluoroquinolones. The early report came from the Middle East and Central Asia in 2014, followed by Ghana in 2016 [10, 11]. European Committee on Antimicrobial Susceptibility Testing (EUCAST) database from 2015 revealed that 6% of Salmonella isolates were resistant to ciprofloxacin. In 2015 as well, the U.S. National Antimicrobial Resistance Monitoring System (NARMS) announced the rising number of Salmonella with decreasing susceptibility to ciprofloxacin from < 0.5 to 3.5% since 1996 [9]. Hence, the antibiotic-resistant priority list published by the World Health Organization (WHO) in 2017 showed that fluoroquinolone-resistant Salmonella was declared in the “high” category, which meant that novel antibiotics were urgently required to be discovered as alternatives to cure typhoid patients [12]. In the meantime, a study by Moehario et al. 2019 in Indonesia showed a contrasting result that 87.5–100% of S. Typhi from Jakarta and Tangerang was yet sensitive to first-line antibiotics and fluoroquinolones [13]. Lugito and Cucunawangsih also revealed the similar condition in Karawaci, Tangerang, that the resistance rate of S. Typhi isolates to ciprofloxacin and levofloxacin was low [14]. As the capital city, Jakarta is the largest city in Indonesia and Southeast Asia, with over 10 million people inhabited. It is surrounded by satellite towns, namely Bogor, Depok, Tangerang, and Bekasi [15]. Many slums can be found around Jakarta, and these circumstances promote S. Typhi dissemination and infection.

Researchers have identified several point mutations in gyrA, gyrB, parC, and parE, part of the QRDR. Another mechanism that plays a role in fluoroquinolone-resistant properties is plasmid-mediated. Many plasmid-mediated quinolone resistance (PMQR) genes have been identified i.e. qnrA, qnrB, qnrS, qnrC, and qnrD [9]. A study by Koirala et al. 2012 [16] and Tack et al. 2019 [17] revealed a point mutation in gyrA, gyrB, and parC. According to a study in Italy by García-Fernández et al. 2015, mutations were detected in gyrA, gyrB, parC, and parE [18]. Qian et al. 2020 in China also reported some mutations in all of the QRDR regions [19].

The different situation revealed in Jakarta was possibly caused by the varied length of S. Typhi genome. Two studies conducted by Thong et al. 1994 and 1996 have revealed different genome fragment patterns and lengths of isolates from Malaysia and Papua New Guinea using Pulsed-field Gel Electrophoresis (PFGE) and three restriction endonuclease enzymes [20]. Variation in fragment patterns and genome lengths was reported...
further by a study by Moehario et al. 2009 using the PFGE method. The S. Typhi isolates from several different areas in Indonesia appeared to have distinct genome lengths ranging from 1.495–4.561 kb and were divided into four clusters. They were from Makassar, Jakarta, Jayapura, and one cluster consisted of diverse areas [21]. On the other hand, the average genome length of 4.300 S. Typhi at Typhi Pathogenwatch is 4.7 Mbp [22].

The distinct genome length, global resistance pattern, and the widespread use of antibiotics are predicted to be the main factors that induce S. Typhi’s resistance to antibiotics, including fluoroquinolones. The hotspot sequences of gyrA, gyrB, parC, and parE from Indonesia’s local S. Typhi strains have not been thoroughly studied. At the same time, most S. Typhi isolates in Jakarta and the neighboring towns are still susceptible to fluoroquinolones. This study aimed to examine the hotspot region of the genes encoding fluoroquinolone-resistant gyrA, gyrB, parC, and parE in the local Jakarta S. Typhi strains based on the fluoroquinolones susceptibility.

Results
Isolate identification and antibiotic susceptibility profile
A total of 28 S. Typhi isolates from Jakarta and its surroundings collected from 2015 to 2021 were retrieved from −20 °C storage. These isolates originally came from patients with typhoid fever and were treated in hospitals across Jakarta and the surroundings. All of the isolates were re-identified and confirmed as S. Typhi. Antibiotic susceptibility from 28 isolates was determined following the guideline from CLSI 2011, CLSI 2020, and EUCAST 2020 [23–25]. All isolates (100%) were susceptible to nalidixic acid, levofloxacin, and moxifloxacin. In contrast, the hotspot region of the genes encoding fluoroquinolone-resistant gyrA, gyrB, parC, and parE in the local Jakarta S. Typhi strains based on the fluoroquinolones susceptibility.

Table 1 Antibiotic susceptibility profile of S. Typhi isolates in Jakarta

| Antibiotic          | Sensitive (n = 28) | Intermediate (n = 28) | Resistant (n = 28) |
|---------------------|-------------------|----------------------|-------------------|
| Ciprofloxacin (CLSI 2020) | 27 (96.4%) | 1 (3.6%) | 0 |
| Levofloxacin (EUCAST 2022) | 28 (100%) | 0 | 0 |
| Nalidixic acid (CLSI 2011) | 28 (100%) | 0 | 0 |
| Moxifloxacin (EUCAST 2022) | 28 (100%) | 0 | 0 |

The DNA sequencing results were aligned with the reference sequence S. Typhi Ty2 (NCBI GenBank AE014613.1). The results showed that the hotspot gyrA sequences from 28 S. Typhi isolates were identical to the reference sequence (Fig. 5). Similar results were also found in the hotspot of gyrB, parC, and parE sequences (Fig. 6, Fig. 7, and Fig. 8). Point mutations in these hotspots were not detected in all genes, not even from the one isolate with intermediate ciprofloxacin susceptibility. The sequence data generated in this study have been submitted and are available at NCBI GenBank with the accession numbers listed in Additional file 1.

Codons 82, 83, 87, 119, and 133 in gyrA have been reported to have point mutations related to fluoroquinolones resistance. However, our results showed that the amino acid obtained from hotspot gyrA sequences in these codons were aspartate (Asp), serine (Ser), aspartate (Asp), alanine (Ala), glycine (Gly), respectively, in all 28 S. Typhi isolates, which were in accordance with the reference (Fig. 5).

In the amino acid sequences of gyrB, codons 426, 435, 464, 465, 466, and 468 have been reported to have point mutations related to fluoroquinolones resistance. Our study showed contrast results that the amino acid obtained from hotspot gyrB sequences in these codons were aspartate (Asp), glycine (Gly), serine (Ser), glutamine (Gln), glutamic acid (Glu), and alanine (Ala), respectively in all 28 S. Typhi isolates, which were in accordance to the reference (Fig. 6).

Similar results were also seen in parC. The most reported codons in parC with point mutations related to fluoroquinolones resistance are codon 79, 80, 84, 92, and 106. On the contrary, our results showed that the amino acid in the hotspot parC sequences were identical with the reference. They were aspartate (Asp), serine (Ser), glutamic acid (Glu), proline (Pro), and tryptophan (Trp), respectively in all 28 S. Typhi isolates (Fig. 7).

Although mutations in parE related to fluoroquinolones resistance were rarely reported, some researchers have found point mutations in codons 364, 420, 434, 444, and 493. On the other hand, our results showed identical amino acids in the hotspot parE sequences compared to the reference, which was alanine (Ala), aspartate (Asp), tyrosine (Tyr), isoleucine (Ile), and serine (Ser), respectively, in all 28 S. Typhi isolates (Fig. 8).
Discussion

Although multiple countries have announced the distribution of fluoroquinolone-resistant S. Typhi and the related QRDR mutations, the local S. Typhi strain in Indonesia, especially Jakarta, still has good susceptibility to fluoroquinolones.
Fig. 3 Electrophoresis of PCR product from *S.* Typhi hotspot parC. *M* = marker 1 kb, marker size from bottom to top (bp): 250, 500, 750, 1,000, 1,500, 2,000, 2,500, 3,000, 4,000, 5,000, 6,000, 8,000, 10,000. Numbers above the gel correspond to the isolate number in Additional file 1. All of the hotspot parC amplicons showed the expected fragments, i.e. 564 bp.

Fig. 4 Electrophoresis of PCR product from *S.* Typhi hotspot parE. *M* = marker 1 kb, marker size from bottom to top (bp): 250, 500, 750, 1,000, 1,500, 2,000, 2,500, 3,000, 4,000, 5,000, 6,000, 8,000, 10,000. Numbers above the gel correspond to the isolate number in Additional file 1. All of the hotspot parE amplicons showed the expected fragments, i.e. 688 bp.
Sequence mutations in the *gyrA* from fluoroquinolone-resistant *S. Typhi* have been reported from regions with and without endemic typhoid fever. Mutations frequently detected in the hotspot sequences of *gyrA* are shown in Table 2. These mutations commonly occurred and appeared to be related to the resistance to fluoroquinolones. According to the data collected from several studies, some *S. Typhi* isolates from different countries shared
similar mutations in the hotspot sequences of \textit{gyrA}. The mutation in Asp82Asn was found solely in Italy [18]. The point mutation in Ser83Phe was found in Congo [17], Italy [18], India [26–28], Nepal [16, 27], Bangladesh [29], and other South Asian countries [27]. Ser83Tyr and Asp87Asn were distributed in similar countries as...
Ser83Phe [17, 18, 27–29] with an additional country from China for Asp87Asn [19]. Asp87 had two other mutations, they were Asp87Val, which was found in Nepal [16], and Asp87Gly in Congo [17], China [19], and Bangladesh [29]. Mutations in Ala119Glu, Glu133Gly, and Gly133Val, that were found in Italy. Other isolates were isolated from Italian residents that came from Italian, India, and Iran [30], respectively. Dissimilar identification in the amino acid 133 of gyrA was observed in the study by Qian et al. 2020 [19] and Hamidian et al. 2011 [30]. According to the sequence data of gyrA S. Typhi from NCBI GenBank, the amino acid at position 133 is glycine instead of glutamic acid, as mentioned by Qian et al. 2020 [19]. The amino acid Glu133 was identified in gyrA from other S. enterica serotypes. Further investigation is needed to confirm the amino acid at position 133. In contrast to the stated reports above, our study showed that both nalidixic acid and ciprofloxacin intermediate S. Typhi isolates.

A similar situation also occurred in gyrB, another QRDR gene. Gly435 had three mutations, Gly435Ala, Gly435Glu, and Gly435Val, that were found in Italy. These isolates were from patients with travel histories to Bangladesh and India. Other isolates were isolated from Italian residents that came from Italian, India, and

Table 2  Mutations found in the hotspot gyrA of S. Typhi

| Mutation    | Susceptibility Profile | Study |
|-------------|-----------------------|-------|
| Asp82Asn    | NAL (R)               | [18]  |
| Ser83Phe    | OFX (R), CIP (R), NAL (R) | [16]  |
|             | CIP (DS)              |       |
|             | CIP (RS), NAL (R)     |       |
|             | NAL (I) CIP (S)       |       |
|             | CIP (R), NAL (R)      | [18]  |
|             | NAL (I) CIP (S)       | [18]  |
|             | NAL (I) CIP (S)       | [18]  |
|             | NAL (R), CIP (S)      | [19]  |
|             | NAL (R), CIP (I)      | [19]  |
|             | OFX (R), CIP (R), NAL (R) | [27]  |
|             | CIP (DS), NAL (R)     |       |
|             | CIP (R), NAL (R)      | [28]  |
|             | CIP (R), NAL (R)      | [28]  |
|             | CIP (R), NAL (R)      | [28]  |
|             | OFX (R), CIP (R), NAL (R) | [27]  |
|             | CIP (DS), NAL (R)     |       |
|             | CIP (DS), NAL (R)     | [29]  |
|             | NAL (I), CIP (S)      | [18]  |
|             | NAL (R), CIP (I)      | [19]  |
|             | NAL (R), CIP (I)      | [19]  |
|             | NAL (R), CIP (I)      | [19]  |
|             | NAL (R), CIP (I)      | [19]  |
|             | NAL (R), CIP (I)      | [19]  |
|             | CIP (R), NAL (R)      | [29]  |
|             | CIP (R), NAL (R)      | [29]  |
|             | CIP (R), NAL (R)      | [29]  |
|             | CIP (R), NAL (R)      | [29]  |
|             | CIP (R), NAL (R)      | [29]  |
|             | Asp87Asn              |       |
|             | CIP (R), NAL (R)      | [29]  |
|             | NAL (R), CIP (I)      | [19]  |
|             | NAL (R), CIP (I)      | [19]  |
|             | NAL (R), CIP (I)      | [19]  |
|             | NAL (R), CIP (I)      | [19]  |
|             | CIP (R), NAL (R)      | [29]  |
|             | OFX (R), CIP (R), NAL (R) | [27]  |
|             | CIP (DS), NAL (R)     |       |
|             | CIP (R), NAL (R)      | [28]  |
|             | CIP (R), NAL (R)      | [28]  |
|             | CIP (R), NAL (R)      | [28]  |
| Asp87Val    | OFX (R), CIP (R), NAL (R) | [16]  |
| Asp87Gly    | CIP (DS)              |       |
|             | NAL (R), CIP (I)      | [19]  |
|             | NAL (R), CIP (I)      | [19]  |
|             | NAL (R), CIP (I)      | [19]  |
|             | CIP (R), NAL (R)      | [29]  |
| Ala119Glu   | CIP (DS)              | [17]  |
| Glu133Gly   | NAL (R), CIP (S)      | [19]  |
| Gly133Glu   | NAL (DS)              | [30]  |

* Double mutations in one isolate: gyrA Ser83Phe and gyrB Gly435Ala; gyrA Ser83Phe and gyrB Gly435Glu; gyrA Ser83Phe and gyrB Gly435Val

A similar situation also occurred in gyrB, another QRDR gene. Gly435 had three mutations, Gly435Ala, Gly435Glu, and Gly435Val, that were found in Italy. These isolates were from patients with travel histories to Bangladesh and India. Other isolates were isolated from Italian residents that came from Italian, India, and
Bangladesh [18]. In Congo and France, the researcher observed mutation in Ser464Tyr [17, 31]. In the Indian subcontinent, Ser464Phe was reported by Britto et al. 2020 [27]. Despite mutations in amino acids Gln 465Leu, Glu466Asp, and Ala 468Glu, some S. Typhi isolates in France showed an interesting profile that was pansusceptible, including fluoroquinolones [31]. Meanwhile, in Congo, Glu466Asp was identified in isolates with decreased susceptibility to ciprofloxacin [17]. Ser426Gly was detected in S. Typhi isolates from China only [19]. However, according to the current survey, the amino acid at position 426 was found to be an aspartic acid. Further investigation is needed to confirm the serine amino acid at position 426 of gyrB. Although many reports on mutations have been reported earlier, we detected the contrary results from the current study with no point mutations at the hotspot gyrB regions, which were Asp426, Gly435, Ser464, Gln465, Glu466, and Ala468. All of them were consistent with the reference sequences. The mutations identified in the hotspot sequences of gyrB earlier are summarized in Table 3.

Sequence mutations related to fluoroquinolone-resistant S. Typhi in parC gene have also been reported, although they were less common than gyrA and gyrB. Table 4 showed the mutations detected in the hotspot sequences of parC.

Only five mutations have been identified in parC. A point mutation in the amino acid Asp79Gly was found in China from isolates resistant to nalidixic acid but susceptible to ciprofloxacin, as reported by Qian et al. 2020. They also detected mutation in Glu84Lys from isolates resistant to ciprofloxacin and nalidixic acid [19].

### Table 3 Mutations found in the hotspot gyrB of S. Typhi

| Mutation     | Susceptibility Profile | Study |
|--------------|------------------------|-------|
| Ser426Gly    | CIP (S), NAL (R)       | [19]  |
| Gly435Ala    | NAL (R), CIP (R)       | [18]  |
| Gly435Glu    | NAL (R) CIP (R), NAL (I) CIP (S) | [18] |
| Gly435Val    | NAL (R), CIP (R)       | [18]  |
| Ser464Tyr    | CIP (DS)               | [17]  |
|              | NAL (S), CIP (DS)     | [31]  |
| Ser464Phe    | OFX (R), CIP (R), NAL (R) | [27] |
| Gln465Leu    | Pansusceptible        | [31]  |
| Glu466Asp    | CIP (DS)               | [17]  |
|              | Pansusceptible        | [31]  |
| Ala468Glu    | Pansusceptible        | [31]  |

1 Double mutations: gyrA Gln133Gly and gyrB Ser426Gly
2 Double mutations: gyrA Ser83Phe and gyrB Gly435Ala
3 Double mutations: gyrA Asp87Asn and gyrB Gly435Glu; Four mutations in one isolate: gyrA Ser83Phe, gyrA Asp87Asn, gyrB Gly435Glu, and parC Ser80lle
4 Double mutations: gyrA Ser83Phe and gyrB Gly435Val

### Table 4 Mutations found in the hotspot parC of S. Typhi

| Mutation      | Susceptibility Profile | Study |
|---------------|------------------------|-------|
| Asp79Gly      | NAL (R) CIP (S)        | [19]  |
| Ser80lle      | OFX (R), CIP (R), NAL (R) | [16] |
| Glu84Lys      | CIP (R), NAL (R)       | [19]  |
| Glu84Gly      | OFX (R), CIP (R), NAL (R) | [27] |
| Glu92Lys      | NAL (R) CIP (R), NAL (I) CIP (S) | [29] |
| Trp106Gly     | NAL (R), CIP (R)       | [26]  |

1 Three mutations: gyrA Ser83Phe, gyrA Asp87Val, and parC Ser80lle
2 Double mutations: gyrA Ser83Phe and parC Glu84Gly
3 Three mutations: gyrA Ser83Phe, gyrA Asp87Gly, parC Glu92Lys, and increased efflux pump activity for nalidixic acid

Nepal, Koirala et al. 2012 identified a mutation in Ser80lle from isolate resistant to ofloxacin, ciprofloxacin, and nalidixic acid [16]. Chiou et al. 2014 found a mutation in Glu92Lys from an isolate originating from Bangladesh [29]. The glutamic acid at position 92 should be examined since the amino acid proline was considered at this codon. Gopal et al. 2016 showed a mutation in Trp106Gly of parC in S. Typhi isolate from India resistant to ciprofloxacin and nalidixic acid [26]. The hotspot parC regions of all S. Typhi isolates from the current study were in line with the reference, in which the codons 79, 80, 84, 92, and 106 were aspartate, serine, glutamic acid, proline, and tryptophan, respectively.

The mutations in gyrA, gyrB, and parC have been mainly reported as the reason for quinolone resistance in S. Typhi. On the other hand, only a few reports mentioned the mutations in parE regions. The list of reported mutations in hotspot parE is shown in Table 5. Accou-Demartin et al. 2011 in France observed mutation at Asp420Asn in parE from isolates resistant to nalidixic acid but susceptible to ciprofloxacin [31]. Garcia-Fernández et al. 2015 in Italy reported a mutation in amino acid Ser493Phe in S. Typhi isolated from a patient that traveled to India with resistance pattern intermediate susceptibility to nalidixic acid but susceptible to ciprofloxacin [18]. Two mutations in Ile444Ser and Tyr434Ser of parE were found in China by Qian et al. 2020 from S. Typhi isolates resistant to nalidixic acid and intermediate susceptibility to ciprofloxacin [19]. In India, Britto et al. 2020 identified one mutation in parE Ala364Val from isolates resistant to ciprofloxacin, ofloxacin, and nalidixic acid [27]. Contrastingly, the present study showed amino acids in hotspots parE were Ala64, Asp420, Tyr434, Ile444, and Ser493, which were identical to the reference NCBI GenBank (AE014613.1), both in susceptible and intermediate susceptibility isolates to ciprofloxacin.
Table 5 Mutations found in the hotspot parE of S. Typhi

| Mutation  | Susceptibility Profile | Study |
|-----------|------------------------|-------|
| Ala364Val | OFX (R), CIP (R), NAL (R) | [27]\a |
| Asp420Asn | CIP (S), NAL (R) | [31]\a |
| Tyr434Ser | CIP (I), NAL (R) | [19]\a |
| Ile444Ser | CIP (I), NAL (R) | [19]\ab |
| Ser493Phe | NAL (I), CIP (S) | [18]\a |

\(\text{a Double mutations: gyrA Ser83Phe and parE Ala364Val; gyrA Ser83Tyr and parE Ala364Val; gyrA Asp87Asn and parE Ala364Val}\
\(\text{b Double mutations: gyrA Ser83Phe and parE Asp420Asn}\
\(\text{c Four mutations: gyrA Ser83Phe, gyrA Glu133Gly, parE Ile444Ser, and parE Tyr434Ser}\
\(\text{d Three mutations: gyrA Ser83Phe, gyrA Glu133Gly, and parE Ile444Ser}\
\(\text{e Double mutations in one isolate: gyrA Ser83Tyr and parE Ser493Phe}\

Multiple research across Indonesia supported our results on the susceptibility profile of the local S. Typhi strains in Jakarta. S. Typhi isolated from 2002 to 2008 in Jakarta and its surroundings showed good susceptibility to ciprofloxacin that was about two decades ago [32] and interestingly a similar profile of susceptibility was found in S. Typhi isolated in the late 2010s to 2020s [13, 14]. However, S. Typhi isolates collected in Sulawesi, an island located in the East of Indonesia, showed an increasing number of S. Typhi resistant to ciprofloxacin [33], and ofloxacin [34].

The distinct strain of S. Typhi could be another possible reason for the difference in the susceptibility profile of S. Typhi isolates from Jakarta. The study carried out by Chiou et al. 2014 about the genetic relationship of S. Typhi from Taiwan, Indonesia, Vietnam, and Bangladesh showed that antibiotic susceptibility is related to clonal variation. Isolates from Bangladesh and Vietnam were closely related with high resistance to nalidixic acid and ciprofloxacin. Isolates from Indonesia were closely related to those from Taiwan and were distant from isolates from Bangladesh and Vietnam. Almost all isolates from Indonesia and Taiwan were sensitive to fluoroquinolones, with a low resistance to nalidixic acid [29]. In 2019, Wang et al. also reported that isolates from Indonesia shared the same genotype as the indigenous strain in Taiwan, which was in agreement with the study by Chiou et al. 2014. All isolates in the clade have good susceptibility to ciprofloxacin [35]. Further, the study by Ingle et al. 2019 found three clonalities of S. Typhi isolates from Indonesia. One clonality shared the same genotype with isolates from the Philippines, France, and the USA, while the other was also detected in Peru and Bangladesh. All of the S. Typhi isolates in these two clonalities were sensitive to fluoroquinolones and had no QRDR mutations, except the isolates from Bangladesh and Peru had a mutation in gyrA [36]. Baker et al. 2008 also reported that no DNA gyrase mutations were detected in S. Typhi isolates from Indonesia, although fluoroquinolone-resistant H58 strains have been introduced from neighboring countries [37].

Fluoroquinolones target and convert topoisomerase IV and DNA gyrase. The binding of the antibiotics to both DNA-enzyme complexes thus inhibits the enzyme activity [38]. Mutation in QRDR results in disturbance in replication and separation of DNA, which ultimately will cause cell lysis and cell death [39]. Other mechanisms of fluoroquinolones resistance in bacteria are mediated by plasmids, the so-called PMQR, and chromosome-mediated efflux pump resistance. The expression of PMQR genes has been reported by Qian et al. 2020 through the existence of aac(6′)-ib-cr-4, qnrS1 and qnrB4 in some S. Typhi isolates that were resistant to nalidixic acid and ciprofloxacin [18]. According to Ingle et al. 2019, there were no PMQR genes reported in S. Typhi isolates from Indonesia [36]. The existence of PMQR genes in Indonesia has not been reported yet and there are limited reports about the role of efflux pump in fluoroquinolone-resistant S. Typhi as well.

Conclusion

Despite surging cases and mutations in QRDR genes of fluoroquinolone-resistant S. Typhi reported worldwide, the overall local strain S. Typhi in Jakarta and surroundings showed good susceptibility to fluoroquinolones. Point mutations were not detected in the hotspot gyrA, gyrB, parC, and parE areas of all isolates, and no amino acid replacements in all reported codons. All DNA sequences from this study were identical to the reference sequence S. Typhi Ty2 (NCBI GenBank AE014613.1). The absence of point mutations in all hotspots indicated that the phenotypic profile of S. Typhi local strains in their susceptibility to ciprofloxacin was consistent with the genotypic characteristic. More research on PMQR genes and efflux pump might be worth to find out the alternative resistance mechanism on intermediate resistance isolate from this research. Further study on resistant local strains S. Typhi, if any, would shed more information about mechanisms of resistance to fluoroquinolones.

Methods

This research was conducted in the Laboratory of Microbiology and Biomolecular, School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia. This study was approved by Ethical Committee of School of Medicine and Health Science, Atma Jaya Catholic University of Indonesia (Ethical approval number: 08/03/KEP-FKIKUAJ/2021 and 9/08/KEP-FKIKUAJ/2021).
Specimen collection and bacterial identification
In this study, 28 S. Typhi isolates from 2015 to 2021 were used. Most of them were recovered from the storage of the Laboratory of Microbiology, School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia. Two isolates were received from hospitals in Jakarta and surroundings. Identification of S. Typhi was performed previously using Microbact 12A (Oxoid®) and standard microbiological procedures. Isolates were inoculated in Brain Heart Infusion Broth (BHIB) and Blood Agar (BA). Biochemical test was performed with Triple sugar iron agar (TSA) and Xylose-lysine-deoxycholate agars (XLD). Positive S. Typhi isolate was characterized by the presence of black precipitate and black-centered colony, respectively. Isolates were further identified using Gram staining and serotyping by slide agglutination test with commercial antiserum O9 and antisera O12 (Difco®). According to the White-Kaufmann Le-Minor scheme classification, S. Typhi was identified with positive agglutination. Isolates were then preserved on Nutrient Agar (NA) slant at room temperature. All inoculated media were incubated for 24 h at 35 °C.

Antibiotic susceptibility test
Antibiotic susceptibility test for ciprofloxacin 5 μg (CIP), nalidixic acid 30 μg (NAL), levofloxacin 5 μg (LVX), and moxifloxacin 5 μg (MXF) was performed with Kirby Bauer or disk diffusion method on Mueller-Hinton agar according to the guideline from Clinical and Laboratory Standards Institute CLSI M100 [23]. The breakpoints of nalidixic acid and ciprofloxacin adhered to the standard from CLSI 2011 and CLSI 2020, respectively [23, 24]. Meanwhile, the breakpoints of levofloxacin and moxifloxacin complied with the data from EUCAST 2022 [25]. Escherichia coli ATCC 25922 was used as the reference for the antibiotic susceptibility test.

DNA extraction
The genomic DNA from all isolates was extracted using QIAmp DNA Mini Kit (Qiagen®) from overnight culture on NA slant according to the instruction from the kit. The extracted DNA was stored in the elution buffer provided by the kit and placed at −20 °C. The presence of the genomic DNA was confirmed by electrophoresis in 0.5% (w/v) agarose gel at 90 V for 60–90 minutes.

Amplification of gyrA, gyrB, parC, and parE
The hotspot regions (gyrA, gyrB, parC, and parE) listed in Table 2 – Table 5 were amplified by polymerase chain reaction (PCR) using the primers shown in Table 6. These primers were specifically designed for this research according to the reference sequences from NCBI GenBank. S. Typhi Ty2 (AE014613.1). Hotspot regions were chosen based on the frequent mutations related to fluoroquinolones resistance. PCR was performed using Taq PCR Master Mix Kit (Qiagen®) as specified by the manufacturer’s manual with a final reaction volume of 50 μl containing 8 μl (≤1 μg/reaction) of DNA template. The annealing temperature was 60 °C followed by 35x cycles and had been optimized prior to the PCR amplification.

The presence of the targeted fragments was confirmed by electrophoresis, with 4 μl of the PCR product and 1 μl of loading dye loaded in 1.5% (w/v) agarose gel submerged in 1x Tris Boric acid EDTA (TBE). The gel was run at 90 V for 60–90 minutes and stained with Flurosafe (1st Base®). The DNA Ladder (Geneaid®) was used to determine the PCR product’s molecular size. The DNA fragment was visualized by gel documentation system (Biorad®). Confirmation of gel electrophoresis results was done by 1st Base®.

DNA sequencing
All PCR products were sequenced by the 1st Base®, Malaysia, using the Sanger sequencing method. SeqTrace and Unipro UGENE were used to analyze the sequences, and the results were compared to the reference sequence (NCBI GenBank AE014613.1).

Abbreviations
S: Sensitive; I: Intermediate; R: Resistant; DC: Decreased susceptibility.

Supplementary Information
The online version contains supplementary material available at https://doi.org/10.1186/s12866-022-02666-z.

Additional file 1: Table S1. NCBI Genbank accession numbers and other information related to all hotspot sequences from S. Typhi isolates in Jakarta. Table S2. Electrophoresis of PCR product from S. Typhi hotspot gyrA and gyrB with the expected fragments of 381 bp and 513 bp, respectively. Figure S1. Original electrophoresis image of PCR product respectively.

Table 6 Primer sequences of gyrA, gyrB, parC, and parE

| Genes | Size (bp) | Sequences |
|-------|-----------|-----------|
| gyrA  | F 381     | 5′- AAAATCTGCGGCGTGTGTTG-3′ |
|       | R         | 5′- TCACCTCGTAGTTGCGGC-3′ |
| gyrB  | F 513     | 5′- AGGTCGTAGTCGCGGTTGTT-3′ |
|       | R         | 5′- GCGTTGCGGTTGATGCTACT-3′ |
| parC  | F 564     | 5′- GATCATGAGCTGGCGGTTCG-3′ |
|       | R         | 5′- GGCCCCTGAGATGCATACCG-3′ |
| parE  | F 688     | 5′- CGCTTATGCTCTCCTCGG-3′ |
|       | R         | 5′- CGCTTCTTCTTTTCCCGTC-3′ |
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Authors’ contributions
IN: conduct research, primer design, data analysis, manuscript preparation. IMN: primer design, PCR product, sequencing analysis, and review the manuscript. AV: primer design, PCR product and sequencing analysis. ACMN, ET, and WOG: isolate collection. LHM: research grand design, determine methods, data analysis, advisory of the research and manuscript. The authors read and approved the final manuscript.

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Availability of data and materials
All data generated during this study are included in this published article and available in NCBI Genbank with the links provided in the supplementary information files.

Declarations
Ethics approval and consent to participate
This study was reviewed and approved by Ethical Committee School of Medicine and Health Sciences of Atma Jaya Catholic University of Indonesia, No. 08/03/KEP-FKIKUJA/2021 and No. 9/08/KEP-FKIKUJA/2021.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no conflict of interests.

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