Russian Journal of Infection and Immunity = Infektsiya i immunitet
2020, vol. 10, no. 1, pp. 99–110

Инфекция и иммунитет
2020, Т. 10, № 1, с. 99–110

THE ANTIMICROBIAL SUSCEPTIBILITY, RESISTANCE MECHANISMS AND PHYLOGENETIC STRUCTURE OF *S.* TYPHI ISOLATED IN 2005–2018 IN THE RUSSIAN FEDERATION

S.A. Egorova[a], K.V. Kuleshov[b], L.A. Kaftyreva[a,c], Z.N. Matveeva[a]

[a] *St. Petersburg Pasteur Institute, St. Petersburg, Russian Federation*
[b] *Central Research Institute for Epidemiology, Moscow, Russian Federation*
[c] *North-Western State Medical University named after I.I. Mechnikov, St. Petersburg, Russian Federation*

Abstract. Here we present current global epidemiological and microbiological trends for typhoid fever, as well as describe antimicrobial susceptibility and resistance mechanisms of *S.* Typhi. The data on examining 299 *S.* Typhi isolates collected in 2005–2018 in the Russian Federation were analyzed from the Russian *S.* Typhi Reference Center. It was found that *S.* Typhi population consisted of the isolates with different resistance phenotypes and mechanisms as well as genetic heterogeneity. Moreover, antimicrobial susceptibility was detected in as low as 10.4% *S.* Typhi strains, whereas 89.6% isolates showed fluoroquinolone resistance (including 7.3% high-level resistance) and 3.0% — multidrug resistance to ampicillin, chloramphenicol, trimethoprim/sulfamethoxazole, tetracycline and fluoroquinolones. All strains preserved susceptibility to extended-spectrum cephalosporins and azithromycin. Fluoroquinolone low-level resistance in *S.* Typhi was due to single nucleotide substitutions in the *gyrA*: Asp87Asn (78.7%) Ser83Tyr (5.0%) and Ser83Phe (3.2%). In addition, a plasmid-mediated low-level fluoroquinolone resistance *(qnrS)* was found in one isolate. In contrast, a fluoroquinolone high-level resistance in *S.* Typhi was due to accumulation of three single nucleotide substitutions in the genes *gyrA* (Asp87Asn+Ser83Phe) and *parC* (Ser80Ile). In multidrug resistant *S.* Typhi isolates, pHCM1 plasmids of incompatibility group IncHI1B(R27) (consisted of $bla_{\text{TEM-1}}$, *catA1, dfrA7* and *tetB*) and single nucleotide substitutions Ser83Tyr or Asp87Asn in gene *gyrA* were detected. The data of phylogenetic reconstruction based on the analysis of core single-nucleotide variations among examined and previously sequenced *S.* Typhi genomes, demonstrated that more than 80.0% of *S.* Typhi isolated in Russia were referred to the Asian genotype as they belonged to subclade 4.3.1 (by Wong et al.) or dominant H58 clade (H58 haplotype by Roumagnac et al.). More than 60.0% isolates in this dominant phylogenetic group possessed a fluoroquinolone low-level resistance due to *gyrA* Asp87Asn. Less than 20.0% of *S.* Typhi strains isolated in Russia phylogenetically belonged to the subclades other than 4.3.1 (non-H58) and differed from the major *S.* Typhi population by lacked antibiotic resistance or exerted fluoroquinolone resistance due to *gyrA* Ser83Phe. The study data allowed to expand our understanding on genetic diversity in *S.* Typhi strains isolated recently and pinpoint features of phylogenetic structure for *S.* Typhi population in the Russian Federation.

Key words: *typhoid fever, S. Typhi, antimicrobial resistance, SNV, fluoroquinolones, nucleotide substitutions.*

Адрес для переписки:
Егорова Светлана Александровна
197101, Россия, Санкт-Петербург, ул. Мира, 14,
ФБУН НИИ эпидемиологии и микробиологии имени Пастера.
Тел.: 8 (812) 232-48-83. Факс: 8 (812) 232-92-17.
E-mail: egorova72@mail.ru

Contacts:
Svetlana A. Egorova
197101, Russian Federation, St. Petersburg, Mira str., 14,
St. Petersburg Pasteur Institute.
Phone: +7 (812) 232-48-83. Fax: +7 (812) 232-92-17.
E-mail: egorova72@mail.ru

Библиографическое описание:
Егорова С.А., Кулешов К.В., Кафтырева Л.А., Матвеева З.Н. Чувствительность к антибиотикам, механизмы резистентности и филогенетическая структура популяции S. Typhi, выделенных в 2005–2018 гг. в Российской Федерации // Инфекция и иммунитет. 2020. Т. 10, № 1. С. 99–110. doi: 10.15789/2220-7619-ASM-1171

Citation:
Egorova S.A., Kuleshov K.V., Kaftyreva L.A., Matveeva Z.N. The antimicrobial susceptibility, resistance mechanisms and phylogenetic structure of S. Typhi isolated in 2005–2018 in the Russian Federation // Russian Journal of Infection and Immunity = Infektsiya i immunitet, 2020, vol. 10, no. 1, pp. 99–110. doi: 10.15789/2220-7619-ASM-1171

DOI: http://dx.doi.org/10.15789/2220-7619-ASM-1171

ЧУВСТВИТЕЛЬНОСТЬ К АНТИБИОТИКАМ, МЕХАНИЗМЫ РЕЗИСТЕНТНОСТИ И ФИЛОГЕНЕТИЧЕСКАЯ СТРУКТУРА ПОПУЛЯЦИИ *S.* TYPHI, ВЫДЕЛЕННЫХ В 2005–2018 гг. В РОССИЙСКОЙ ФЕДЕРАЦИИ

Егорова С.А.[1], Кулешов К.В.[2], Кафтырева Л.А.[1,3], Матвеева З.Н.[1]

[1] ФБУН НИИ эпидемиологии и микробиологии имени Пастера, Санкт-Петербург, Россия
[2] ФБУН Центральный НИИ эпидемиологии Роспотребнадзора, Москва, Россия
[3] ФГБОУ ВО Северо-Западный государственный медицинский университет им. И.И. Мечникова МЗ РФ, Санкт-Петербург, Россия

Резюме. В статье представлены современные глобальные эпидемиологические и микробиологические тенденции брюшного тифа, описаны чувствительность и механизмы резистентности к антибиотикам. Приведены результаты исследования 299 штаммов *S.* Typhi, выделенных в 2005–2018 гг. в Российской Федерации, из коллекции российского референс-центра по мониторингу возбудителя брюшного тифа. Популяция штаммов *S.* Typhi, характеризовалась различными фенотипами и механизмами резистентности к антибиотикам и генетической неоднородностью. Чувствительными к антибиотикам были 10,4% штаммов, 89,6% штаммов характеризовались устойчивостью к фторхинолонам (7,3% штаммов — устойчивостью высокого уровня), 3,0% — множественной устойчивостью к ампициллину, хлорамфениколу, триметоприм/сульфаметоксазолу, тетрациклину и фторхинолонам. Все штаммы сохраняли чувствительность к цефалоспоринам расширенного спектра и азитромицину. Устойчивость низкого уровня к фторхинолонам у штаммов *S.* Typhi обусловлена однонуклеотидными заменами в гене *gyrA*: Asp87Asn (78,7%), Ser83Tyr (5,0%) и Ser83Phe (3,2%). У одного штамма выявлена плазмидоопосредованная устойчивость низкого уровня к фторхинолонам (ген *qnrS*). Устойчивость высокого уровня к фторхинолонам обусловлена сочетанием трех однонуклеотидных замен: в гене *gyrA* (Asp87Asn+Ser83Phe) и *parC* (Ser80Ile). У штаммов с множественной устойчивостью выявлены плазмиды pHCM1 группы несовместимости IncHI1B(R27), которые включали гены bla_{TEM-1}, *catA1*, *dfrA7* и *tetB*, и однонуклеотидные замены Ser83Tyr и Asp87Asn в гене *gyrA*. По результатам филогенетической реконструкции, проведенной на основе анализа коровых однонуклеотидных вариаций среди исследуемых и ранее секвенированных геномов *S.* Typhi из разных регионов мира (порядка 1700 штаммов), показано, что более 80,0% российских штаммов относились к азиатскому генотипу, поскольку принадлежали к филогенетической линии гаплотипа H58 (Roumagnac et al.) или субкладе 4.3.1 (Wong et al.). Более, чем 60,0% штаммов этого генотипа были идентичны по фенотипу и механизму резистентности: устойчивость низкого уровня к фторхинолонам, обусловленная мутацией *gyrA* Asp87Asn. Менее 20,0% исследуемых штаммов филогенетически относились к другим субкладам (не 4.3.1) и отличались от основной популяции возбудителя брюшного тифа отсутствием резистентности к антибиотикам, либо имели резистентность к хинолонам, обусловленную однонуклеотидной заменой *gyrA* Ser83Phe. Результаты исследования позволили расширить представление о генетическом разнообразии штаммов *S.* Typhi, выделенных за последние годы на территории РФ, и определить особенности популяционной структуры возбудителя брюшного тифа.

Ключевые слова: *брюшной тиф, S. Typhi, устойчивость к антибиотикам, SNP, фторхинолоны, нуклеотидные замены.*

Introduction

Typhoid fever is registered worldwide and has not yet been eradicated globally. According to the most recent WHO estimates, between 11 and 21 million cases and 128,000 to 161,000 typhoid-related deaths occur annually worldwide (https://www.who.int/immunization/diseases/typhoid/en). The real typhoid fever incidence is difficult to assess due to the lack of reliable laboratory diagnostic in typhoid-endemic countries (especially in Africa), and among infants and young children. Some population-based studies showed a wide variation in the typhoid fever incidence (from single cases to > 500 per 100,000 per year), both at the global level and within individual countries [10, 25].

Typhoid fever continues to be a serious public health problem in the regions of sub-Saharan Africa, South and South-East Asia, and Oceania with the lowest population coverage with safety water and good sanitation facilities. In these countries the urbanization, which leads to overcrowding, increases the probability of outbreaks. Also the serious problem is that typhoid fever is common among children under 5 years of age in many endemic countries. The lack of appropriate laboratory facilities and poor access to the hospital-level healthcare are the important reasons of the delaying in effective antimicrobial treatment, the increasing the frequency of complications, deaths and chronic carriers. In economy developed countries, the typhoid fever is usually registered in tourists returning from typhoid endemic countries (India, Pakistan, Nepal, Bangladesh, Indonesia, etc.). Often typhoid fever is imported by the labour migrants from the endemic countries.

In 1972 epidemic *S.* Typhi isolates resistant to first-line antibiotics (ampicillin, chloramphenicol and trimethoprim/sulfametoxazole) were isolated (so called “multi drug resistant *S.* Typhi”, MDRST) [29]. The number of MDRST has increased rapidly

in South Asia and South Africa. Thus, in Vietnam, the first MDRST was isolated in 1993, but in 2005 almost 90.0% of *S.* Typhi isolated in this country were MDRST, so these antibiotics have lost their importance in the treatment of typhoid fever [45]. In the UK, MDRST ranged from 20.0 to 40.0% in the 1990s, and about 90.0% of MDRST cases were associated with travel to Pakistan and India [45]. Fluoroquinolone (ciprofloxacin and ofloxacin) has become the drug of choice following the emergence of MDRST [14], but the spread of isolates with decreased susceptibility to ciprofloxacin has limited their effectiveness. In recent years, the proportion of fluoroquinolones resistant *S.* Typhi with low-level resistance (MIC of ciprofloxacin 0.12–0.5 mg/l) reaches 70.0–80.0%. The uncontrolled access to fluoroquinolones has led to the rapid spread of resistant *S.* Typhi in South-East Asia. Several global studies conducted in 1995–2012 in eight Asian countries, cover about 80.0% of world's typhoid fever cases (India, Vietnam, Nepal, Bangladesh, Cambodia, Laos, Thailand, China), showed that the MDRST proportion decreased from 16.0 to 37.0%. In Vietnam and India the fluoroquinolone low-level resistant *S.* Typhi increased to 97.0%, in other countries — to 65.0%; moreover, there was a dramatically rapid increasing in such resistance from 4.0 to 97.0% [9, 19].

The gradual decline of MDRST proportion in typhoid-endemic Asian countries in recent years is likely due to the change in the antibiotic treatment of typhoid fever. Antibiotic change has decreased the selective pressure and caused the elimination of plasmids carrying the multidrug resistance genes. At the same time, MDRST proportion is high in the countries of West Africa: from 20.0 to 60.0% of *S.* Typhi isolated in Nigeria, Cameroon, Guinea in 2000–2013 were multidrug resistant due to plasmids IncHI1, but quinolone resistance due to *gyrA* mutations was rare found [7, 22].

In developed countries with mainly imported cases of the typhoid fever, the situation with antimicrobial resistance in *S.* Typhi repeats the situation in the "source" endemic countries. According to CDC in the USA the proportion of resistant *S.* Typhi increased from 25.6% (in 2002) to 75.5% (in 2014) and the level of quinolone resistant *S.* Typhi is constantly growing (from 23.6% in 2002 to 77.6% in 2014), MDRST was about 12.0% [8]. The travel persons had the highest chance to be infected by resistant *S.* Typhi in the Indian subcontinent countries: 65.0% of patients in USA and 32.0% in France infected in these countries had a quinolone resistant *S.* Typhi [8, 45].

In 2003 the World Health Organization published the guidelines that recommended azithromycin, ceftriaxone, or cefixime for the treatment of quinolone-resistant *S.* Typhi and *S.* Paratyphi A infections [48]. Extended-spectrum cephalosporins (ceftriaxone and cefixime) are commonly used for typhoid fever in children and caused by MDRST or fluoroquinolone resistant *S.* Typhi. Since 2007 in Asia (India, Kuwait, Nigeria, Korea, Pakistan, Bangladesh) and Africa (Nigeria, Congo) *S.* Typhi producing both ESBL (mainly CTX-M15 and SHV-12) and AmpC cephalosporinases (CMY-2 and ACC-1) have been isolated. Single isolates of *S.* Typhi isolated in European countries (Germany, Norway, the Netherlands, Spain) and the United States were imported from Asia [3–6, 16–18, 20, 34–40].

So, *S.* Typhi has acquired the resistance to almost all antibiotics used to the treatment of typhoid fever at the different time. This pathogen has adapted to the antimicrobial selective pressure by the various molecular mechanisms: the emergence of chromosomal mutations or the acquisition of mobile genetic elements containing resistance genes. The widespread of *S.* Typhi resistant to the fluoroquinolones and the emergence of isolates resistant to extended-spectrum cephalosporins mean that the choice of antibiotics for the treatment of typhoid fever is limited.

The classical subtyping techniques used by public health laboratories such as phage typing or pulsed-field gel electrophoresis are phylogenetically naive (don't reflect phylogenetic relatedness among strains) and have limited discriminatory power to support an objective picture of the diversity of strains in global scale and evolution of this pathogen. Whole genome analysis showed that the global *S.* Typhi population is rather young, highly clonal and originated from a common ancestor existed so recently that multiple mutations have not yet accumulated (15,000–150,000 years ago) [2, 21, 24]. According to Enterobase (http://enterobase.warwick.ac.uk) more than 95.0% of the *S.* Typhi studied by MLST of 7 housekeeping genes belong to two genetically closely related sequence types ST1 and ST2.

To evaluate the phylogenetic relatedness in the higly clonal pathogen population, the genotyping method, based on the detection in the compared genomes of the whole range of core single-nucleotide variations (SNV) located both in coding and non-coding genome regions, is commonly used. The principle of SNV-typing of *S.* Typhi isolates is currently widely used to evaluate the pathogen population structure and to determine the relation between single or group strains.

In 2015, Wong et al. [46] carried out a large-scale study using a whole genome analysis of 1832 isolates of *S.* Typhi isolated in 63 countries. Phylogenetic analysis showed that most isolates belonged to the recently defined haplotype H58 [41, 46]. All these isolates contained the nucleotide substitution C2348902T in the position relative to the reference genome *S.* Typhi CT18. Moreover, the strains of H58 haplotype formed the two main sublineages I and II. Taking into account the time and place of *S.* Typhi isolation, their phylogenetic position and early facts of the intercontinental transmission, it was suggested that the earliest reservoir for H58 strains was the re-

gion of South Asia with further spread to South-East and West Asia, East Africa (Kenya, Tanzania, Malawi) and South Africa [41, 46].

Wong et al. [47] proposed an algorithm for the rapid phylogenetic classification of the strain genome. This analysis is based on the detection of specific nucleotide polymorphisms causing the branch divergence on a pre-reconstructed phylogenetic tree [46]. In practice, the researcher analyzes only one isolate genomic data and understands its phylogenetic position on the global phylogenetic tree. According to this analysis, the global *S.* Typhi population is divided into 4 primary cluster (1–4), which in turn are divided into 16 clades and 49 subclade [47]. Although this analysis does not allow to establish the exact phylogenetic position of the isolate, but allows to determine the geographical region of the isolate origin.

The Russian Federation isn't typhoid-endemic country, and according to the official registration the incidence is low (0.03–0.1 per 100,000 per year) and sporadic. In the last ten years (2009–2018) in Russia 320 cases of typhoid fever were registered as sporadic cases, group cases (2–4 persons) and several outbreaks (15–20 persons). The occurrence of outbreaks was possible due to hygiene and sanitation violations in the canteen (where the Asian cook was a *S.* Typhi carrier) and in the hostel for the Asian labour migrants. Annually the typhoid fever cases are imported from endemic areas (Central, South and South-East Asia) by the Russians tourists, labour migrants and foreign students returned to Russian universities after the holiday in Asia home countries. In general, in 2008–2018 the typhoid fever was imported to Russia from 13 countries: Tajikistan, Uzbekistan, Kyrgyzstan, Azerbaijan, Abkhazia, Bangladesh, Cambodia, India, Pakistan, Nepal, Egypt, Madagascar and the United Arab Emirates.

The aim of this study was to evaluate the antimicrobial susceptibility and phylogenetic structure of the *S.* Typhi population isolated in Russia in 2005–2018.

Materials and methods

The study included 299 isolates from the collection of the Russian *S.* Typhi Reference Center isolated in 21 administrative regions of the Russian Federation in 2005–2018: St. Petersburg and Leningrad region, Moscow, Ivanovo, Ryazan, Arkhangelsk, Tula, Smolensk, Voronezh, Orel, Novgorod, Ulyanovsk, Irkutsk, Kaliningrad, Kemerovo, Tomsk, Kirov, Krasnoyarsk, Khabarovsk, Khanty-Mansi and in the Jewish Autonomous region.

The set of antimicrobials for testing was selected in order to reflect the importance for treatment and surveillance: the drugs of choice or alternative for the treatment of typhoid fever (ciprofloxacin, azithromycin, cefotaxime, ampicillin, chloramphenicol, trimethoprim/sulfametoxazole); the indicators of clinically important resistance mechanisms (amoxicillin/clavulanic acid, ceftazidime, nalidixic acid, pefloxacin); the antibiotics critical important for the public health that may be needed for future treatment of MDRST (meropenem); the additional epidemiological markers for outbreak investigation (aminoglycosides, tetracycline).

Antimicrobial susceptibility testing was made by disk diffusion method and E-test with Mueller–Hinton agar and discs of Oxoid and E-tests of bioMérieux, according to EUCAST (http://www.eucast.org/fileadmin /src/media/PDFs/ EUCAST_files/Breakpoint_tables/v_8.0_Breakpoint_Tables.pdf) and Russian clinical guidelines "Antimicrobial susceptibility testing of microorganisms", version 2015 (http://www.antibiotic.ru/minzdrav/files/docs/clrec-dsma2015.pdf). The fluoroquinolones were tested by disk diffusion method (with nalidixic acid and pefloxacin) and E-test (MIC of ciprofloxacin). We used the following breakpoints for the category "resistant" to fluoroquinolones: inhibition zone of pefloxacin < 24 mm, nalidixic acid < 16 mm, and MIC of ciprofloxacin > 0.06 mg/l. Susceptibility of *S.* Typhi to azithromycin was determined by E-test, the isolates with MIC ≤ 16.0 mg/l were interpreted as "susceptible".

Plasmid-mediated fluoroquinolone resistance (genes *qnr* S, A, B and *aac(6')-Ib-cr*) was detected in 299 *S.* Typhi isolates by PCR according to previously published protocols [32, 38].

Whole genome sequencing of 117 *S.* Typhi was performed on the device MiSeq (Illumina, USA) with MiSeq Reagent Kit v3 600 cycles. Genomic DNA was isolated by the DNeasy Blood & Tissue Kit (Qiagen, Germany). Genome libraries were prepared using MiSeq Nextera XT (Illumina, USA). Genome assembly and analysis was performed using CLC Genomics Workbench 8.0 (Qiagen, Germany). Detection of chromosomal mutations (in *gyrA*, *gyrB*, *parC* and *parE* genes), acquired resistance genes and plasmids was made using the online-services ResFinder and PlasmidFinder (https://cge.cbs.dtu.dk).

To reconstruct the global phylogenetic tree, we analyzed a set of 1683 *S.* Typhi isolates, which included both Russian isolates and isolates sequenced in previous studies. Thus, the set of *S.* Typhi isolates under our investigation was characterized by the wide isolation time period (from 1905 to 2013) and broad geographical origin (63 countries, 6 continents (Asia, Africa, North and South America, Europe, Australia and Oceania).

The detection of orthological SNV was performed using the previously developed algorithm of data analysis [27]. The nucleotide sequence of *S.* Typhi CT18 strain (NCBI acc. AL513382) was used as a reference genome. The resulting matrix of orthologous SNV was used for phylogenetic reconstruction in RAxML software, the model GTR+I was used as

a model of nucleotide substitutions. Bootstrap analysis was carried out with the number of repetitions 1000. The phylogenetic tree visualization was carried out in the program Figtree v1.3.1.

Additionally, Russian *S.* Typhi isolates were analyzed by Genotyphi software (https://github.com/katholt/genotyphi) according to the aurthor's instructions.

Results

All S. Typhi isolates gave the good bacterial grows and formed the typical colonies on selective and non-selective Russian and foreign media for Enterobacterales represented in the Russian market. *S.* Typhi had typical biochemical activities and serological formula (9, 12, Vi: d:-). All isolates had a well-developed Vi-antigen; moreover, 36 isolates (12.0%) had a highly developed Vi-antigen, which did not allow to identify the O-group. All isolates were well lysed by liquid therapeutic bacteriophage for Salmonella of groups A, B, C, D, E (produced by Microgen, Russian Federation).

Antimicrobial susceptibility of *S.* Typhi

The *S.* Typhi population was represented by both antimicrobial susceptible (31 isolates, 10.4%) and resistant (268 isolates, 89.6%) isolates. The proportion of isolates resistant to fluoroquinolone was 89.6%, to ampicillin, chloramphenicol, trimethoprim/sulfametoxazole and tetracycline — by 2.7%. *S.* Typhi resistant to extended-spectrum cephalosporins, carbapenems, aminoglycosides and azithromycin were not detected (tabl. 1).

So, in our study 89.6% of *S.* Typhi isolates were found to be resistant to fluoroquinolones — the drugs of choice for the treatment of typhoid fever. 82.3% of *S.* Typhi had the low-level resistance with MIC of ciprofloxacin 0.125–0.5 mg/l, 7.3% of isolates — the high-level resistance with MIC 8.0–32.0 mg/l. All *S.* Typhi were susceptible to azithromycin with MIC_{90} 8.0 mg/l, seven isolates had "critical" MIC 16.0 mg/l.

The *S.* Typhi population divided into 3 resistance phenotypes: susceptible to all tested antibiotics (31 isolates, 10.4%), resistant only to fluoroquinolones (260 isolates, 86.9%) and multidrug resistant, MDRST (to fluoroquinolones, ampicillin, chloramphenicol, trimethoprim/sulfamethoxazole, tetracycline) (8 isolates, 2.7%).

Susceptible isolates of *S.* Typhi were isolated in 9 regions of the Russian Federation: St. Petersburg (2005–2012, 2014), Leningrad region (2009, 2014), Moscow (2011), Irkutsk (2005, 2012), Orel (2009), Voronezh (2015), Novgorod (2009), Ulyanovsk (2010) and Kemerovo (2012). Fluoroquinolone resistant *S.* Typhi were isolated annually in all regions of Russia.

Eight *S.* Typhi had multidrug resistance: to fluoroquinolones (low-level resistance, MIC ciprofloxa-

Table 1. Antimicrobial susceptibility of *S.* Typhi strains isolated in Russia in 2005–2018 (n = 299)

Antimicrobials		Category (S — susceptible, R — resistant)	Number of isolates		
			n	%	95% CI
Beta-lactames	Aminopenicillines	S	291	97,3	94,8–98,6
		R	8	2,7	1,4–5,2
	Extended spectrum cephalosporines	S	299	100	98,7–100
		R	0	0	0–1,3
	Carbapenems	S	299	100	98,7–100
		R	0	0	0–1,3
Fluoroquinolones		S	31	10,4	7,4–14,3
		R	268	89,6	85,7–92,6
		low-level resistant*	246	82,3	77,5–86,2
		high-level resistant**	22	7,3	4,9–10,9
Aminoglycosides (genta-, tobra-, amikacin)		S	299	100	98,7–100
		R	0	0	0–1,3
Chloramphenicol		S	291	97,3	94,8–98,6
		R	8	2,7	1,4–5,2
Trimethoprim/ sulfametoxazole		S	291	97,3	94,8–98,6
		R	8	2,7	1,4–5,2
Tetracycline		S	290	97,0	98,1–99,9
		R	9	3,0	1,6–5,6
Azithromycin		S MIC_{50} — 4,0 mg/l MIC_{90} — 8,0 mg/l	299	100	98,7–100
		R	0	0	0–1,4

Note. * MIC of ciprofloxacin 0,125–0,5 mg/l; ** MIC of ciprofloxacin 4,0–32,0 mg/l.

cin 0.12–0.5 mg/l), aminopenicillins (MIC > 256.0 mg/l), chloramphenicol (MIC > 256.0 mg/l), tetracycline (MIC 64.0–128.0 mg/l) and trimethoprim/sulfamethoxazole (MIC > 32.0 mg/l). Six MDRST were isolated in 2005 and 2006 in St. Petersburg, Leningrad and Irkutsk oblast. During next six years (from 2007 to 2012) MDRST were not detected in Russia. In 2013 and 2015 two MDRST were isolated in St. Petersburg. The emergence of MDRST in Russia was associated with the import by labor migrants from Central Asia (Tajikistan and Uzbekistan).

Molecular resistance mechanisms of *S.* Typhi

Fluoroquinolone resistance molecular mechanisms of 117 *S.* Typhi were detected by FesFinder. Resistance phenotypes and molecular mechanisms of *S.* Typhi with different levels of fluoroquinolone resistance are presented in tabl. 2. No mutations in chromosomal genes *gyrA*, *gyrB*, *parC* and *parE* or acquired resistance plasmid genes were found in susceptible *S.* Typhi. The group of fluoroquinolone resistant *S.* Typhi (94 isolates) had different mutations in *gyrA* and *parC*. Isolates with low-level resistance had a single nucleotide substitution in *gyrA*, the most frequent in codon 83 of *gyrA* Asp87Asn (78.7%). *S.* Typhi with these phenotype and mutation were isolated annually in all region of Russia. Three *S.* Typhi with single nucleotide substitution in *gyrA* Ser83Phe were isolated in St. Petersburg and Arkhangelsk (according epidemiological data *S.* Typhi from Arkhangelsk was imported to Russia from India in 2012). In five *S.* Typhi the substitution in *gyrA* Ser83Tyr was detected: one isolate with additional multidrug resistance was isolated in St. Petersburg in 2006, other four *S.* Typhi were isolated in 2011 in St. Petersburg, Irkutsk, Tula and Arkhangelsk. So, the identical fluoroquinolone resistance phenotype (low-level resistance) in *S.* Typhi was caused by three different single nucleotide substitutions in *gyrA*.

All studied *S.* Typhi with fluoroquinolone high-level resistance had three single nucleotide substitutions simultaneously: in *gyrA* (Ser83Phe + Asp87Asn) and *parC* (Ser80Ile). *S.* Typhi isolates with fluoroquinolone high-level resistance were isolated in 2005–2018 in seven regions of Russia: St. Petersburg (2007, 2013), Kaliningrad (2011, 2012), Smolensk (2012), Voronezh (2014), Kirov (2015), Arkhangelsk (2015) and Khanty-Mansiysk (2016). In all cases, the patients were infected travelling to India (tourists, Indian students of Russian universities).

Plasmid-mediated fluoroquinolone resistance *(qnrS)* was found in one *S.* Typhi isolate with "paradox" phenotype of quinolones resistance: MIC of ciprofloxacin 0.25 mg/l (low-level resistance), but susceptibility to nalidixic acid (MIC 4.0 mg/l), which is common for plasmid-mediated fluoroquinolone resistance.

Multidrug resistance in 8 *S.* Typhi isolates was mediated by the plasmid pHCM1 (incompatibility group IncHI1B(R27)) PST6, which included genes bla_{TEM-1}, *catA1*, *dfrA7* and *tetB*. Fluoroquinolone low-level resistance in these isolates was associated with single nucleotide substitutions in *gyrA* Ser83Tyr or Asp87Asn.

Phylogenetic structure of *S.* Typhi population isolated in Russia in 2005–2018

We constructed the global *S.* Typhi phylogeny by SNV analysis of 1683 isolates of *S.* Typhi including 92 *S.* Typhi isolated in the Russian Federation with different antimicrobial resistance phenotypes and mechanisms.

In the global phylogenetic tree Russian *S.* Typhi isolates were clustered into several phylogenetic groups. The most of them (82.6%) belonged to the dominant H58 haplotype of *S.* Typhi (fig., tabl. 3). The phylogenetic lineage of H58 was heterogeneous: *S.* Typhi isolates were clustered into three phylogenetic groups (designated as G1, G2 and G3), and five isolates had individual genotypes (designated as S1-S5). 60.0% isolates of H58 belonged to the group G3 and had identical resistance phenotype (fluo-

Table 2. Phenotypes and molecular mechanisms of quinolones resistance in *S.* Typhi strains isolated in Russia in 2005–2018 (n = 117)

Phenotypes	MIC, mg/l		Single nucleotide substitutions (amino acid substitutions) in *gyrA* and *parC*	Plasmid-mediated resistance	Number of strains	
	Nalidixic acid	Ciprofloxacin			n	% in resistant strains studied (n = 94)
Susceptible n = 23	0,75–4,0	0,004–0,023	Not detected	Not detected	23	–
Fluoroquinolone low-level resistance n = 83	24 — ≥ 256,0	0,094–0,25	*gyrA* (Asp87Asn)	Not detected	74	78,7
	≥ 256,0	0,19–0,25	*gyrA* (Ser83Tyr)	Not detected	5	5,3
	≥ 256,0	0,19	*gyrA* (Ser83Phe)	Not detected	3	3,2
	4,0	0,25	Not detected	qnrS1	1	1,1
Fluoroquinolone high-level resistance n = 11	≥ 256,0	8,0 — ≥ 32,0	*gyrA* (Ser83Phe+ Asp87Asn) + *parC* (Ser80Ile)	Not detected	11	11,7

Amino acids: Ser — Serine, Asp — Aspartic acid, Asn — Asparagine, Phe — Phenylalanine, Ile — Isoleucine, Tyr — Tyrosine.

roquinolone low-level resistance) and resistance mechanism (single nucleotide substitution in *gyrA* Asp87Asn).

The phylogenetic group G1 (11.2%) included *S.* Typhi with fluoroquinolone high-level resistance mediated by three single nucleotide substitutions: *gyrA* (Ser83Phe+Asp87Asn) + *parC* Ser80Ile. The *S.* Typhi isolates of the phylogenetic group G2 (7.8%) had the identical resistance mechanism (fluoroquinolone low-level resistance due to *gyrA* Asp87Asn), but two isolates also had additional multidrug resistance associated with the plasmid IncHI1B(R27). Five *S.* Typhi H58 isolates with individual genotypes (S1-S5) were susceptible to antibiotics or had fluoroquinolone low-level resistance due to *gyrA* Ser83Tyr — the single nucleotide substitution, which was not found in other phylogenetic groups.

The described phylogenetic groups of H58 clade included *S.* Typhi isolated in different regions of Russian during all years under study. Epidemiological data was agreed with phylogenetic analysis as well as antimicrobial susceptibility. The isolates from the same outbreaks (St. Petersburg 2006, Moscow 2013) or group cases (Kaliningrad 2012, Irkutsk 2016) were clustered together in one phylogenetic group and had identical antimicrobial resistance patterns due to identical single nucleotid substitutions.

In groups of non-H58 *S.* Typhi almost all isolates were susceptible to antibiotics except one isolate with fluoroquinolone low-level resistance due to the single nucleotide substitution in *gyrA* Ser83Phe, detected in other phylogenetic groups only in combinations with other single nucleotide substitution. Some isolates were clustered into phylogenetic groups (designated as G4-G6), others had individual genotypes (fig., tabl. 3).

Additional analysis of sequenced *S.* Typhi genomes by Genotyphi software showed that Russian *S.* Typhi population was represented by the isolates of all four primary clusters, but mainly — by the cluster 4 (83.7%) (tabl. 3). Within cluster 4, the majority of isolates (82.6%) belonged to subclade 4.3.1. According to Wong et al. [47] progentor *S.* Typhi isolates of this subclade are originated from the countries in South-East and South Asia. It should be noted that all isolates of the subclade 4.3.1. belonged to the phylogenetic lineage of H58 haplotype defined by the global phylogeny. At the same time, Russian isolates of the subclade 4.3.1. were further clustered in two genetic clusters. The cluster 4.3.1.1 (68.5%) mainly included the isolates with fluoroquinolone low-level resistance due to *gyrA* Asp87Asn, and the cluster 4.3.1.2 (14.1%) — the isolates with fluoroquinolone low-level resistance due to non-common nucleotide substitution in *gyrA* Ser83Tyr, and the isolates with high-level resistance due to three single nucleotide substitutions: *gyrA* (Ser83Phe+Asp87Asn) and *parC* (Ser80Ile). Furthermore, within cluster 4, one antimicrobial susceptible *S.* Typhi isolate (Voronezh, 2015) belonged to subclade 4.1.1 and was probably of African origin.

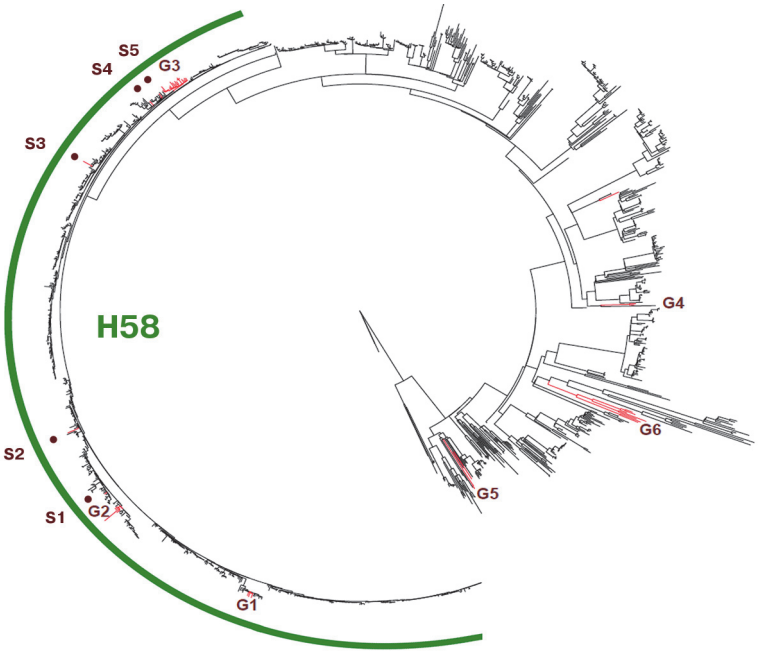

Figure. The global phylogenetic tree constructed on the basis of the identified ortological SNV in 1683 *S.* Typhi genomes

The tree was reconstructed by the maximum likelihood method implemented in the RAxML. The phylogenetic lineage related to haplotype H58 is highlighted in green. The tree branches with Russian *S.* Typhi isolates are marked in red. If several Russian *S.* Typhi isolates were clustered together, they were designated as a phylogroup "G", if individually — as "S". Description of phylogroup of Russian isolates given in Table 3.

Table 3. Characteristics of *S.* Typhi strains isolated in Russia in 2005–2018 by the resistance mechanisms and phylogenetic analysis (n = 92)

Phylogroups		Resistance genotypes	Number of strains	Place and year of isolation	Geographic origin of isolates in reference set (microreact.org/project/styphi)
Wong et al. [47]	Global phylogeny				
1.2.1	nonH58_G6	WT	8	St. Petersburg 2009 and 2011; Leningrad region 2009; Moscow 2011; Tomsk 2015; Kyrgyzstan 2010; Kazakhstan 2012	South-East Asia (100%) — Vietnam
2.0.2	nonH58	*gyrA* (Ser83Phe)	1	St. Petersburg 2017	North America (50%) — Mexico North Africa (50%) — Algeria, Tunisia
2.3.2	nonH58_G5	WT	2	Kemerovo 2012	West Africa (33%) — Nigeria, Mali South America (27%) — Argentina South-East Asia (20%) — Vietnam, Thailand North America (13%) — Mexico West Asia (7%) — Turkey
	nonH58_S7	WT	1	Ulyanovsk 2010	
3.0.1	nonH58_G4	WT	2	St. Petersburg 2010 and 2011	North Africa (50%) — Morocco South Asia (50%) — Pakistan
3.0.2	nonH58_S6	*gyrA* (Ser83Phe)	1	St. Petersburg 2012	South Asia (100%) — India
4.1.1	nonH58	WT	1	Voronezh 2015	Southern Africa (78%) — Malawi South Africa (11%) West Africa (6%) — Mauritania Central Africa (6%) — Cameroon
4.3.1.1.	H58_G3	*gyrA* (Asp87Asn)	54	St. Petersburg 2006 (outbreak), 2007, 2010–2012, 2014 and 2017; Moscow 2011 and 2013 (outbreak); Kaliningrad 2011 and 2012; Khabarovsk 2012; Voronezh 2014; Irkutsk 2015	South-East Asia (50%) — Vietnam, Laos, Cambodia South Asia (26%) — India, Bangladesh, Pakistan, Nepal, Sri Lanka, Afghanistan East Africa (10%) — Tanzania, Kenia Southern Africa (9%) — Malawi
	H58_G2	*gyrA* (Asp87Asn)	5	St. Petersburg 2008; Khanty-Mansiysk 2009; Jewish Autonomous region 2011; Irkutsk 2017	
		gyrA (Asp87Asn) + p IncHI1B(R27)	2	St. Petersburg 2013 and 2015	
	H58_S2	WT	1	Irkutsk 2012	
	H58_S4	WT	1	St. Petersburg 2011	
4.3.1.2	H58_G1	*gyrA* (Ser83Phe+ Asp87Asn) + *parC* (Ser80Ile) *gyrA* (Ser83Phe+ Asp87Asn) + *parC* (Ser80Ile) + p IncI	9 1	Kaliningrad 2011 and 2012; Smolensk 2011; Kirov 2015; Khanty-Mansiysk 2016; Voronezh 2017; Krasnoyarsk 2017; St. Petersburg 2018 Arkhangelsk 2015	
	H58_S1	*gyrA* (Ser83Tyr) + p IncHI1B(R27)	1	St. Petersburg 2006	
	H58_S3	*gyrA* (Ser83Tyr)	1	Arkhangelsk 2011	
	H58_S5	*gyrA* (Ser83Tyr)	1	St. Petersburg 2011	

WT — wild type, susceptible to fluoroquinolones and other antibiotics.

Primary cluster 1, subclade 1.2.1 included eight antimicrobial susceptible *S.* Typhi isolates, also belonged to the same phylogenetic group (G6). It is interesting to note that some susceptible *S.* Typhi from our collection, isolated in Kyrgyzstan and Kazakhstan in 2010 and 2012, also belonged to this subclade. According to Wong et al. [47] the *S.* Typhi isolates of subclade 1.2.1 originate from countries in South-East Asia.

The primary clusters 2 and 3 and their subclades in our study were presented by single *S.* Typhi isolates full susceptible to antibiotics or with fluoroquinolone low-level resistance due to *gyrA* Ser83Phe (not detected as single substitution in other clusters).

Discussion

The *S.* Typhi population isolated in 2005–2018 in St. Petersburg and 20 other regions of the Russian Federation consisted of the isolates with different resistance phenotypes and mechanisms and genetically heterogeneous. Only 10.4% *S.* Typhi were susceptible to antibiotics, 89.6% *S.* Typhi had fluoroquinolone resistance (7.3% with high-level resistance), which is a clinical fail predictor of using the fluoroquinolones for the empirical treatment of typhoid fever in Russia. Evaluation of the level of fluoroquinolone resistance in *S.* Typhi is important for choice of antimicrobial treatment of typhoid fever. There is clinical evidence of the ciprofloxacin poor clinical efficacy in typhoid fever, caused by *S.* Typhi with low-level resistance [11]. In this case, it is recommended to use alternative antibiotics: cephalosporins or azithromycin [13, 47]. But the some authors showed the high efficiency of gatifloxacin (the last generation fluoroquinolone) in treatment of typhoid fever when *S.* Typhi population had a high proportion of isolates with low-level resistance to ciprofloxacin [12, 44]. The fluoroquinolones (regardless of the drug or dosage) should not be used for *S.* Typhi with fluoroquinolone high-level resistance. The interpretive criteria for *S.* Typhi and ciprofloxacin differs from other Enterobacterales. According to EUCAST *S.* Typhi should be consider as "resistant" with MIC of ciprofloxacin > 0.06 mg/l. For the disc diffusion method, pefloxacin disc should be used instead of ciprofloxacin.

The level of fluoroquinolone resistance in Enterobacterales (MIC of ciprofloxacin) depends on the resistance mechanisms. The primary targets for the fluoroquinolones are the subunits of DNA gyrase (GyrA and GyrB) and the topoisomerase IV (ParC and ParE). Nonsynonymous single nucleotide substitutions in the quinolone resistance-determining regions of chromosome genes *gyrA*, *gyrB*, *parC* and *parE* decrease the fluoroquinolone susceptibility [1, 9, 19, 26, 28, 31, 42]. Single nucleotide substitutions, mainly in *gyrA*, leads to low-level resistance (MIC of ciprofloxacin 0.12–0.5 mg/l), the most common nucleotide substitutions in *S.* Typhi are in the codons 83 and 87 of *gyrA*, leading to amino acid substitutions Ser83Phe or Asp87Asn. Some plasmid-mediated mechanisms are associated with fluoroquinolone low-level resistance: in *S.* Typhi the genes *qnrS, qnrB* and aac(6')-Ib-cr are rarely described [15, 23, 30, 34, 43]. The emergence of high-level resistance (MIC of ciprofloxacin 1.0 mg/l and more) is always associated with the combination of several resistance mechanisms: the accumulation of single nucleotide substitutions in chromosomal genes or the acquisition of additional plasmid-mediated resistance genes by an isolate that already has any chromosomal resistance mutations.

Despite of identical fluoroquinolone resistance phenotype (low-level resistance) Russian *S.* Typhi population had different single nucleotide substitutions in *gyrA*: Asp87Asn (78.7%), Ser83Tyr (5.0%) and Ser83Phe (3.2%). High-level fluoroquinolone resistance was due to accumulation of three single nucleotide substitutions: *gyrA* (Asp87Asn+Ser83Phe) and *parC* (Ser80Ile). Plasmid-mediated fluoroquinolone low-level resistance *(qnrS)* was found in only one isolate. So, the leading fluoroquinolones resistance mechanism in Russian *S.* Typhi population is single nucleotide substitution in *gyrA* Asp87Asn.

About 3.0% of *S.* Typhi isolates had multidrug resistance to the antibiotics used for treatment of typhoid fever (fluoroquinolones, chloramphenicol, ampicillin, trimethoprim/sulfametoxazole). MDRST phenotype was a result of the acquisition plasmid-mediated resistance genes ($bla_{\text{TEM-1}}$, *catA1*, *dfrA7* and *tetB*) by the isolates that already had a chromosomal mutation in *gyrA* Ser83Tyr or Asp87Asn. It also complicates the antibiotic choice for the therapy of typhoid fever caused by such isolates.

Taking into account the wide spread of fluoroquinolone resistant *S.* Typhi and the lack of the resistance to extended-spectrum cephalosporins and azithromycin, these antibiotics can be considered as the drugs of first choice for the treatment of typhoid fever in Russia. EUCAST has no criteria for the interpretation of azithromycin for Enterobacterales, and it is proposed to use the "epidemiological cut off value" and consider as "susceptible" to azithromycin the isolates with MIC ≤ 16.0 mg/l. Antimicrobial susceptibility testing of azithromycin by disc diffusion method isn't possible due to the lack of interpretation criteria for this method, as well as uncertain test results [33].

More than 80.0% of *S.* Typhi isolates, imported to the Russian Federation in 2005–2018, belonged to successful international Asian clone — "subclade 4.3.1" by Wong et al. [47] or dominant H58 clade of *S.* Typhi [41, 46] and with high probability originated from the countries of South-East and South Asia. In Russia, this dominant phylogenetic group mainly included the isolates with the same resistance phenotype and mechanisms: about 60.0% had fluoroquinolone low-level resistance due to the single nucleotide substitution in *gyrA* Asp87Asn. All *S.* Typhi isolates with fluoroquinolone high-level resistance (due tree single nucleotide substitutions in *gyrA* and

parC) and MDRST isolates also belonged to subclade 4.3.1. The isolates of this subclade caused the typhoid fever cases in different years in all regions of the Russian Federation. According the epidemiological data in many cases the patients were infected travelling to India (the tourists and Indian students of Russian universities). Only single *S*. Typhi isolates belonged to subclades other then 4.3.1 and differed by full antimicrobial susceptibility or the mutations non common for Russian *S*. Typhi population.

Based on the results of this study, the Russian *S*. Typhi Reference Center Database was created.

As well as in other economic developed countries, the cases of the typhoid fever in the Russian Federation are mainly caused by resistant *S*. Typhi imported by the tourists, students or labor migrants from typhoid-endemic South-East Asia countries. In this situation the patients with fever and diarrhea who visited these countries within the incubation period (14–21 days) should be obligatory blood and faces examined for *S*. Typhi. Despite of the only sporadic typhoid fever cases registered annually in Russia, the antimicrobial resistance of *S*. Typhi is a serious problem in our country.

Список литературы/References

1. Accou-Demartin M., Gaborieau V., Song Y., Roumagnac P., Marchou B., Achtman M., Weill F.-X. Salmonella enterica Serotype Typhi with nonclassical quinolone resistance phenotype. *Emerg. Infect. Dis., 2011, vol. 17, no. 6, pp. 1091–1094. doi: 10.3201/eid1706.101242*
2. Achtman M., Wain J., Weill F.-X., Nair S., Zhou Z., Sangal V., Krauland M.G., Hale J.L., Harbottle H., Uesbeck A., Dougan G., Harrison L.H., Brisse S., S. Enterica MLST Study Group. Multilocus sequence typing as a replacement for serotyping in Salmonella enterica. *PLoS Pathogens, 2012, vol. 8, no. 6: e1002776. doi: 10.1371/journal.ppat.1002776*
3. Ahamed Riyaaz A.A., Perera V., Sivakumaran S., de Silva N. Typhoid fever due to extended spectrum β-lactamase-producing Salmonella enterica serovar Typhi: a case report and literature review. *Case Reports in Infect. Dis., 2018: 4610246. doi: 10.1155/2018/4610246*
4. Akinyemi K.O., Iwalokun B.A., Alafe O.O., Mudashiru S.A., Fakorede C. blaCTX-M-I group extended spectrum beta lactamase-producing Salmonella typhi from hospitalized patients in Lagos, Nigeria. *Infect. Drug. Resist., 2015, vol. 11, no. 8, pp. 99–106. doi: 10.2147/IDR.S78876*
5. Akinyemi K.O., Iwalokun B.A., Oyefolu A.O., Fakorede C.O. Occurrence of extended-spectrum and AmpC β-lactamases in multiple drug resistant Salmonella isolates from clinical samples in Lagos, Nigeria. *Infect. Drug. Resist., 2017, vol. 10, pp. 19–25. doi: 10.2147/IDR.S123646*
6. Al Naiemi N., Zwart B., Rijnsburger M.C., Roosendaal R., Debets Ossenkopp Y.J., Mulder J.A., Fijen C.A., Maten W., Vandenbroucke-Grauls C.M., Savelkoul P.H. Extended-spectrum-beta-lactamase production in a Salmonella enterica serotype Typhi strain from the Philippines. *J. Clin. Microbiol., 2008, vol. 46, pp. 2794–2795. doi: 10.1128/JCM.00676-08*
7. Baltazar M., Ngandjio A., Holt K.E., Lepillet E., Pardos de la Gandara M., Collard J.M., Bercion R., Nzouankeu, A., Le Hello S., Dougan G., Fonkoua M.C., Weill F.-X. Multidrug-resistant Salmonella enterica serotype Typhi, Gulf of Guinea Region, Africa. *Emerg. Infect. Dis., 2015, vol. 21, no. 4, pp. 655–659. doi: 10.3201/eid2104.141355*
8. CDC. National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS): Human Isolates Surveillance Report for 2015 (Final Report). Atlanta, Georgia: U.S. Department of Health and Human Services, CDC, 2018.
9. Chau T.T., Campbell J.I., Galindo C.M., Van Minh Hoang N., Diep T.S., Nga T.T., Van Vinh Chau N., Tuan P.Q., Page A.L., Ochiai R.L., Schultsz C., Wain J., Bhutta Z.A., Parry C.M., Bhattacharya S.K., Dutta S., Agtini M., Dong B., Honghui Y., Anh D.D., Canh do G., Naheed A., Albert M.J., Phetsouvanh R., Newton P.N., Basnyat B., Arjyal A., La T.T., Rang N.N., Phuong le T., Van Be Bay P., von Seidlein L., Dougan G., Clemens J.D., Vinh H., Hien T.T., Chinh N.T., Acosta C.J., Farrar J., Dolecek C. Antimicrobial drug resistance of Salmonella enterica serovar typhi in Asia and molecular mechanism of reduced susceptibility to the fluoroquinolones. *Antimicrob. Agents Chemother., 2007, vol. 51, no. 12, pp. 4315–4323. doi: 10.1128/AAC.00294-07*
10. Crump J.A., Luby S.P., Mintz E.D. The global burden of typhoid fever. *Bull. World Health Organ., 2004, vol. 82, pp. 346–353.*
11. Crump J.A., Kretsinger K., Gay K. Clinical response and outcome of infection with Salmonella enterica serotype Typhi with decreased susceptibility to fluoroquinolones: a United States FoodNet multicentre retrospective study. *Antimicrob. Agents Chemother., 2008, vol. 52, pp. 1278–1284. doi: 10.1128/AAC.01509-07*
12. Dolecek C., Tran T.P., Nguyen N.R., Le T.P., Ha V., Phung Q.T., Doan C.D., Nguyen T.B., Duong T.L., Luong B.H., Nguyen T.B., Nguyen T.A., Pham N.D., Mai N.L., Phan V.B., Vo A.H., Nguyen V.M., Tran T.T., Tran T.C., Schultsz C., Dunstan S.J., Stepniewska K., Campbell J.I., To S.D., Basnya, B., Nguyen V.V., Nguyen V.S., Nguyen T.C., Tran T.H., Farrar J. A multi-center randomised controlled trial of gatifloxacin versus azithromycin for the treatment of uncomplicated typhoid fever in children and adults in Vietnam. *PLoS One, 2008, vol. 3, no. 5: e2188. doi: 10.1371/journal.pone.0002188*
13. Effa E.E., Bukirwa H. Azithromycin for treating uncomplicated typhoid and paratyphoid fever (enteric fever). *Cochrane Database Syst. Rev., 2011, vol. 10: CD006083. doi: 10.1002/14651858.CD006083.pub3*
14. Effa E.E., Lassi Z.S., Critchley J.A., Garner P., Sinclair D., Olliaro P.L., Bhutta Z.A. Fluoroquinolones for treating typhoid and paratyphoid fever (enteric fever). *Cochrane Database Sys.t Rev., 2011, vol. 10: CD004530. doi: 10.1002/14651858.CD004530.pub4*
15. Geetha V.K., Yugendran T., Srinivasan R., Harish B.N. Plasmid-mediated quinolone resistance in typhoidal Salmonellae: a preliminary report from South India. *Indian J. Med. Microbiol., 2014, vol. 32: pp. 31–34. doi: 10.4103/0255-0857.124292*
16. Gokul B.N., Godfred A. Menezes G.A., Belgode N., Harish B.N. ACC-1 β-Lactamase– producing Salmonella enterica Serovar Typhi, India. *Emerg. Infect. Dis., 2010, vol. 16, no. 7, pp. 1170–1171. doi: 10.3201/eid1607.091643*
17. González-López J., Piedra-Carrasco N., Salvador F., Rodríguez V., Sánchez-Montalvá A., Planes A.M., Molina I., Larrosa M.N. ESBL-producing Salmonella enterica serovar Typhi in traveler returning from Guatemala to Spain. *Emerg. Infect. Dis., 2014, vol. 20, no. 11, pp. 1918–1920. doi: 10.3201/eid1607.091643*

18. Gul D., Potter R.F., Riaz H., Ashraf S.T., Wallace M.A., Munir T., Ali A., Burnham C.-A., Dantas G., Andleeb S. Draft genome sequence of a Salmonella enterica serovar Typhi strain resistant to fourth-generation cephalosporin and fluoroquinolone antibiotics. *Genome Announc., 2017, vol. 5, no. 42: e00850–17. doi: 10.1128/genomeA.00850-17*
19. Gupta R., Gaind R., Wain J., Deb M., Singh L.C., Basir S.F. Characterization of non-classical quinolone resistance in Salmonella enterica serovar Typhi: Report of a novel mutation in gyrB gene and diagnostic challenges. *Biomol. Detect. Quantif., 2015, vol. 2, pp. 30–34. doi: 10.1016/j.bdq.2015.01.003*
20. Hendriksen R.S., Leekitcharoenphon P., Mikoleit M., Jensen J.D., Kaas R.S., Roer L., Joshi H.B., Pornruangmong S., Pulsrikarn C., Gonzalez-Aviles G.D., Reuland E.A., Al Naiemi N., Wester A.L., Aarestrup F.M., Hasman H. Genomic dissection of travel-associated extended-spectrum-beta-lactamase-producing Salmonella enterica serovar typhi isolates originating from the Philippines: a one-off occurrence or a threat to effective treatment of typhoid fever? *J. Clin. Microbiol., 2015, vol. 53, no. 2, pp. 677–680. doi: 10.1128/JCM.03104-14*
21. Holt K.E., Parkhill J., Mazzoni C.J., Roumagnac P., Weill F.-X., Goodhead I., Rance R., Baker S., Maskell D.J., Wain J., Dolecek C., Achtman M., Dougan G. High-throughput sequencing provides insights into genome variation and evolution in Salmonella Typhi. *Nat. Genet., 2008, vol. 40, no. 8, pp. 987–993. doi: 10.1038/ng.195*
22. International Typhoid Consortium, Wong V.K., Holt K.E., Okoro C., Baker S., Pickard D.J., Marks F., Page A.J., Olanipekun G., Munir H., Alter R., Fey P.D., Feasey N.A., Weill F.-X., Le Hello S., Hart P.J., Kariuki S., Breiman R.F., Gordon M.A., Heyderman R.S., Jacobs J., Lunguya O., Msefula C., MacLennan C.A., Keddy K.H., Smith A.M., Onsare R.S., De Pinna E., Nair S., Amos B., Dougan G., Obaro S. Molecular surveillance identifies multiple transmissions of typhoid in West Africa. *PLoS Negl. Trop. Dis., 2016, vol. 10, no. 9: e0004781. doi: 10.1371/journal. pntd.0004781*
23. Keddy K.H, Smith A.M, Sooka A., Ismail H., Oliver S. Fluoroquinolone-resistant typhoid, South Africa. *Emerg. Infect. Dis., 2010, vol. 16, pp. 879–880. doi: 10.3201/eid1605.091917*
24. Kidgell C., Reichard U., Wain J., Linz B., Torpdahl M., Dougan G. Salmonella Typhi, the causative agent of typhoid fever, is approximately 50,000 years old. *Infect. Genet. Evol., 2002, vol. 2, no. 1, pp. 39–45. doi: 10.1016/S1567-1348(02)00089-8*
25. Kothari A., Pruthi A., Chugh T.D. The burden of enteric fever. *J. Infect. Dev. Ctries., 2008, vol. 2, no. 4, pp. 253–259. doi: 10.3855/jidc.218*
26. Kuijpers L., Phe T., Veng C.H., Lim K., Ieng S., Kham C., Fawal N., Fabre L., Le Hello S., Vlieghe E., Weill F.-X., Jacobs J., Peetermans W.E. The clinical and microbiological characteristics of enteric fever in Cambodia, 2008–2015. *PLoS Negl. Trop. Dis., 2017, vol. 11, no. 9: e0005964. doi: 10.1371/journal. Pntd.0005964*
27. Kuleshov K.V., Kostikova A., Pisarenko S.V., Kovalev D.A., Tikhonov S.N., Savelieva I.V., Saveliev V.N., Vasilieva O.V., Zinich L.S., Pidchenko N.N., Kulichenko A.N., Shipulin G.A. Comparative genomic analysis of two isolates of Vibrio cholerae O1 Ogawa El Tor isolated during outbreak in Mariupol in 2011. *Infect. Genet. Evol., 2016, vol. 44, pp. 471–478. doi: 10.1016/j.meegid.2016.07.039*
28. Matono T., Morita M., Yahara K., Lee K., Izumiya H., Kaku M., Ohnishi M. Emergence of resistance mutations in Salmonella enterica serovar Typhi against fluoroquinolones. *Open Forum Infect. Dis., 2017, vol. 4, no. 4: ofx230. doi: 10.1093/ofid/ofx230*
29. Mirza S., Beeching N., Hart C. Multi-drug resistant typhoid: a global problem. *J. Med. Microbial., 1996, vol. 44, pp. 317–319. doi: 10.1099/00222615-44-5-317*
30. Nüesch-Inderbinen M., Abgottspon H., Sägesser G., Cernela N., Stephan R. Antimicrobial susceptibility of travel-related Salmonella enterica serovar Typhi isolates detected in Switzerland (2002–2013) and molecular characterization of quinolone resistant isolates. *BMC Infect. Dis., 2015, vol. 15: 212. doi: 10.1186/s12879-015-0948-2*
31. Okanda T., Haque A., Ehara T., Huda Q., Ohkusu K., Miah R.A., Matsumoto T. Characteristics of resistance mechanisms and molecular epidemiology of fluoroquinolone-nonsusceptible Salmonella enterica serovar Typhi and Paratyphi A isolates from a Tertiary Hospital in Dhaka, Bangladesh. *Microb. Drug. Resist., 2018, vol. 24, no. 10. doi: 10.1089/mdr.2018.0039*
32. Park C.H., Robicsek A., Jacoby G.A., Sahm D., Hooper D.C. Prevalence in the United States of aac(6')-Ib-cr encoding a ciprofloxacin-modifying enzyme. *Antimicrob. Agents Chemother., 2006, vol 50, no. 11, pp. 3953–3955. doi: 10.1128/AAC.00915-06*
33. Parry C.M., Thieu N.T.V., Dolecek C., Karkey A., Gupta R., Turner P., Dance D., Maude R.R., Ha V., Tran C.N., Thi P.L., Be B.P.V., Phi L.T.T., Ngoc R.N., Ghose A., Dongol S., Campbell J.I., Thanh D.P., Thanh T.H., Moore C.E., Sona S., Gaind R., Deb M., Anh H.V., Van S.N., Tinh H.T., Day N.P., Dondorp A., Thwaites G., Faiz M.A., Phetsouvanh R., Newton P., Basnyat B., Farrar J.J., Baker S. Clinically and microbiologically derived azithromycin susceptibility breakpoints for Salmonella enterica serovars Typhi and Paratyphi A. *Antimicrob. Agents Chemother., 2015, vol. 59, no. 5, pp. 2756–2764. doi: 10.1128/AAC.04729-14*
34. Pfeifer Y., Matten J., Rabsch W. Salmonella enterica serovar Typhi with CTX-M β-lactamase, Germany. *Emerg. Infect. Dis., 2009, vol. 15, pp. 1533–1535. doi: 10.3201/eid1509.090567*
35. Phoba M.F., Barbé B., Lunguya O., Masendu L., Lulengwa D., Dougan G., Wong V.K., Bertrand S., Ceyssens P.J., Jacobs J., Van Puyvelde S., Deborggraeve S. Salmonella enterica serovar Typhi producing CTX-M-15 extended spectrum β-lactamase in the Democratic Republic of the Congo. *Clin. Infect. Dis., 2017, vol. 65, no. 7, pp. 1229–1231. doi: 10.1093/cid/cix342*
36. Pokharel B.M., Koirala J., Dahal R.K., Mishra S.K., Khadga P.K., Tuladhar N.R. Multidrug-resistant and extended-spectrum beta-lactamase (ESBL)-producing Salmonella enterica (serotypes Typhi and Paratyphi A) from blood isolates in Nepal: surveillance of resistance and a search for newer alternatives. *Int. J. Infect. Dis., 2006, vol. 10, no. 6, pp. 434–438. doi: 10.1016/j.ijid.2006.07.001*
37. Ramachandran A., Shanthi M., Sekar U. Detection of blaCTX-M extended spectrum beta-lactamase producing Salmonella enterica serotype Typhi in a tertiary care centre. *J. Clin. Diagn. Res., 2017, vol. 11, no. 9: DC21-DC24. doi: 10.7860/JCDR/2017/30150.10637*
38. Robicsek A., Strahilevitz J., Sahm D.F., Jacoby G.A., Hooper D.C. Qnr prevalence in ceftazidime-resistant Enterobacteriacae isolates from the United States. *Antim. Agents Chemot., 2006, vol. 50, no. 8, pp. 2872–2874. doi: 10.1128/AAC.01647-05*
39. Rodrigues C., Kapil A., Sharma A., Devanga Ragupathi N.K., Inbanathan F.Y., Veeraraghavan B., Kang G. Whole genome shotgun sequencing of cephalosporin-resistant Salmonella enterica serovar Typhi. *Genome Announc., 2017, vol. 5: e01639-16. doi: 10.1128/genomeA.01639-16*
40. Rotimi V., Jamal W., Pal T., Sovenned A., John Albert M. Emergence of CTX-M-15 type extended-spectrum b-lactamase-producing Salmonella spp. in Kuwait and the United Arab Emirates. *J. Med. Microbiol., 2008, vol. 57, pp. 881–886. doi: 10.1099/jmm.0.47509-0*

41. Roumagnac P., Weill F.-X., Dolecek C., Baker S., Brisse S., Chinh N.T., Le T.A., Acosta C.J., Farrar J., Dougan G., Achtman M. Evolutionary history of Salmonella Typhi. *Science, 2006, vol. 314, no. 5803, pp. 1301–1304. doi: 10.1126/science.1134933*
42. Song Y., Roumagnac P., Weill F.-X., Wain J., Dolecek C., Mazzoni C.J., Holt K.E., Achtman M. A multiplex single nucleotide polymorphism typing assay for detecting mutations that result in decreased fluoroquinolone susceptibility in Salmonella enterica serovars Typhi and Paratyphi A. *J. Antimicrob. Chemoth., 2010, vol. 65, no. 8, pp. 1631–1641. doi: 10.1093/jac/dkq175*
43. Tadesse G., Tessema T.S., Beyene G., Aseffa A. Molecular epidemiology of fluoroquinolone resistant Salmonella in Africa: a systematic review and meta-analysis. *PLoS One, 2018, vol. 13, no. 2: e0192575. doi: 10.1371/journal. pone.0192575*
44. Thompson C.N., Karkey A., Dongol S., Arjyal A., Wolbers M., Darton T., Farrar J.J., Thwaites G.E., Dolecek C., Basnyat B., Baker S. Treatment response in enteric fever in an era of increasing antimicrobial resistance: an individual patient data analysis of 2092 participants enrolled into 4 randomized, controlled trials in Nepal. *Clin. Infect. Dis., 2017, vol. 64, no. 11, pp. 1522–1531. doi: 10.1093/cid/cix185*
45. Weill F.-X. La fievre typhoide n'est plus aussi simple a soigner. *Med. Sci., 2010, vol. 26, pp. 969–975. doi: 10.1051/medsci/20102611969*
46. Wong V.K., Baker S., Pickard, D.J., Parkhill J., Page A.J., Feasey N.A., Kingsley R.A., Thomson, N.R., Keane J.A., Weill F.-X., Edwards D.J., Hawkey J., Harris S.R., Mather A.E., Cain A.K., Hadfield J., Hart P.J., Thieu N.T., Klemm E.J., Glinos D.A., Breiman R.F., Watson C.H., Kariuki S., Gordon M.A., Heyderman R.S., Okoro C., Jacobs J., Lunguya O., Edmunds W.J., Msefula C., Chabalgoity J.A., Kama M., Jenkins K., Dutta S., Marks F., Campos J., Thompson C., Obaro S., MacLennan C.A., Dolecek C., Keddy K.H., Smith A.M., Parry C.M., Karkey A., Mulholland E.K., Campbell J.I., Dongol S., Basnyat B., Dufour M., Bandaranayake D., Naseri T.T., Singh S.P., Hatta M., Newton P., Onsare, R.S., Isaia L., Dance D., Davong V., Thwaites G., Wijedoru L., Crump J.A., De Pinna E., Nair S., Nilles E.J., Thanh D.P., Turner P., Soeng S., Valcanis M., Powling J., Dimovski K., Hogg G., Farrar J., Holt K.E., Dougan G. Phylogeographical analysis of the dominant multidrug-resistant H58 clade of Salmonella Typhi identifies inter-and intracontinental transmission events. *Nat. Genet., 2015, vol. 47, no. 6, pp. 632–641. doi: 10.1038/ng.3281*
47. Wong V.K., Baker S., Connor T.R., Pickard D., Page A.J., Dave J., Murphy N., Holliman R., Sefton A., Millar M., Dyson Z.A., Dougan G., Hol K.E., International Typhoid Consortium. An extended genotyping framework for Salmonella enterica serovar Typhi, the cause of human typhoid. *Nat. Commun., 2016, vol. 7: 12827. doi: 10.1038/ncomms12827*
48. World Health Organization (WHO). The diagnosis, treatment and prevention of typhoid fever. WHO/V&B/03.07. *Geneva: WHO; 2003*

Авторы:

Егорова С.А., к.м.н., старший научный сотрудник лаборатории кишечных инфекций ФБУН НИИ эпидемиологии и микробиологии имени Пастера, Санкт-Петербург, Россия;
Кулешов К.В., к.б.н., старший научный сотрудник лаборатории молекулярной диагностики и эпидемиологии кишечных инфекций ФБУН ЦНИИ эпидемиологии, Москва, Россия;
Кафтырева Л.А., д.м.н., зав. лабораторией кишечных инфекций ФБУН НИИ эпидемиологии и микробиологии имени Пастера, Санкт-Петербург, Россия; профессор кафедры медицинской микробиологии ФГБОУ ВО Северо-Западный государственный медицинский университет им. И.И. Мечникова МЗ РФ, Санкт-Петербург, Россия;
Матвеева З.Н., к.м.н., ведущий научный сотрудник лаборатории кишечных инфекций ФБУН НИИ эпидемиологии и микробиологии имени Пастера, Санкт-Петербург, Россия.

Authors:

Egorova S.A., PhD (Medicine), Senior Researcher, Laboratory of Enteric Infections, St. Petersburg Pasteur Institute, St. Petersburg, Russian Federation;
Kuleshov K.V., PhD (Biology), Senior Researcher, Laboratory of Enteric Diseases Epidemiology and Molecular Diagnostic, Central Research Institute for Epidemiology, Moscow, Russian Federation;
Kaftyreva L.A., PhD, MD (Medicine), Professor, Head of the Laboratory of Enteric Infections, St. Petersburg Pasteur Institute, St. Petersburg, Russian Federation;
Matveeva Z.N., PhD (Medicine), Leading Researcher, Laboratory of Enteric Infections, St. Petersburg Pasteur Institute, St. Petersburg, Russian Federation.

Поступила в редакцию 26.03.2019
Отправлена на доработку 11.04.2019
Принята к печати 11.03.2020

Received 26.03.2019
Revision received 11.04.2019
Accepted 11.03.2020