

PREVALENCE AND ANTIBIOTICS SUSCEPTIBILITY OF THERMOTOLERANT *CAMPYLOBACTER* spp. ISOLATED FROM HUMANS AND CHICKENS IN THE REPUBLIC OF GUINEA



R. Balde^a, Z.N. Matveeva^b, L.A. Kaftyreva^{b,c}, M.A. Makarova^{b,c}

^a Research Institute of Applied Biology of Guinea, Kindia, Republic of Guinea

^b St. Petersburg Pasteur Institute, St. Petersburg, Russian Federation

^c I.I. Mechnikov North-Western State Medical University, St. Petersburg, Russian Federation

Abstract. *Background.* The issue of diarrheal diseases remains relevant for modern health care in all countries. Campylobacteriosis is the most common infectious disease with foodborne transmission and poultry meat is a transmission factor. *Materials and methods.* 724 items of faeces sampled from patients with diarrheal syndrome and 283 samples of faeces of chickens raised on private farms and five poultry farms in the province were studied. For bacteriological method were used selective media. Traditional routine tests (cell morphology, cytochrome oxidase, catalase, hydrolysis of sodium hippurate and indoxyl acetate) and MALDI-ToF mass spectrometry was performed for identification. The susceptibility of strains to antibiotics was analysed using the disc-diffusion method. Results were interpreted according to the EUCAST criteria, versions 2019–2022. *Results.* *Campylobacter* spp. was cultured in 65 out of 724 faecal samples from patients with acute diarrhoea, of them 83.08% were identified as *C. jejuni*, and 16.92% as *C. coli*. Of the 237 *Campylobacter* strains from chicken were identified as *C. jejuni* (54.0%), as *C. coli* (46.0%). *Campylobacter* spp. strains from humans were resistant to tetracycline (40.0%), to erythromycin (6.15%), to ciprofloxacin (12.31%). The strains from chickens kept on farms, were resistant to tetracycline in 42.55%, to ciprofloxacin — in 22.70% and to erythromycin — in 11.35%. The strains from chickens kept on private farms were resistant to tetracycline in 4.17%, to ciprofloxacin — in 1.04%, all strains were sensitive to erythromycin. *Conclusion.* Thus, due to the widespread prevalence of *Campylobacter* spp., infectious diseases they cause remain a topical issue. Studying the resistance to antibiotics in *Campylobacter* spp. among poultry could allow to develop new approaches to confirming the significance of their foodborne nature and to improve the national disease prevention system.

Key words: *Campylobacter* spp., *Campylobacter* infection, Africa, foodborne infections, poultry farms, antibiotic resistance.

РАСПРОСТРАНЕННОСТЬ И ЧУВСТВИТЕЛЬНОСТЬ К АНТИБИОТИКАМ ТЕРМОТОЛЕРАНТНЫХ *CAMPYLOBACTER* spp., ВЫДЕЛЕННЫХ ОТ ЛЮДЕЙ И ПТИЦ В РЕСПУБЛИКЕ ГВИНЕЯ

Балдэ Р.¹, Матвеева З.Н.², Кафтырева Л.А.^{2,3}, Макарова М.А.^{2,3}

¹ Институт прикладной биологии, г. Киндия, Гвинейская Республика

² ФБУН НИИ эпидемиологии и микробиологии имени Пастера, Санкт-Петербург, Россия

³ ФБГБОУ ВО Северо-Западный государственный медицинский университет имени И.И. Мечникова, Санкт-Петербург, Россия

Резюме. *Введение.* Проблема диарейных заболеваний остается актуальной для современного здравоохранения всех стран. Кампилобактериоз является наиболее распространенным инфекционным заболеванием,

Адрес для переписки:

Макарова Мария Александровна
197101, Россия, Санкт-Петербург, ул. Мира, 14,
ФБУН НИИ эпидемиологии и микробиологии имени Пастера.
Тел.: 8 921 631-31-83. E-mail: makmaria@mail.ru

Contacts:

Maria A. Makarova
197101, Russian Federation, St. Petersburg, Mira str., 14,
St. Petersburg Pasteur Institute.
Phone: +7 921 631-31-83. E-mail: makmaria@mail.ru

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передающимся через пищевые продукты, а мясо птицы — общепризнанный фактор передачи. Цель исследования: оценка распространенности термотолерантных *Campylobacter* spp. в Гвинейской Республике у пациентов с диарейным синдромом и кур при различных типах содержания. *Материалы и методы.* Изучено 724 пробы испражнений пациентов с диарейным синдромом и 283 пробы помета кур, содержащихся в личных хозяйствах и птицефермах. Для бактериологического метода использовали селективные питательные среды. Идентификацию штаммов *Campylobacter* spp. проводили традиционными рутинными тестами (морфология клеток, оксидазный и каталазный тесты, гидролиз гиппурата натрия и индоксилатацетата) и методом MALDI-ToF масс-спектрометрией. Чувствительность штаммов к антибиотикам определяли диско-диффузионным методом. Результаты интерпретировали в соответствии с критериями EUCAST версий 2019–2022 гг. *Результаты.* *Campylobacter* spp. был выявлен в 65 из 724 пробах испражнений пациентов с острой диареей, из них 83,08% были идентифицированы как *C. jejuni* и 16,92% — как *C. coli*. Из 237 штаммов *Campylobacter* spp., выделенных из помета кур были идентифицированы *C. jejuni* 54,0% и *C. coli* 46,0%. Кампилобактерии, выделенные от людей были устойчивы к тетрациклину 40,0%, к эритромицину 6,15%, к ципрофлоксацину 12,31%. Штаммы, выделенные от промышленной птицы, были устойчивы к тетрациклину 42,55%, к ципрофлоксацину 22,70% и эритромицину 11,35%. Штаммы, выделенные от домашних кур, характеризовались резистентностью к тетрациклину — 4,17%, к ципрофлоксацину — 1,04%, все штаммы были чувствительны к эритромицину. *Выводы.* В связи с широкой распространенностью *Campylobacter* spp., вызываемые ими инфекционные заболевания остаются актуальной проблемой. Изучение устойчивости к антибиотикам *Campylobacter* spp. среди домашней птицы может позволить разработать новые подходы к подтверждению значимости их как фактора передачи и усовершенствовать национальную систему профилактики кампилобактериоза.

Ключевые слова: *Campylobacter*, кампилобактериоз, Африка, инфекции, передающиеся с пищевыми продуктами, птицефабрики, устойчивость к антибиотикам.

Introduction

The issue of diarrheal diseases remains relevant for modern health care in all countries. This is due to the wide range of diverse pathogens that cause diarrheal diseases, their wide distribution, as well as significant socio-economic impact. According to the World Health Organization (WHO) Expert Committee, they occupy the fourth place on the “importance scale” of the Global Burden of Disease and are included in the list of emergent foodborne infections affecting over 500 million people every year, of which 220 million are children under 5 years old [13].

Campylobacter is among the main causes of gastroenteritis worldwide and has increased in both developed and developing countries over the last 10 years. It accounts for 8.5% of the total number of diarrheal diseases reported [8, 12, 14].

The genus *Campylobacter* was first reported in 1886 by Theodor Escherich who discovered these microorganisms in a deceased child during an outbreak of “children’s cholera” and described them as uncultivated spiral-shaped bacteria. At the beginning of the 20th century, in humans learned of a widespread *Campylobacter* distribution among animals and their significance in reproductive system pathologies. In 1906, veterinarians McFadyean and Stockman found *Campylobacter* in smears from the uterine mucosa of a pregnant sheep as “a large number of unusual microorganisms”; in 1913, similar microorganisms were sampled from an aborted cow foetus and thus named *Vibrio fetus*. In 1927, Smith and Orcutt named a group of bacteria sampled from cattle faeces in diarrhoea *Vibrio jejuni*. Seventeen years later, in 1944, Doyle sampled bacteria from the faeces of pigs with diarrhoea

that differed in biochemical properties from previously isolated *Vibrio jejuni* and classified them as *Vibrio coli*. *Campylobacter* (*V. fetus*) were first sampled from human blood in 1947 [8]. Initially, all the above bacteria were assigned to the genus *Vibrio* and, despite having significant differences in biological properties from the “true” *Vibrio* spp., they were classified as an independent genus *Campylobacter* only in 1963. In 1969, Dekeyser first sampled *Campylobacter* from the faeces of patients with diarrhoea by direct membrane filtration on a selective agar medium. The development and increased use of selective media for the sampling of *Campylobacter* in the late 1970s and early 1980s led, on the one hand, to the recognition of the significance of said microorganisms as those causing acute intestinal conditions in humans, and, on the other hand, to the improvement of laboratory diagnostic methods and discovery of new species [5].

As of December 2022, the genus *Campylobacter* includes 43 species, and almost half of them may cause various human diseases, including gastroenteritis. In countries with developed laboratory diagnostics of campylobacteriosis, thermotolerant *Campylobacter* species *C. jejuni* and *C. coli* are considered the most significant causative agents of gastroenteritis. Other species of this *Campylobacter* group, *C. lary*, *C. concisus*, *C. ureolyticus* and *C. upsaliensis*, may cause diarrhoea too, but less often [8, 17].

Campylobacter infection is characterised by its impact on the gastrointestinal tract. It may manifest as enteritis, enterocolitis, colitis or gastroenterocolitis and result in serious gastrointestinal or extraintestinal complications [5, 27]. Immunocompromised humans (patients with AIDS, cancer, etc.), as well as infants are most vulnerable to complications. An acute infec-

tion can have serious long-term consequence, including the peripheral neuropathies, Guillain–Barré syndrome (GBS) and Miller–Fisher syndrome (MFS), and functional bowel diseases, such as irritable bowel syndrome (IBS). GBS occurs in one in 1000 cases in people who have had campylobacteriosis. Older males get sick more often than females [3].

Campylobacteriosis is diagnosed based on the results of faeces examination using laboratory diagnostic methods, that is, bacteriology, molecular and immunology tests aimed at identifying the pathogen or its antigens and genetic markers [2, 21]. In countries that established observation practices for foodborne infections, it was found that *C. jejuni* is the main cause of foodborne outbreaks and one of the most important zoonotic pathogens capable of causing human diseases [4, 13, 20].

Epidemiological features of campylobacteriosis are studied in detail in most industrialised countries, as they record large outbreaks with foodborne transmission type. In the European Union, including the European Economic Area (EU/EEA), 30 countries reported 129 960 confirmed cases of campylobacteriosis in 2021. The overall recording rate was 44.5 per 100 000 population [16]. Despite the decrease in the incidence of *Campylobacter* infection over the past 3 years in a range of countries in North, Central and South America, thermotolerant *Campylobacter* spp. are the leading causative agents of bacterial diarrhoea in Europe, as well as in Australia and New Zealand. The number of confirmed cases in the European Union in 2020 reached 121 000 cases, whereas the incidence was 40.4‰ [1, 26].

Epidemiological data from a number of countries of Africa, Asia and the Middle East is incomplete; however, it shows that *Campylobacter* infection is relevant for these regions as well [23]. The results of 10-year studies (1997–2007) conducted using the molecular method based on RT-PCR in Blantyre (Malawi, Africa) showed that *Campylobacter* are often causative agents of diarrheal diseases in children; *C. jejuni* and *C. coli* were detected in every fifth child hospitalised with diarrhoea and in 14% of the cases where examinations found no signs of an acute intestinal infection, while *C. jejuni* accounted for up to 85% of all cases of campylobacteriosis [18]. These results are confirmed by another study conducted in Moramanga (Madagascar), in which the proportion of *Campylobacter* spp. was 8.9% in faecal samples of children with diarrheal syndrome, and 9.4% in children without diarrhoea [22]. From 2005 to 2009, 5443 strains of *Campylobacter* spp. were sampled from the faeces of children with diarrhoea at the Red Cross Children’s Hospital in Cape Town (South Africa), of which 40% were *C. jejuni*; the second most common species were *C. concisus* (24.6%) [23]. In general, it can be concluded that *C. jejuni* and other species of the genus *Campylobacter* are significant for children in most regions of Africa.

Reducing disease risks and preventing campylobacteriosis in the population are primarily associated with the idea of reservoirs/factors of transmission of infectious agents [6]. The most important reservoir/factor of transmission of *C. jejuni* and *C. coli* pathogens for humans is industrial poultry: chickens, turkeys, ducks, geese, etc., among which the leading place is occupied by broiler chickens raised on poultry farms [9]. Numerous epidemiological studies have shown that *Campylobacter* infection caused by chicken meat consumption is more often recorded in urban residents than in rural residents [26]. However, there is evidence that other types of *Campylobacter* are often sampled from chickens in various regions. This is due to the high level of *Campylobacter* spp. among broiler chickens. On poultry farms *Campylobacter* are found in the environment including soil, water sources, dust, building surfaces and air [11]. International trade in broiler chickens, industrial poultry products and feed contributes to the overall burden of *Campylobacter* infection. In Switzerland 71% of campylobacteriosis cases were caused by poultry products [25, 26]. Given that *C. jejuni* strains survive in chicken faeces up to six days after isolation, they can be a potential source of environmental pollution, and the use of poultry manure as fertiliser is a factor in human infection. According to the Food Standards Agency in the UK, 72.9% of chicken carcasses were contaminated with *Campylobacter* spp. between 2014 and 2015, with 18.9% of them characterised by significant contamination (> 10 000 CFU/g) [16, 19].

Considering the above, the purpose of this study was to assess the prevalence of thermotolerant *Campylobacter* in the Republic of Guinea among patients of various ages with diarrheal syndrome and chickens with various types of livestock management.

Materials and methods

The study was conducted in the period from 2019 to 2022 in the province of Kindia (Republic of Guinea), in a laboratory of Guinea-Russian Research Centre of Epidemiology and Prevention of Infectious Diseases (Kindia, Republic of Guinea).

724 items of faeces sampled from patients with diarrheal syndrome were studied, among them 73 from children aged 0 to 5, 127 from children aged 6 to 17, and 524 from humans aged 18 and older, as well as 283 samples of faeces of chickens raised on private farms and five poultry farms in the province. The samples were delivered to the laboratory in a Cary–Blair Transport Medium in a refrigerated container in 4–8 hours.

For bacteriological method, the following media (Oxoid, UK) were used: 1. *Campylobacter* Blood-Free Selective Agar Base and CCDA Selective Supplement; 2. Selective medium carbon agar and a Selective Supplement (cefoperazone and teicoplanin); 3. Blood agar Muller–Hinton Agar, with 5% Defibrinated Horse Blood (E&O Laboratories

limited) and culture growth supplement to increase *Campylobacter* aerotolerance. Inoculation on the blood agar was performed using cellulose acetate filters (Sartorius Stedim Biotech) with a pore diameter of 0.45 μm . The cultures were incubated in a micro-aerobic atmosphere at 42°C for 48 hours.

Traditional routine tests based on the determination of key phenotypic features were used for primary identification: cell morphology and Gram staining, production of cytochrome oxidase and catalase, hydrolysis of sodium hippurate and indoxyl acetate. The second identification level was performed using MALDI-ToF mass spectrometry (Bruker Daltonik MALDI Biotyper).

The susceptibility of thermotolerant *Campylobacter* strains to antimicrobial agents was determined by disc-diffusion method using Muller-Hinton Agar (Oxoid), 5% Defibrinated Horse Blood (E&O Laboratories limited) and 20 mg/l of β -NAD. Results were interpreted according EUCAST criteria, versions 2019–2022 (https://www.eucast.org/ast_of_bacteria/previous_versions_of_documents).

In parallel with the culture method, faeces samples from patients with diarrheal syndrome was examined by PCR method with fluorescence in situ hybridization using the Russian reagent kit AmpliSense® OKI screen-FL to identify and differentiate the DNA of *Campylobacter* microorganisms (thermophilic *Campylobacter* spp.).

Statistical processing of results. The obtained data were processed using the computer program Excel (Microsoft Office). Fisher's exact test was used to assess the statistical significance of differences in indicators (frequency, proportion). Differences were considered statistically significant at a 95% confidence interval ($p < 0.05$).

Results

Thermotolerant *Campylobacter* spp. was cultured in 65 out of 724 faecal samples from patients with acute diarrhoea (8.98%). In children under 5 years old, they were found three times more often than in adults (20.55% vs 7.06%, respectively), $p \leq 0.05$ (Table 1). Molecular markers of thermotolerant *Campylobacter* were detected in 72 samples (9.94%).

Thermotolerant *Campylobacter* were found in 237 out of 283 (83.75%) samples of chicken intestinal contents, regardless of the livestock management type (personal

farming or poultry farms). In chickens raised free-range on personal farming, *Campylobacter* spp. was found in 96 out of 132 samples studied (72.73%). In poultry farm broilers, thermotolerant *Campylobacter* was detected in 141 out of 151 samples, which was 93.38%. The use of membrane filters and non-selective media made it possible to identify three strains of closely related microorganisms (*Arcobacter cryaerophilus*) in the samples studied, which will not be discussed in this paper since they are not pathogenic to humans.

Of the 237 *Campylobacter* strains, 128 were identified as *C. jejuni* and 109 as *C. coli*, representing 54.0% and 46.0%, respectively. Identification using classical tests of six strains of *C. jejuni* showed questionable results after the hippurate hydrolysis test. The use of MALDI-ToF mass spectrometry and PCR with species-specific primers allowed for the correct culture identification.

To assess the prevalence of resistance strains of *Campylobacter* spp., were conducted a screening of sampled cultures for clinically significant drugs. Were studied 302 strains of thermotolerant *Campylobacter* spp. sampled from humans (65 strains), as well as from chicken intestinal contents (237 strains) of chickens kept in different livestock management types: 96 strains from personal farming and 141 strains from five poultry farms (Table 3). 212 strains (70.20%) of *Campylobacter* spp. were susceptible to all antibiotics whereas 90 (29.80%) were resistant to one or several agents.

When it comes to the general population of strains, *Campylobacter* spp. strains sampled from humans were resistant to tetracycline (40.0%), $p \leq 0.05$, significantly more often. The proportion of strains resistant to erythromycin and ciprofloxacin was 6.15% and 12.31%, respectively. At the same time, there were no significant differences in the levels of resistance to these drugs ($p \geq 0.05$).

Among the strains sampled from the intestinal contents of chickens kept on poultry farms, strains resistant to tetracycline were significantly more common as they accounted for (42.55%), $p \leq 0.05$. As for fluoroquinolones which had previously been widely used in veterinary medicine (enrofloxacin), 22.70% of strains were resistant; 11.35% were resistant to erythromycin. No significant differences were identified.

The proportion of strains sampled from the faeces of chickens kept on private farms resistant to tetracycline was 4.17%, whereas the proportion of strains resistant to ciprofloxacin amounted to 1.04%. At the same time, all strains remained susceptible to erythromycin.

The analysis of combined resistance showed that 18.46% of strains sampled from humans were characterized by resistance to two antibiotics: 8 to tetracycline and ciprofloxacin, 4 to erythromycin and tetracycline. Strains from livestock kept on poultry farms with phenotypes of combined resistance were sampled almost twice as often (1.84).

Table 1. Frequency of sampling *Campylobacter* spp. in patients of various ages

Age	Total samples	Frequency of findings, n (%)	95% CI
0–5	73	15 (20.55%)	12.87–31.18
6–17	127	13 (10.23%)	6.08–16.73
18 and older	524	37 (7.06%)	5.17–9.58
Total	724	65 (8.98%)	7.11–11.28

Table 2. Frequency of findings for *C. jejuni* and *C. coli* sampled from humans and intestinal contents of chickens kept on personal farming and poultry farms

Type of <i>Campylobacter</i>	Humans n (%)	95% CI	Chickens personal farming n (%)	95% CI	Chickens poultry farms n (%)	95% CI
<i>C. jejuni</i>	54 (7.46%)	5.76–9.61	46 (34.85%)	27.25–43.30	82 (54.31%)	46.35–62.04
<i>C. coli</i>	11 (1.52%)	0.85–2.70	50 (37.88%)	30.06–46.39	59 (39.07%)	31.65–47.03
Not found	659 (90.61%)	88.72–92.89	36 (27.27%)	20.40–35.43	10 (6.62%)	3.63–11.76
Total	724 (100%)	99.47–100	132 (100%)	67.79–82.27	151 (100%)	58.37–73.29

Table 3. Antimicrobial resistance of *Campylobacter* spp. strains sampled in Kindia, Republic of Guinea, 2019–2022

Antibiotic	Humans (n = 65) n (%)	Chickens personal farming (n = 96) n (%)	Chickens poultry farms (n = 141) n (%)	Total (n = 302) n (%)
Tetracycline	26 (40.00%)	4 (4.17%)	60 (42.55%)	90 (29.80%)
Erythromycin	4 (6.15%)	0 (0%)	16 (11.35%)	20 (6.62%)
Ciprofloxacin	8 (12.31%)	1 (1.04%)	32 (22.70%)	41 (13.58%)

Discussion

Bacteria of the genus *Campylobacter* are among the leading causative agents of acute intestinal infections of bacterial etiology in residents of developed countries, exceeding in some regions the frequency of registration of salmonellosis and escherichiosis. In a third of cases, they are the cause of “travelers’ diarrhea” among residents of economically developed countries visiting regions with a high degree of circulation of *Campylobacter* spp. among the population, animals and environmental objects [15]. According to the latest estimates of the World Health Organization, campylobacteriosis is one of the most common infectious diseases with foodborne transmission. Campylobacteriosis is registered in all age groups, most often among children aged from one year to 3–5 years; a relative increase in cases of disease is observed in older children and young people (compared to other age categories) [4, 11, 18, 23].

Our studies showed that the campylobacteriosis accounted for 8.98% in the etiological structure of diarrheal diseases in individuals residing in the Republic of Guinea in 2019–2022. Analysis of the age structure confirmed that thermotolerant *Campylobacter* are common pathogens among the child population: *C. jejuni* and *C. coli* were detected in one in five children under 5 ages. *C. jejuni* (83.08%) were significantly predominant in the *Campylobacter* infection structure compared to *C. coli* (16.92%), $p \leq 0.05$.

The incidence of *Campylobacter* colienteritis, as well as the frequency of detection of thermotolerant campylobacters in chickens in different countries varies very widely. Thus, in the countries of the European Union, where monitoring has been carried out for many years, the incidence is at the level of 61.4–66.5‰, varying from < 5.8‰ in Bulgaria, Latvia, Portugal to 230.0‰ in the Czech Republic. Poultry meat is a transmission factor in campylobacteriosis. The frequency of detecting *Campylobacter* spp.

in chickens in different countries varies in wide ranges [3, 16, 19, 25]. As our studies have shown, the level of *Campylobacter* spp. among chickens was high (82.57%) and ranged from 70.58% in chickens kept free-range on personal farming to 93.37% in broilers kept on poultry farms. There were no significant differences in the species structure: *C. jejuni* and *C. coli* were distinguished with almost the same frequency of 54.0% and 46.0% ($p \geq 0.05$). If we talk about the frequency of detection of *Campylobacter* in chickens, our data are consistent with the results of other authors [9, 11, 14], however, sometimes comparison is difficult due to differences in methodological approaches to research. In our work, we assessed the distribution of thermotolerant campylobacters in the chicken population, while most modern studies deal with the frequency and intensity of contamination of chicken meat, i.e. product prepared for shipment to the consumer [19].

In clinical practice, for the treatment of moderate and severe forms of campylobacteriosis, the prescription of broad-spectrum antibiotics is regulated, among which the drugs of choice are macrolides, and mainly azithromycin. Along with antibiotics of this group, aminoglycosides, quinolones, tetracyclines, chloramphenicol, nitrofurans and carbapenems are recognized as alternative and effective therapeutic drugs. Fluoroquinolones, previously widely used for the treatment of campylobacteriosis, contributed to the development of resistance to this group of antibiotics in 50–84% of circulating strains of *Campylobacter* spp., which made them unsuitable for therapeutic purposes. In recent years, the clinical ineffectiveness of ongoing antibacterial therapy has been accompanied by the emergence of a large number of resistant strains. A feature of the formation of resistance in *Campylobacter* is not only the rapid onset of the effect of insensitivity of strains to the action of antibiotics, but also the multiple nature of this phenomenon. In countries where surveillance of campylobacteriosis pathogens has been carried out in recent years, it has been noted that the population of *Campylobacter* spp.

is dominated by strains characterized by multidrug resistance [12, 24, 26]. In 2017, WHO published a list of 12 bacterial priority pathogens that pose the greatest threat to human health. *Campylobacter* spp. due to the need for the creation of new AMPs, those resistant to fluoroquinolones are classified as a group of microorganisms with a high level of priority (<https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed>).

Thermophilic *Campylobacter* spp. are among the most difficult microorganisms to cultivate. In the laboratory diagnosis of campylobacteriosis, the most difficult task is to isolate a pure culture of the pathogen from stool samples due to their massive concomitant microbial contamination. In recent years, the use of molecular research methods has been considered not as an alternative, but as a mandatory addition to regulated diagnostic regimens for acute intestinal infections, allowing for the rapid and effective identification of pathogens of acute intestinal infections, including thermophilic *Campylobacter* spp. At the same time, it does not imply species identification and determination of sensitivity to antibiotics [2, 7, 10].

Conclusion

Thus, due to the widespread prevalence of thermotolerant *Campylobacter* spp., infectious diseases caused by them remain a topical issue. Successful use of molecular diagnostic methods along with traditional culture inoculation methods makes it possible to effectively assess the prevalence of *Campylobacter* in poultry and to enact effective control strategies to prevent campylobacteriosis in individuals resid-

ing in the Republic of Guinea. Studying the distribution and resistance to antibiotics in the population of *C. jejuni* and *C. coli* among poultry could make it possible to develop new approaches to confirming the significance of their foodborne nature and to improve the national disease prevention system to reduce the risk of contamination with *Campylobacter* pathogens through industrial poultry products as well as infection burden levels in the population.

Additional information

Author contributions. For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used Conceptualization, L.K. and R.B.; methodology, M.M.; software, R.B. and M.M.; validation, Z.M., M.M. and L.K.; formal analysis, R.B.; investigation, M.M.; resources, R.B. and L.K.; data curation, M.M. and Z.M.; writing — original draft preparation, Z.M.; writing — review and editing, L.K.; visualization, Z.M.; supervision, M.M.; project administration, L.K.; funding acquisition, L.K. All authors have read and agreed to the published version of the manuscript.

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Авторы:

Балдэ Р., научный сотрудник отдела бактериологии НИИ прикладной биологии Гвинеи, г. Киндия, Гвинейская Республика;

Матвеева З.Н., к.м.н., ведущий научный сотрудник лаборатории кишечных инфекций ФБУН НИИ эпидемиологии и микробиологии имени Пастера, Санкт-Петербург, Россия;

Кафтырева Л.А., д.м.н., ведущий научный сотрудник группы эпидемиологии брюшного тифа 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, Санкт-Петербург, Россия;

Макарова М.А., д.м.н., ведущий научный сотрудник лаборатории кишечных инфекций ФБУН НИИ эпидемиологии и микробиологии имени Пастера, Санкт-Петербург, Россия; доцент кафедры медицинской микробиологии ФГБОУ ВО Северо-Западный государственный медицинский университет им. И.И. Мечникова, Санкт-Петербург, Россия.

Authors:

Balde R., Researcher, Department of Bacteriology Research Institute of Applied Biology of Guinea, Kindia, Republic of Guinea; **Matveeva Z.N.**, PhD (Medicine), Leading Researcher, Laboratory of Enteric Infections, St. Petersburg Pasteur Institute, St. Petersburg, Russian Federation;

Kaftyreva L.A., DSc (Medicine), Leading Researcher, Typhoid Epidemiology Research Group, St. Petersburg Pasteur Institute, St. Petersburg, Russian Federation; Professor, Department of Medical Microbiology, I.I. Mechnikov North-Western State Medical University, St. Petersburg, Russian Federation;

Makarova M.A., DSc (Medicine), Senior Researcher, Laboratory of Enteric Infections, St. Petersburg Pasteur Institute, St. Petersburg, Russian Federation; Associate Professor, Department of Medical Microbiology, I.I. Mechnikov North-Western State Medical University, St. Petersburg, Russian Federation.