

DIFFICULTIES IN IDENTIFICATION OF *COMAMONAS KERSTERSII* STRAINS ISOLATED FROM INTESTINAL MICROBIOTA OF RESIDENTS OF REPUBLIC OF GUINEA AND RUSSIAN FEDERATION

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Abstract. The *Comamonas* genus, described in 1985, included one species — *Comamonas terrigena*. At present, the *Comamonas* genus includes 21 species. The ability of *Comamonas* to survive in environmental objects (samples of water, soil and plants), including hospital environment and medical equipment, allows them to be considered as opportunistic microorganisms. The purpose of the research was to study the biological properties and antimicrobial susceptibility of *Comamonas kerstersii* strains isolated from fecal samples of healthy people living in Saint Petersburg (Russian Federation) and the Republic of Guinea. The study was carried out in the laboratory of enteric infections of the St. Petersburg Pasteur Institute. 1532 fecal samples were obtained from residents of St. Petersburg and 46 samples from residents of the Republic of Guinea. The generic and species identification of the isolated microorganisms was carried out using routine biochemical tests, the NEFERM test²⁴ commercial test system (MIKROLATEST, Erba Rus, Russia) and Vitek 2 Compact (BioMerieux). The difficult for identification microorganisms were studied by MALDI-TOF mass spectrometry, on a “Microflex LRF” mass-spectrometer. Antimicrobial susceptibility was determined by the gradient method with MICEvaluator™ (OXOID, UK) on Müller–Hinton agar (Russia). In the intestine microbiota of 1532 St. Petersburg residents among the opportunistic microorganisms two strains of *C. kerstersii* were isolated (0.13%). In a survey of 46 residents of the Republic of Guinea in 8 cases (17.4%) *C. kerstersii* were isolated. The identification of *C. kerstersii* strains using various methods and biochemical tests has shown different results: routine biochemical tests allowed the strains to be assigned to the group of non-fermenting gram-negative bacteria; NEFERM test²⁴ kit identified all strains as *C. testosteroni*; VITEC 2 referred all strains to *C. testosteroni*; using mass spectrometry with a coincidence ratio of 2.19–2.33, all strains were identified as *C. kerstersii*. It should be taken into account that the *C. kerstersii* bacteria cannot be identified by the tests of NEFERM test²⁴ kit and the GN VITEC 2 card, since only the *C. testosteroni* is represented in the databases of both methods. The taxonomic base of mass spectrometry includes reference spectra of five species of *Comamonas* from 21 known ones, including *C. kerstersii*. Strains isolated from the inhabitants of the Republic of Guinea were unsusceptible to at least one tested antibiotic: ciprofloxacin (4 strains), tetracycline (5 strains) and trimethoprim/sulfamethoxazole (5 strains), almost all strains being

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simultaneously unsusceptible to several antibiotics: ciprofloxacin and trimethoprim/sulfamethoxazole (2 strains), tetracycline and trimethoprim/sulfamethoxazole (2 strains), ciprofloxacin and tetracycline (1 strain). One strain was unsusceptible to ciprofloxacin, tetracycline, and trimethoprim/sulfamethoxazole.

Key words: *microbiome of the intestines, non-fermenting bacteria, Comamonas kerstersii, MALDI-TOF, antimicrobial resistance.*

ВЫДЕЛЕНИЕ И ОСОБЕННОСТИ ИДЕНТИФИКАЦИИ *COMAMONAS KERSTERSII* ПРИ ИЗУЧЕНИИ МИКРОБИОТЫ КИШЕЧНИКА ЖИТЕЛЕЙ РОССИЙСКОЙ ФЕДЕРАЦИИ И ГВИНЕЙСКОЙ РЕСПУБЛИКИ

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Резюме. Род *Comamonas*, описанный в 1985 году, включал один вид — *Comamonas terrigena*. В настоящее время род *Comamonas* включает 21 вид. Способность бактерий рода *Comamonas* выживать в объектах внешней среды (пробах воды, почвы и растений), в том числе во влажных условиях госпитальной среды и на медицинском оборудовании, позволяет считать их условно-патогенными микроорганизмами. Цель исследования состояла в изучении биологических свойств и чувствительности к антибиотикам штаммов *Comamonas kerstersii*, выделенных из проб испражнений здоровых людей, проживающих в Санкт-Петербурге и Гвинейской Республике. Работа проводилась в лаборатории кишечных инфекций ФБУН НИИ эпидемиологии и микробиологии имени Пастера, Санкт-Петербург. Исследованы 1532 пробы фекалий, полученных от жителей Санкт-Петербурга и 46 проб жителей Гвинейской Республики. Родовую и видовую идентификацию выделенных микроорганизмов проводили, используя рутинные биохимические тесты; коммерческую тест-систему «НЕФЕРМ тест24» (MIKROLATEST, Эрба Рус, РФ) и бактериологический анализатор Vitek 2 Compact (BioMerieux). Трудноидентифицируемые микроорганизмы исследовали масс-спектрометрическим методом на MALDI-TOF масс-спектрометре «Microflex LRF». Чувствительность к антибиотикам определяли методом градиентных концентраций с полосками M.I.C.Evaluator™ (OXOID, Великобритания) на агаре Мюллера–Хинтон (НИЦФ, Россия). В составе микробиоты кишечника у 1532 жителей Санкт-Петербурга были выделены 2 штамма *C. kerstersii* (0,13%). При обследовании 46 жителей Гвинейской Республики в 8 случаях (17,4%) были выделены *C. kerstersii*. Идентификация штаммов *C. kerstersii* с использованием различных методов дала различные результаты: рутинные биохимические тесты позволили отнести штаммы к группе неферментирующих грамотрицательных бактерий; «НЕФЕРМ тест24» идентифицировал штаммы как *C. testosteroni*; анализатор VITEC 2 отнес штаммы к *C. testosteroni*; методом масс-спектрометрии с коэффициентом совпадения 2,19–2,33 все штаммы идентифицированы как *C. kerstersii*. Следует учесть, что бактерии *C. kerstersii* не могут быть идентифицированы тестами, входящими в набор «НЕФЕРМ тест24» и карту GN VITEC 2, так как в базах данных обоих методов представлена характеристика только *C. testosteroni*. Таксономическая база масс-спектрометрии включает эталонные спектры пяти видов *Comamonas* из 21 известных, в том числе и *C. kerstersii*. Штаммы, выделенные от жителей Гвинейской Республики, характеризовались нечувствительностью хотя бы к одному тестируемому антибиотику: ципрофлоксацину (4 штамма), тетрациклину (5 штаммов) и триметоприм/сульфаметоксазолу (5 штаммов), причем практически все штаммы характеризовались одновременно нечувствительностью к нескольким АМП: ципрофлоксацину и триметоприм/сульфаметоксазолу (2 штамма), тетрациклину и триметоприм/сульфаметоксазолу (2 штамма), ципрофлоксацину и тетрациклину (1 штамм). Один штамм был нечувствителен одновременно к ципрофлоксацину, тетрациклину и триметоприм/сульфаметоксазолу.

Ключевые слова: *микробиота кишечника, неферментирующие бактерии, Comamonas kerstersii, MALDI-TOF, антимикробная резистентность.*

Introduction

The group of non-fermenting bacteria includes chemo-organotrophic Gram-negative microorganisms belonging to different genera and families, united in one group due to the lack of ability to carry out fermentation processes (utilization of carbohydrates in anaerobic conditions). In group of non-fermenting bacteria *Pseudomonas* and *Acinetobacter* genera have the most important clinical significance as agents

of health-care associated infections. In the taxonomy of pseudomonads, significant changes have taken place in recent years: bacteria that previously belonged to *Pseudomonas* are now divided into five main clusters, which are separated into independent genera or families, on the basis of homology studies using the rRNA hybridization method [18]. The first group includes bacteria of the *Pseudomonas* genus. Microorganisms belonging to the third rRNA homologous group are classified as a family

of *Comamonadaceae*, including *Comamonas*, *Delftia* and *Acidovorax* genera. The *Comamonas* genus, described in 1985, included one species — *Comamonas terrigena* [10]. Later, a detailed study of strains belonging to this genus allowed them to be divided into three distinct species: *C. terrigena*, *C. aquatica*, *C. kerstersii* [22]. At present, the *Comamonas* genus includes 21 species [14]. Bacteria of the *Comamonas* genus — aerobic, non-spore-forming gram-negative rods, mobile due to the presence of polar flagella, oxidase and catalase positive, grow well on simple and differential diagnostic nutrient media, do not oxidize or ferment carbohydrates, optimum growth temperature is 35–40°C. The ability of bacteria of the *Comamonas* genus to survive in environmental objects (samples of water, soil and plants), including hospital environment and medical equipment, allows them to be considered as opportunistic microorganisms [15].

The purpose of the research was to study the biological properties and antimicrobial susceptibility of *C. kerstersii* strains isolated from fecal samples of healthy people living in Saint Petersburg (Russian Federation) and the Republic of Guinea.

Materials and methods

The study was carried out in the laboratory of enteric infections of the St. Petersburg Pasteur Institute. 1532 fecal samples were obtained from residents of St. Petersburg and 46 samples from residents of the Republic of Guinea. The residents were clinically healthy and showed no signs of gastrointestinal disease during the 6 months preceding the study. Microorganisms (families of Enterobacteriaceae, Staphylococcus spp., yeast-like fungi of the genus Candida, non-fermenting bacteria, etc.) in Ig of feces were determined by the quantitative bacteriological method on various selective and differential-diagnostic media [1, 3]. The generic and species identification of the isolated microorganisms was carried out using routine biochemical tests, the NEFERM test24 commercial test system (MIKROLATEST, Erba Rus, Russia), the results were interpreted using the software supplied by the manufacturer. The difficult for identification microorganisms were studied by mass spectrometry. The spectra of the cells samples were acquired in linear positive mode using the "Microflex LRF" (Bruker Daltonik, Germany) mass spectrometer. Each spectrum was acquired using the FlexControl 3.3 software (Bruker Daltonics, Germany) in an automatic mode. The identification was carried out on the basis of the MALDI Biotyper 3.1 (Bruker Daltonics, Germany) software by comparing the mass spectra of each test sample with the reference spectra data from the taxonomic base and calculating the coincidence coefficients presented as scores. In addition, the identification of strains was carried out using the GN card for gram-negative microorganisms of the bacteriological analyzer Vitek 2 Compact (BioMerieux, France).

Antimicrobial susceptibility of *C. kerstersii* strains (minimum inhibitory concentrations, MIC) was determined by the gradient concentration method with MICEvaluator™ (OXOID, UK) strips on Müller-Hinton agar (Russia). The test included antibiotics from the group of expanded-spectrum cephalosporins (ceftazidime, cefotaxime, cefepime), carbapenems (imipenem, meropenem), fluoroquinolones (ciprofloxacin), aminoglycosides (gentamicin, amikacin), tetracycline, chloramphenicol and trimethoprim/sulfamethoxazole. Interpretation of the results was carried out according to the CLSI M100-S27 guidelines, Table 2B-5 "Minimal Inhibitory Concentration Breakpoints (µg/ml) for Other Non-Enterobacteriaceae", where we found the breakpoints of the MIC for microorganisms not belonging to the *Enterobacteriaceae* family: *Pseudomonas* spp. and other non-fermenting glucose, Gram-negative rods (with the exception of *P. aeruginosa*, *Acinetobacter* spp., *Burkholderia cepacia*, *B. mallei*, *B. pseudomallei* and *Stenotrophomonas maltophilia*, *Aeromonas* spp., and *Vibrio* spp.) [9]. Interpretation criteria according to CLSI M100-S27 are: ceftazidime, cefotaxime and cefepime: S ≤ 8.0 mg/l, R ≥ 32.0 mg/l; imipenem and meropenem: S ≤ 4.0 mg/l, R ≥ 16.0 mg/l; ciprofloxacin: S ≤ 1.0 mg/l, R ≥ 4.0 mg/l; gentamycin: S ≤ 4.0 mg/l, R ≥ 16.0 mg/l; amikacin: S ≤ 16.0 mg/l, R ≥ 64.0 mg/l; tetracycline: S ≤ 4.0 mg/l, R ≥ 16.0 mg/l; chloramphenicol: S ≤ 8.0 mg/l, R ≥ 32.0 mg/l; trimethoprim / sulfamethoxazole: S ≤ 2/38 mg/l, R ≥ 4/76 mg/l.

Results

In the intestine microbiota of 1532 St. Petersburg residents among the opportunistic microorganisms, *Klebsiella* spp. and *Staphylococcus aureus* were isolated most frequently, the detection rate was 20 and 16.7%, respectively. Findings of non-fermenting bacteria were less often. *Pseudomonas aeruginosa* was found only in 0.9% of cases. Other species of the *Pseudomonas* genus (*P. monteilii*, *P. putida*) were found in single cases. Two strains of *C. kerstersii* were also isolated (0.13%).

In a survey of 46 residents of the Republic of Guinea from the opportunistic microorganisms *Staphylococcus aureus* (34.8%) and *Klebsiella* spp. were also detected with the highest frequency (26.1%). In 8 cases (17.4%) non-fermenting bacteria were isolated, which were identified as *C. kerstersii*.

In the intestine microbiota of all the residents, *C. kerstersii* strains were found in the amounts exceeding the normal quantities (in St. Petersburg residents it was 10⁶ CFU/g, in the residents of the Republic of Guinea — from 10⁶ to 10⁹ CFU/g). In all cases, *C. kerstersii* strains were found in association with other opportunistic microorganisms (*Klebsiella* spp., *Candida* spp., *Hafnia alvei*, non-fermenting bacteria, *Staphylococcus aureus*, *Enterobacter* spp.). The identi-

Table. MIC of antibiotics for *Comamonas kerstersii* strains

No.	MIC, mg/ml										
	ceftazidim	cefotaxim	cefepim	imipenem	meropenem	ciprofloxacin	gentamicin	amikacin	tetracycline	chloramphenicol	trimethoprim/ sulfamethoxazol
1	2.0	4.0	1.5	0.125	0.125	> 32.0	2.0	4.0	8.0	3.0	> 32.0
2	1.0	1.0	0.38	0.125	0.125	0.06	2.0	3.0	128.0	1.0	> 32.0
3	0.75	0.75	0.38	0.125	0.125	8.0	2.0	4.0	4.0	1.5	> 32.0
4	0.5	0.75	0.25	0.125	0.125	4.0	2.0	4.0	4.0	1.5	> 32.0
5	0.75	0.5	0.25	0.125	0.125	0.06	2.0	3.0	8.0	1.0	0.38
6	0.75	0.75	0.38	0.125	0.125	0.06	2.0	3.0	6.0	1.5	> 32.0
7	0.75	1.0	0.5	0.19	0.19	4.0	2.0	4.0	8.0	1.0	1.5
8	0.75	1.0	0.38	0.125	0.125	0.12	2.0	3.0	4.0	1.5	0.25
9	0.75	1.0	0.38	0.125	0.19	0.12	2.0	4.0	4.0	1.5	0.38

fication of *C. kerstersii* strains using various methods and biochemical tests has shown different results:

1. Routine biochemical tests allowed the strains to be assigned to the group of non-fermenting bacteria (gram-negative, mobile, catalase and oxidase-positive, non-fermenting carbohydrates bacteria).
2. Using the tests included in the NEFERM test 24 kit, the strains were identified as *C. testosteroni*.
3. Using the test card of the GN analyzer VITEC 2, strains were referred to *C. testosteroni*.
4. Using mass spectrometry, all strains were identified as *C. kerstersii* with a score of 2.19–2.33.

It should be taken into account that the *C. kerstersii* bacteria cannot be identified by the tests included in the NEFERM test24 kit and the GN VITEC 2 card, since only the *C. testosteroni* is represented in the databases of both methods. The taxonomic base of mass spectrometry includes reference spectra of five species of *Comamonas* from 21 known ones, including *C. kerstersii*.

Minimal inhibition concentration (MIC) of antibiotics of different groups of *C. kerstersii* strains is given in the table. The MIC of ceftazidime ranged from 0.75 to 2 mg/l; cefotaxime — from 0.75 to 4 mg/l; cefepime — from 0.25 to 1.5 mg/l; imipenem and meropenem — from 0.125 to 0.19 mg/l; ciprofloxacin — from 0.06 to > 32 mg/l; tetracycline — from 4 to 128 mg/l; chloramphenicol — from 1 to 3 mg/l, trimethoprim/sulfamethoxazole — from 0.25 to > 32 mg/l. MIC of gentamicin for all strains was 2 mg/l, of amikacin — 3–4 mg/l. Two strains of *C. kerstersii* isolated from residents of Saint Petersburg (No. 8 and 9 in the table), according to the CLSI criteria, were susceptible to all tested antibiotics.

Strains isolated from the residents of the Republic of Guinea were unsusceptible to at least one tested antibiotic: ciprofloxacin (4 strains), tetracycline

(5 strains) and trimethoprim/sulfamethoxazole (5 strains), almost all strains were simultaneously unsusceptible to several antibiotics: ciprofloxacin and trimethoprim/sulfamethoxazole (2 strains), tetracycline and trimethoprim/sulfamethoxazole (2 strains), ciprofloxacin and tetracycline (1 strain). One strain was unsusceptible to ciprofloxacin, tetracycline, and trimethoprim/sulfamethoxazole.

Discussion

Microorganisms of the *Comamonas* genus are most often isolated from various environmental objects, such as wetland, humus, open water, contents of the termites intestine, less often from humans. For a long time, bacteria of this genus have not been considered pathogenic to humans, however, in recent years, reports of the isolation of *C. kerstersii* and *C. testosteroni* from patients with invasive infections have appeared, and the publications on etiological significance of *C. testosteroni* are more numerous [6, 7, 12, 13, 16, 20, 21].

C. kerstersii strains were isolated from patients with catheter-associated infections (bacteremia), peritonitis, appendicitis, postoperative complications in the form of septic infections, meningitis, etc. In Argentina in 2010–2015 *C. kerstersii* in association with other opportunistic microorganisms were isolated from the psoas abscess, and in a case of pelvic peritonitis, from the abdominal fluid in 12 patients with acute peritonitis that developed as a result of gangrenous or perforated appendicitis [4, 5]. In the UK for two years, *C. kerstersii* were isolated from 27 hospitalized patients with diarrhea. The authors suggested that the carriage of this microorganism in the intestine can occur more often than was previously thought, since modern identification methods were not available [8].

Identification of bacteria of the *Comamonas* genus presents significant difficulties. This is due to the relatively recent definition of them in a separate ge-

nus, the isolation of these bacteria almost always in the microbial associations as well as the difficulties of their differentiation from other *Pseudomonas* species. Until recently, the identification of strains was based on the study of biochemical activity, for correct identification it was necessary to study at least 11 substrates. Test systems and microbiological analyzers used in laboratory practice do not always allow to identify these hard-to-identify bacteria and to differentiate *C. kerstersii* species from *C. testosteroni*, since only one species, *C. testosteroni*, is included in the databases of many methods.

As the databases expand, the identification capacity of the methods will include a wider range of microorganisms, and other types of *Comamonas*. Publications about etiological significance of *C. kerstersii* appeared shortly after the method of Matrix-Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) mass spectrometry was used to identify microorganisms in bacteriological laboratories and the reliability of the identification results was confirmed by molecular genetic methods [4, 5, 8, 17].

In connection with the foregoing, many authors believe that the etiological role of *C. kerstersii* is currently underestimated, since in the previously described cases of infections associated with isolation of microorganisms of the *Comamonadaceae* family, the identification of strains was carried out by methods that do not allow to differentiate species within the genus.

In 2015–2017 in the intestinal microbiota of 1532 people without signs of acute intestinal pathology living in Saint Petersburg, the strains of *C. kerstersii* were isolated in two patients, while in a survey of 46

residents of the Republic of Guinea this microorganism was detected in 8 individuals. Perhaps the frequent discovery of *C. kerstersii* is associated with the climatic and socio-economic conditions of the Republic of Guinea [19].

The antimicrobial susceptibility testing of *C. kerstersii* as causative agents of septic infections is complicated by the lack of data on the natural resistance of these microorganisms and the criteria for interpreting test results in the Russian Clinical Guidelines “Antimicrobial susceptibility testing of microorganisms” and in the Guidelines of the European Committee on antimicrobial susceptibility testing (EUCAST) [2, 11]. The only guideline that presents the interpretation criteria that can be used for testing of *C. kerstersii* is the document CLSI M100-S27 [9].

The data of a few foreign studies of the antimicrobial susceptibility testing of *C. kerstersii* strains do not allow comparison of the results, as some authors used a disc-diffusion method (not recommended by CLSI) for testing these microorganisms [8] or testing using other interpretation criteria [4, 5]. Nevertheless, the MIC results allow us to conclude that resistance to fluoroquinolones, trimethoprim/sulfamethoxazole and amikacin was noted among *C. kerstersii* strains isolated at different times [4, 5, 17]. Our results confirm the high in vitro activity of the expanded spectrum cephalosporins, carbapenems, aminoglycosides and chloramphenicol against *C. kerstersii* strains. Unsusceptible (to ciprofloxacin, trimethoprim/sulfamethoxazole and tetracycline) *C. kerstersii* strains were isolated only from the residents of the Republic of Guinea.

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