# COMPARATIVE BIOINFORMATICS ANALYSIS OF ANTIMICROBIAL RESISTANCE GENE POOL IN THE GENOMES OF REPRESENTATIVES OF GENUS *CORYNEBACTERIUM*

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Currently, multidrug resistance of bacterial infectious agents poses a serious threat to the global public health. The following *Corynebacterium* strains are of special importance for infections, including hospital-acquired ones: *C. amycolatum*, *C. urealyticum*, *C. striatum*, *C. jeikeium*, *C. aurimucosum*, *C. genitalium* that are resistant to the broad spectrum of antimicrobial drugs. The study was aimed to conduct bioinformatics analysis of the pool of antimicrobial resistance genes in the published genomes of some members of the genus *Corynebacterium*. The data on the whole genome nucleotide sequences of 22 *Corynebacterium* isolates readily available from NCBI GenBank were assessed. Bioinformatics analysis of the whole genome sequences conducted in order to search for antimicrobial resistance genes in the specified genomes was performed using the PATRIC online resource. It was found that the genomes provided comprised various combinations of 25 antimicrobial drug resistance genes. Amino acid substitutions in *GyrA* (positions 87, 88 and 91) were revealed in some *Corynebacterium* strains, through which quinolone/fluoroquinolone resistance could be realized.

**Keywords:** *C. amycolatum*, *C. jeikeium*, *C. striatum*, *C. urealyticum*, *C. aurimucosum*, genomes, antimicrobial resistance genes, *gyrA*, antimicrobial drugs

**Author contribution:** Kulshan TA — study planning, literature review, dealing with molecular genetic data (selection of genomes, genome annotation, comparative analysis of *gyrA* amino acid sequences), data analysis, manuscript writing; Bugaeva IO — study planning, data analysis, interpretation of findings, manuscript writing; Soboleva EF — literature review, data analysis, manuscript writing; Allyanova MS — literature review, analysis of the antimicrobial resistance gene pool in the genomes of corynebacterial strains, dealing with the PATRIC online service; Popov DA — literature review, search for *gyrA* amino acid sequences in the genomes of corynebacterial strains, comparative analysis of amino acid sequences; Shvidenko IG — advising during manuscript writing, data analysis.

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# СРАВНИТЕЛЬНЫЙ БИОИНФОРМАТИЧЕСКИЙ АНАЛИЗ СОСТАВА ГЕНОВ АНТИМИКРОБНОЙ УСТОЙЧИВОСТИ В ГЕНОМАХ ПРЕДСТАВИТЕЛЕЙ РОДА *CORYNEBACTERIUM*

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В настоящее время множественная антимикробная резистентность бактериальных инфекционных агентов представляет серьезную угрозу для мирового здравоохранения. Особое значение в развитии инфекций, в том числе госпитальных, играют следующие виды коринебактерий: *C. amycolatum*, *C. urealyticum*, *C. striatum*, *C. jeikeium*, *C. aurimucosum*, *C. genitalium*, которые устойчивы к большому арсеналу антимикробных препаратов. Целью исследования было проведение биоинформатического анализа спектра генов устойчивости к антимикробным препаратам в опубликованных геномах некоторых представителей рода *Corynebacterium*. Исследованы данные о нуклеотидных последовательностях полных геномов 22 штаммов коринебактерий, представленных в свободном доступе в NCBI GenBank. Биоинформатический анализ полногеномных последовательностей с целью поиска генов антимикробной устойчивости в указанных геномах осуществляли с помощью онлайн-ресурса PATRIC. Установлено, что представленные геномы в различных комбинациях содержали 25 генов устойчивости к антимикробным препаратам. У некоторых штаммов коринебактерий выявлены аминокислотные замены в *GyrA* (позиции 87, 88 и 91), с которыми может быть связана реализация устойчивости к хинолонам/фторхинолонам.

**Ключевые слова:** *C. amycolatum*, *C. jeikeium*, *C. striatum*, *C. urealyticum*, *C. aurimucosum*, геномы, гены антимикробной устойчивости, *gyrA*, антимикробные (противомикробные) препараты

**Вклад авторов:** Т. А. Кульшань — планирование исследования, анализ литературы, работа с молекулярно-генетическими данными (подбор геномов, аннотация генома, сравнительный анализ аминокислотной последовательности гена *gyrA*), аналитическая работа с полученными данными, написание публикации; И. О. Бугаева — планирование исследования, аналитическая работа с полученными данными, интерпретирование результатов, участие в написании публикации; Е. Ф. Соболева — анализ литературы, аналитическая работа с полученными данными, написание публикации; М. С. Аллянова — анализ литературы, анализ состава генов антимикробной устойчивости в геномах штаммов коринебактерий, работа с онлайн-сервисом PATRIC; Д. А. Попов — анализ литературы, поиск аминокислотных последовательностей гена *gyrA* в геномах коринебактерий, сравнительный анализ аминокислотных последовательностей; И. Г. Швиденко — консультирование в ходе написания статьи, аналитическая работа с полученными данными.

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Today, antimicrobial multidrug resistance of bacterial infectious agents poses a serious threat to global public health. Irrational use of antimicrobials for treatment of humans, in the livestock sector and agriculture is a determinant of widespread resistance to drugs among bacteria [1–3].

Selective pressure of antimicrobials on the bacterial population contributes to realization of various resistance mechanisms emerging due to acquisition of genetic determinants of resistance or spontaneous mutations [1, 4–6]. Assessment of evolutionary transformation of bacterial genomes associated with antibiotic resistance contributes to optimization of treatment strategies and preventive measures.

Currently, the greater role played by normal flora members, specifically by members of the genus *Corynebacterium*, in infectious diseases can be associated with the spread of genes responsible for antimicrobial resistance across bacterial genomes. The increasingly frequent isolation of *Corynebacterium*

**Table 1.** *Corynebacterium non diphtheriae* strains, the whole genome nucleotide sequences of which are used in the study

| № | Strain | Year, place, isolation source | GenBank ID: |
|---|---|---|---|
| 1 | *Corynebacterium amycolatum BER245* | 2011, Brazil, human (biomaterial collected from the ear) | CP102778.1 |
| 2 | *Corynebacterium amycolatum ICIS 53* | 2016, Russia, human (vaginal discharge) | MIFV00000000 |
| 3 | *Corynebacterium amycolatum VH6958* | 2016, Spain, human | JAFJMB000000000.1 |
| 4 | *Corynebacterium amycolatum ICIS 9* | 2017, Russia, human (vaginal discharge) | MTPT00000000.1 |
| 5 | *Corynebacterium amycolatum SB-1* | 2019, South Korea, human (skin) | CP120206.1 |
| 6 | *Corynebacterium amycolatum ICIS 99* | 2020, Russia, human (vaginal discharge) | JAIUSU000000000 |
| 7 | *Corynebacterium amycolatum 1189* | n/a, Germany, n/a | CP069513.1 |
| 8 | *Corynebacterium urealyticum DSM 7109* | 1985, Germany, human (urine) | AM942444 |
| 9 | *Corynebacterium urealyticum VH3073* | 2017, Spain, human (urine) | VTFJ00000000 |
| 10 | *Corynebacterium urealyticum 996* | n/a, Germany, n/a | CP065982.1 |
| 11 | *Corynebacterium urealyticum 994* | n/a, Germany, n/a | CP066064.1 |
| 12 | *Corynebacterium striatum 2308* | 2011, Brazil, human (blood) | NRIO00000000.1 |
| 13 | *Corynebacterium striatum 708C* | 2021, UK (synovial fluid) | JASNMG000000000 |
| 14 | *Corynebacterium striatum 824M* | 2022, UK, blood | JASNMH000000000 |
| 15 | *Corynebacterium striatum 1197* | n/a, Germany, n/a | CP069514.1 |
| 16 | *Corynebacterium striatum 1115* | n/a, Germany, n/a | CP068158.1 |
| 17 | *Corynebacterium striatum ATCC 6940* | n/a, human (urogenital tract) | ACGE00000000 |
| 18 | *Corynebacterium jeikeium K411* | 2004, Germany, human (axilla region) | CR931997.1 |
| 19 | *Corynebacterium jeikeium 574* | 2016, USA, human | CP033784.1 |
| 20 | *Corynebacterium jeikeium ATCC 43734* | n/a, human (urogenital tract) | ACYW00000000 |
| 21 | *Corynebacterium aurimucosum UMB7769* | 2013, USA, human (urine) | JASOLN000000000 |
| 22 | *Corynebacterium genitalium ATCC 33030* | n/a, USA, human (urogenital tract) | ACLJ00000000 |

as pathogens, especially in immunocompromised individuals, is indicative of the greater role in the development of infectious complications in patients played by *Corynebacterium* [2].

The following *Corynebacterium* species are of special importance for development of infections: *C. amycolatum* (skin and soft tissue infections, bacteremia, endocarditis, genital infections), *C. urealyticum* (acute and chronic urinary tract infections, urolithiasis), *C. striatum* (true bacteremia, bacterial colonization of prostheses, catheters, breathing tubes, etc.), *C. jeikeium* (bacteremia, endocarditis, pneumonia, skin and soft tissue infections), *C. aurimucosum* (acute and chronic joint infections, diabetic foot ulcer infection), *C. genitalium* (urinary tract infections) [2, 3, 5, 7–14]. Multidrug resistance of some *Corynebacterium* species to β-lactams, macrolides, aminoglycosides, quinolones, tetracyclines and rifampicins, lincosamides, etc., should be noted [1, 4, 12–14].

However, the data on the *Corynebacterium* drug resistance are contradictory, that is why our study was aimed to conduct bioinformatics analysis of the pool of antimicrobial resistance genes in the published genomes of some representatives of the genus *Corynebacterium*.

## METHODS

The study involved data on the whole genome nucleotide sequences of 22 strains of six *Corynebacterium* species (*C. amycolatum*, *C. urealyticum*, *C. striatum*, *C. jeikeium*, *C. aurimucosum*, *C. genitalium*) readily available from NCBI GenBank, isolated in different countries over the years (Table 1).

Bioinformatics analysis of whole genome sequences aimed at the search for antibiotic resistance genes in the specified genomes was performed using PATRIC (Pathosystems Resource Integration Center), Comprehensive Antibiotic Resistance Database (CARD), and Database of Antibiotic-Resistant Organisms (NDARO) [15].

Amino acid sequences of *gyrA* gene were acquired from Genbank. The UGENE (Unipro UGENE) 48.1 software package was used for analysis of *gyrA* amino acid sequences [16]. Amino acid sequence alignment was performed using the MUSCLE tool integrated into UGENE.

## RESULTS

Bioinformatics analysis showed that the genomes provided comprised various combinations of antimicrobial resistance genes. A total of 25 different genes encoding resistance to drugs exhibiting antimicrobial activity were determined (Table 2).

It should be noted that the following genes were significantly less often found in the genomes of studied isolates (Table 3):

1) *tetO* (*tetW*) (encodes resistance to tetracyclines) — was not found in genomes of 19 strains (86.4%);

2) *aph (3')-I, aph (6)-Ic* (encode resistance to aminoglycosides) — were not found in genomes of 14 strains (63.6%);

3) *ermX* (encodes resistance to macrolides, lincosamides, streptogramins) — was not found in genomes of 13 strains (59%);

4) *lsu* (*rplF*) (encodes resistance to fusidic acid) — was not found in genomes of 12 strains (54.5%);

5) *cmx* (encodes resistance to chloramphenicol) — was not found in genomes of eight strains (36,4,3%);

6) *ispC* (*dxr*) (encodes resistance to fosfomycin) — was not found in genomes of seven strains (32%);

7) *gibB* (encodes resistance to aminoglycosides), *oxyR* (encodes resistance to изониазиду), *fabG* (encodes resistance to triclosan) — were not found in genome of one strain (4,5%) (*Corynebacterium striatum 824M*, *Corynebacterium striatum 1197*, *Corynebacterium striatum 708C*, respectively).

However, resistance to aminoglycosides, fusidic acid, fosfomycins was encoded by several genes. In this regard, the

**Table 2.** List of antimicrobial resistance genes found in the genomes of the studied *Corynebacterium non diphtheriae* strains using the PATRIC online resource

| Antimicrobial drugs | Genes encoding antimicrobial resistance |
|---|---|
| Lipopeptides | *pgsA, gdpD (ugpQ, glpQ)* |
| Macrolides, penicillins | *mtrA, mtrB* |
| Macrolides, lincosamides, streptogramins | *ermX* |
| Diaminopyrimidines | *folA (dfr)* |
| Tetracyclines, glycylcyclines | *s10p (rpsJ)* |
| Tetracyclines | *tetO (tetW)* |
| Sulfonamides | *folP* |
| Aminoglycosides | *s12p (rpsL, rpsJ), gibB, aph(3')-I, aph(6)-Ic* |
| Fusidic acid | *ef-G (fusA), lsu (rplF)* |
| Cycloserine | *alr, dlr* |
| Isoniazid | *oxyR* |
| Fosfomycins | *murA, ispC (dxr)* |
| Chloramphenicol | *cmx* |
| Muropirocin | *ileS* |
| Triclosan | *fabG* |
| Bicyclomycin | *rho* |
| Elfamycins | *ef-Tu(tufA)* |

lack of one gene in the genome can not indicate the isolate sensitivity to these antimicrobial substances.

All other genes provided in Table 2 were found in 100% of genomes of 22 Corynebacterium strains.

*C. striatum* 2308 was the strain containing 24 identified antimicrobial resistance genes out of 25. Only the *tetO* (*tetW*) gene was not found in its genome. According to the literature, this strain was isolated in 2011 from the blood culture of a man, who was treated at the hospital in Rio de Janeiro. Based on phenotypic characteristics, it showed sensitivity to tetracycline (MIC 1 mg/L), linezolid (MIC 0.25 mg/L) and vancomycin (MIC 0.5 mg/L) only [12]. The data of bioinformatics analysis we have obtained confirm the phenotypic study results [12]: no *tetO* (*tetW*) gene (tetracycline resistance), no genes encoding resistance to oxazolidones (linezolid) and glycopeptides (vancomycin). It is worth noting that no linezolid and vancomycin resistance genes were found in any of the studied strains. However, the authors point out that this strain showed phenotypic resistance to erythromycin (MIC > 256 mg/L) and clindamycin (MIC > 256 mg/L), as well as to gentamicin (aminoglycoside) (MIC 256 mg/L) [12]. Such phenotypic effects may result from the presence of genes *ermX* and *aph (3')-I*, *aph (6)-Ic*.

*Corynebacterium amycolatum* ICIS 9 extracted from vagina of a healthy woman in 2017 in Russia turned out to be one more strain with the genome showing the lack of gene *ispC* (*dxr*) (fosfomycin resistance) only. However, fosfomycin resistance is also encoded by the *murA* gene, which was found in the genome. The authors of the paper considered *Corynebacterium amycolatum* ICIS 9 as a potential probiotic agent for treatment of vaginal dysbiosis [9–11]. The *Corynebacterium amycolatum* ICIS 9 phenotypic resistance to antimicrobials (amikacin, gentamicin (aminoglycosides), amoxicillin (β-lactams), clarithromycin (macrolide), chloramphenicol, ciprofloxacin (fluoroquinolone) and tetracycline) was determined [9–11]. Indeed, our bioinformatics study showed that the genome of this isolate comprised genes encoding resistance to penicillins, aminoglycosides, macrolides, chloramphenicol, fluoroquinolones, and tetracyclines (Table 2).

As for strains, the genomes of which lack a significant number of antimicrobial resistance genes (6–10 genes), these included the following: *C. amycolatum* ICIS 99, *C. amycolatum* ICIS 53, *C. amycolatum* SB-1, *C. amycolatum* 1189, *C. striatum* 824M, *C. striatum* 708 (Table 3).

The *C. striatum* 708 strain extracted from synovial fluid of a patient in the UK (BioSample: SAMN34403526) comprised the lowest number of antimicrobial resistance genes (19 genes).

Currently, many causes of antimicrobial resistance of microorganisms are distinguished. This phenomenon results not only from the presence of genetic determinants associated with antimicrobial resistance, but also with various mutations in these genes. It has been found that mutations in the short regions of genes *gyrA* and *gyrB* (quinolone resistance-determining regions (QRDR)) encoding A and B subunits of DNA gyrase result in quinolone/fluoroquinolone resistance [9].

In *Corynebacterium*, quinolone/fluoroquinolone resistance results from spontaneous mutations in the gene encoding gyrase A subunit [12, 13]. It has been found that mutations associated with amino acid changes in positions 87, 88 and 91 increase the minimum inhibitory concentrations (MICs) of quinolones/fluoroquinolones. Thus, amino acid substitutions in position 87, Ser (S) to Arg (R), Phe (F), Val (V), in position 88, Ala (A) to Pro (P), in position 91, Asp (D) to Tyr (Y), Gly (G), Ala (A), increased the ciprofloxacin, levofloxacin and moxifloxacin MICs [12, 13]. In this regard we considered it necessary to conduct a molecular genetic analysis of the gene amino acid sequence in 22 studied strains. *GyrA* of *Corynebacterium glutamicum* ATCC 13032 (GenBank ID: NP599264) was used as a reference when performing comparative analysis and determining the amino acid position number [13].

According to the literature data, the *C. striatum* ATCC 6940, *C. jeikeium* ATCC 43734 and *C. urealyticum* DSM 7109 isolates showed quinolone/fluoroquinolone resistance [13]. The *gyrA* amino acid sequences of these strains were used as controls.

The analysis showed that in the *C. striatum* ATCC 6940, *C. jeikeium* ATCC 43734, *C. amycolatum* 1189, *C. aurimucosum* UMB7769, *C. striatum* 1115, *C. urealyticum* 994, *C. urealyticum* 996, *C. urealyticum* DSM 7109, *C. jeikeium* K411, *C. amycolatum* SB-1, *C. genitalium* ATCC 33030 strains, position 87 was occupied by Ser (S), while position 91 was occupied by Asp (D). According to the literature, such gene structure ensured the strains' sensitivity to quinolones/fluoroquinolones, despite the presence of resistance genes [12, 13].

**Table 3.** List of antimicrobial resistance genes not found found in the genomes of the studied *Corynebacterium non diphtheriae* strains

| № | Strain | Antimicrobial resistance genes not found in the genome |
|---|---|---|
| 1 | *Corynebacterium amycolatum BER245* | *ermX, tetO (tetW),ispC (dxr)* |
| 2 | *Corynebacterium amycolatum ICIS 53* | *ermX, tetO (tetW), aph(3')-I, aph(6)-I, lsu (rplF), ispC (dxr), cmx* |
| 3 | *Corynebacterium amycolatum VH6958* | *tetO (tetW), lsu (rplF), ispC (dxr)* |
| 4 | *Corynebacterium amycolatum ICIS 9* | *ispC (dxr)* |
| 5 | *Corynebacterium amycolatum SB-1* | *ermX, tetO (tetW), aph(3')-I, aph(6)-I, lsu (rplF), ispC (dxr), cmx* |
| 6 | *Corynebacterium amycolatum ICIS 99* | *ermX, tetO (tetW), aph(3')-I, aph(6)-I, lsu (rplF), ispC (dxr)* |
| 7 | *Corynebacterium amycolatum 1189* | *ermX, tetO (tetW), aph(3')-I, aph(6)-I, lsu (rplF), ispC (dxr), cmx* |
| 8 | *Corynebacterium urealyticum DSM 7109* | *ermX, tetO (tetW), aph(3')-I, aph(6)-I* |
| 9 | *Corynebacterium urealyticum VH3073* | *tetO (tetW), lsu (rplF)* |
| 10 | *Corynebacterium urealyticum 996* | *ermX, tetO (tetW), lsu (rplF)* |
| 11 | *Corynebacterium urealyticum 994* | *tetO (tetW), lsu (rplF)* |
| 12 | *Corynebacterium striatum 2308* | *tetO (tetW)* |
| 13 | *Corynebacterium striatum 708C* | *mtrA, mtrB, ermX, tetO (tetW), gibB,aph(3')-I, aph(6)-I, lsu (rplF), fabG, cmx* |
| 14 | *Corynebacterium striatum 824M* | *ermX, tetO (tetW),aph(3')-I, aph(6)-I, lsu (rplF), gibB* |
| 15 | *Corynebacterium striatum 1197* | *oxyR* |
| 16 | *Corynebacterium striatum 1115* | *tetO (tetW), aph(3')-I, aph(6)-I, lsu (rplF),cmx* |
| 17 | *Corynebacterium striatum ATCC 6940* | *ermX, tetO (tetW),aph(3')-I, aph(6)-I* |
| 18 | *Corynebacterium jeikeium K411* | *ermX, tetO (tetW), aph(3')-I, aph(6)-I* |
| 19 | *Corynebacterium jeikeium 574* | *tetO (tetW), aph(3')-I, aph(6)-I, lsu (rplF),cmx* |
| 20 | *Corynebacterium jeikeium ATCC 43734* | *ermX, tetO (tetW), aph(3')-I, aph(6)-I* |
| 21 | *Corynebacterium aurimucosum UMB776* | *aph(3')-I, aph(6)-I, lsu (rplF), cmx* |
| 22 | *Corynebacterium genitalium ATCC 33030* | *ermX, tetO (tetW), aph(3')-I, aph(6)-I, cmx* |

Ser (S) replaced with Arg (R) in position 87 was reported in *C. amycolatum* ICIS 53, *C. amycolatum* ICIS 99. As for *C. amycolatum* VH6958 strain, Ala (A) replaced with Pro (P) in position 88 was reported in addition to Ser (S) replaced with Arg (R) in position 87. It is worth paying attention to the *C. amycolatum* BER245 strain, for which Asp (D) replaced with Tyr (Y) in position 91 was reported along with Ser (S) replaced with Arg (R) in position 87. Such mutations dramatically increased the MICs of quinolones/fluoroquinolones [12, 13].

*C. urealyticum* VH3073 had two unique substitutions: 87 — Ser (S)/ Val (V) and 91 — Asp (D)/ Tyr (Y). *C. striatum* 2308, *C. striatum* 708C, *C. striatum* 824M had only one amino acid substitution: 87 — Ser (S)/ Val (V). Moreover, strains carrying unique substitutions were identified: 87 — Ser (S)/ Ile (I), 91 — Asp (D)/ Ala (A) — C. amycolatum ICIS 9; 87 — Ser (S)/ Ile (I), 91 — Asp (D)/ Gly (G) — *C. jeikeium* 574; 87 — Ser (S)/ Phe (F), 91 — Asp (D)/ Gly (G) — *C. striatum* 1197 (Fig. ). The evolutionary significance of these substitutions is to be determined in further studies.

Thus, 11 isolates have position 87 occupied by Ser (S), in 4 strains it is occupied by Val (V), in 4 strains by Arg (R), in 2 strains by Ile (I), in one strain by Phe (F). A total of 21 strains have position 88 occupied by Ala (A), while in one isolate it is occupied by Pro (P). A total of 17 isolates have position 91 occupied by Asp (D), in 2 strains it is occupied by Tyr (Y), in 2 strains by Gly (G), in 1 strain by Ala (A).

To summarize, it is worth noting that double mutations in *gyrA* described in the literature as mutations causing a dramatic increase in MICs of quinolones/fluoroquinolones were found in: *C. amycolatum* VH6958 isolated in 2016 in Spain (BioSample: SAMN18038700) — Ser (S) replaced with (R) in position 87, Ala (A) replaced with Pro (P) in position 88; *C. amycolatum* BER245 isolated in 2011 in Brazil from the patient with otitis — Ser (S) replaced with Arg (R) in position 87, Asp (D) replaced with Tyr (Y) in position 91; *C. urealyticum* VH3073 isolated in 2017 in Spain from the patient's urine (BioSample: SAMN12621417) — Ser (S)/ Val (V) substitution in position 87, Asp (D)/ Tyr (Y) in position 91.

One mutation was found in two strains (*C. amycolatum* ICIS 53, *C. amycolatum* ICIS 99): Ser (S) replaced with Arg (R).

## DISCUSSION

The spread of antimicrobial drug resistance genes by horizontal transfer causes the increase in the number of resistant microorganisms, including opportunistic pathogens. It is worth noting that *Corynebacterium* strains, such as *C. amycolatum* ICIS 53, *C. amycolatum* ICIS 9, *C. amycolatum* ICIS 99 isolates extracted from vaginal discharge of healthy women we have studied, had a large enough pool of antimicrobial resistance genes [9, 11]. In this regard, it is necessary to continuously monitor antimicrobial resistance of bacteria in order to develop effective measures against their growing resistance to antimicrobial drugs. The databases containing information about antibiotic resistance of bacteria will make it possible to compare the results obtained using different methods and estimate the abundance of antimicrobial resistance genes.

Our findings allowed us to single out a core set of antimicrobial resistance genes comprised in the Corynebacterium genomes. These data can be used as potential estimates of the use of antimicrobials for treatment of patients. However, molecular genetic testing should be combined with other methods based on phenotypic assessment of sensitivity to drugs, since the data on phenotypic and genotypic resistance are not always correlated.

Antimicrobial resistance can be associated with various mutations. In particular, quinolone/fluoroquinolone resistance is realized mainly through acquisition of point mutations in the sequence of *gyrA* gene encoding DNA gyrase A subunit, while overexpressed efflux pump can also contribute to acquisition

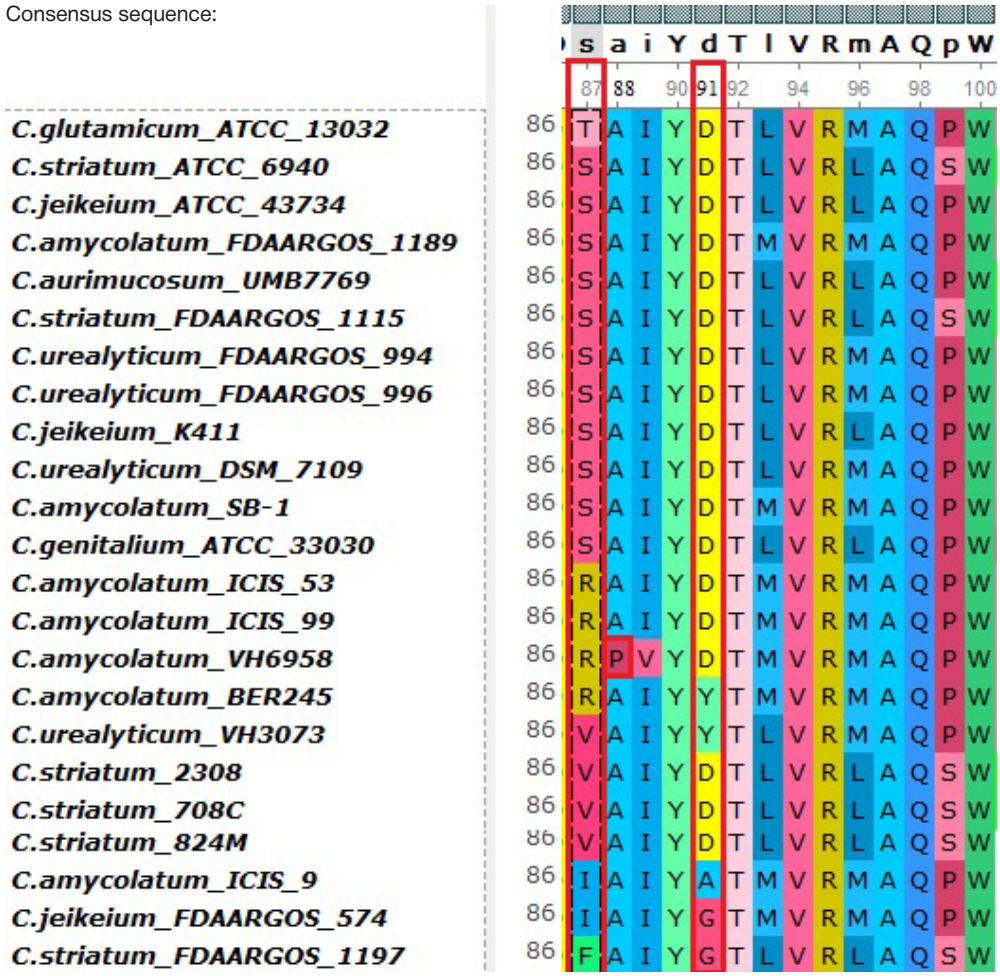


**Fig.** The *gyrA* amino acid sequence of *Corynebacterium non diphtheriae* isolates taken as examples. Positions of point mutations found in the *gyrA* amino acid sequence, which, according to the literature, affect the increase in the quinolone/fluoroquinolone minimum inhibitory concentrations (MICs), are marked with frames

of quinolone resistance [12, 13]. In *C. amycolatum*, alteration in the *GyrA* position 87 ensured resistance to all the tested quinolones/fluoroquinolones [12, 13]. Such substitutions were also observed in the genomes of *C. amycolatum* strains we had assessed. Furthermore, some Corynebacterium strains carried several mutations in the *gyrA* amino acid sequence, which increased the MICs of quinolones/fluoroquinolones [12, 13]. Investigation of genetic variability through mutation is important for the study of evolutionary transformation of bacterial genomes and can be used to develop rapid molecular diagnostic tests.

## CONCLUSIONS

A growing etiological significance of Corynebacterium for infectious diseases, especially as hospital-acquired pathogens among immunocompromized patients having a history of the long-term hospital stay, several courses of antibiotic therapy and treatment with the use of invasive medical devices, determines the need to constantly monitor pathogens. Antimicrobial resistance of bacteria is a major concern: 1) it was found that there was a large pool of antimicrobial resistance genes (25 genes) forming various combinations in the *Corynebacterium* genomes. The presence of gene was correlated to the isolate capability of being resistant to antimicrobial drugs. This represented an important evolutionary effect of the impact of antibiotics on the population structure of microorganisms. It should be noted that antimicrobial resistance is most often encoded by several genes. Variability of antimicrobial resistance determinants emphasizes the need for continuous monitoring of the *Corynebacterium* resistance profiles; 2) mutations were detected in the *gyrA* amino acid sequences of the studied strains (positions 87, 88, 91), which were considered to be associated with quinolone/fluoroquinolone resistance.

The goal of the study was achieved. The limited data on *Corynebacterium*, including molecular genetic data, hamper comparative analysis. Expansion of the range of strains, including ones represented in various databases, will contribute to better understanding of the genome structure, phenotypic characteristics, while identification of the range of antimicrobial resistance genes will expand the knowledge about the directions of antibiotic therapy.

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