BIOLOGICAL PROPERTIES OF *SERRATIA LIQUEFACIENS* 1/2024, ISOLATED FROM CHICKEN

Liliia VYGOVSKA[1], Artem USHKALOV[1], Liubov ZELENA[2], Valerii USHKALOV[1], Cristina SIRBU[4], Yurii VISHOVAN[3]

[1] Department of Veterinary Epidemiology and Animal Health, Faculty of Veterinary Medicine, National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine
[2] Department of Virus Reproduction, Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, Kyiv, Ukraine
[3] Department of Microbiological research Ukrainian Laboratory of Quality and Safety of Agricultural Products of National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine
[4] National Food Safety Agency of the Republic of Moldova

Corresponding author: Valerii Ushkalov, e-mail: ushkalov63@gmail.com

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ABSTRACT

Introduction According to a study by the European Centre for Disease Prevention and Control (ECDC) for 2022-2023, *Serratia* spp. caused 54,406 cases of disease in 28 countries worldwide. *Serratia liquefaciens* is considered the second most frequently isolated organism from human clinical samples after *Serratia marcescens*. The aim of this study was to investigate the biological properties of *S. liquefaciens* isolated from chickens.

Material and methods The study investigated a *Serratia liquefaciens* strain isolated in 2024 from chickens kept in a vivarium with clinical manifestations of gastrointestinal tract disorders. The aim of the study was to examine the strain *S. liquefaciens*. When studying the biological properties of the isolate, morphological characteristics, growth typicality, biochemical properties, pathogenicity for laboratory animals, and antibiotic sensitivity were taken into account.

Results The isolated microorganism formed colonies typical of the *Serratin* genus, and the cells were motile at 37°C. The enzymatic profile matched the known characteristics of *S. liquefaciens*. The strain produced a denser biofilm at 23°C, exhibited pathogenicity in white mice, and showed resistance to several classes of antimicrobial agents. Based on the sequencing of a 16S rRNA gene fragment, the strain was deposited in the NCBI GenBank database under accession number PQ308601.1.

Conclusions The results obtained suggest that the *S. liquefaciens* 1/2024 may be used as a reference strain for the differential diagnosis of enterobacteria, assessment of antimicrobial activity, and application as a laboratory control strain in experimental studies.

Keywords Antibiotic resistance, biofilm, pathogenicity, sequencing, *Serratia* spp.

PROPRIETĂȚILE BIOLOGICE ALE *SERRATIA LIQUEFACIENS* 1/2024, IZOLATĂ DE LA PUI

Introducere Conform unui studiu ECDC pentru perioada 2022-2023, *Serratia* spp. a cauzat 54.406 cazuri de boală în 28 de țări din întreaga lume. *Serratia liquefaciens* este considerată al doilea cel mai frecvent izolat organism din probele clinice umane, după *Serratia marcescens*. Scopul studiului a fost de a investiga proprietățile biologice ale *S. liquefaciens* izolate de la pui.

Material și metode Obiectul cercetării au fost tulpinile de *S. liquefaciens*, izolate în 2024 de la puii crescuți într-un vivariu cu manifestări clinice de afecțiuni ale tractului gastrointestinal. La studierea proprietăților biologice ale izolatului, s-au luat în considerare proprietățile morfologice, culturale, biochimice, patogenitatea pentru animalele de laborator și sensibilitatea la antibiotice.

Rezultate Microorganismul izolat a format colonii tipice genului *Serratia*, iar bacteriile au fost mobile la 37°C. Activitatea enzimatică a izolatului a corespuns caracteristicilor *S. liquefaciens*. Bacteriile au fost capabile să formeze un biofilm mai dens la 23°C, au fost patogene pentru șoarecii albi și au prezentat semne de rezistență multiplă la agenții antimicrobieni. Pe baza rezultatelor secvențierii unui fragment al genei ARNr 16S, datele despre tulpină au fost introduse în GenBank NCBI sub numărul PQ308601.1.

Concluzii Rezultatele obținute oferă motive pentru utilizarea tulpinii *S. liquefaciens* 1/2024 în calitate de standard pentru diagnosticul diferențial al enterobacteriilor, determinarea activității preparatelor antibacteriene, ca tulpină de control, de testare etc.

Cuvinte-cheie Rezistența la antibiotice, biofilm, patogenitate, secvențiere, *Serratia* spp.

INTRODUCTION

S. marcescens is a prominent member of the genus *Serratia* and is of considerable significance in medical practice due to its role in numerous hospital-acquired infections. According to the ECDC study, "Point Prevalence Survey of Healthcare-Associated Infections and Antimicrobial Use in European Acute Care Hospitals" for 2022–2023, *Serratia* spp. accounted for 54,406 cases across 28 countries globally (1).

Infectious illnesses attributable to *Serratia* spp. demand meticulous scrutiny from veterinary and medical practitioners due to the ubiquity of *Serratia* in the environment (soil, water, and the gastrointestinal tracts of diverse animals) and their role as agents of nosocomial infections in humans.

S. marcescens predominantly induces respiratory tract infections in intubated patients, urinary tract infections in individuals with permanent catheters, and surgical wound infections, superinfections, and sepsis in cases involving intravenous catheterization or complicated local infections. It can also induce meningitis, brain abscesses, and several other illnesses, primarily in hospitalized patients, neonates, and individuals with diabetes (2, 3, 4, 5, 6, 7, 8).

Noncompliance with sanitary and hygienic norms and rules when handling plant-based food products increases the risk of *Serratia* spp. accumulation and infection of humans and animals. Soil serves as a reservoir for the pathogen; therefore, vegetables pose a potential risk of infection to animals, plants, and humans (9, 10). Animal products, such as cured ham, smoked bacon, and sausage, if not properly manufactured and handled, can also be contaminated with *Serratia* spp. and serve as a source of infection for consumers (11, 12, 13).

Among *S. marcescens* strains, a significant proportion produces a red pigment, which led to their use as bioindicators in studies of microbial dissemination and transmission. This practice was long facilitated by the misconception that bacteria of this genus were non-pathogenic. The first documented experiment was conducted in 1906 by M. H. Gordon at the request of the British government (14). The most significant tests involving this biological agent were carried out by the U.S. military between 1940 and 1960 to simulate the dissemination of biological weapons in the event of potential bioterrorist attacks. The "bioindicator" was released at military training facilities and in civilian locations, including San Francisco (1950); the Pentagon in Washington, D.C.; Mechanicsburg, Pennsylvania (1950); Panama City, Florida (1951); Point Mugu-Port Hueneme, California (1953); the New York City underground system (1966); and several other sites across the United States. A journalistic investigation into associated illnesses and fatalities led to hearings in the United States Senate in 1977 (14).

The importance of regulating the proliferation of bacteria belonging to the genus *Serratia* is evidenced by the following findings. In one medical center in southern Taiwan, during the period 1999–2003, 69 nonrepetitive bloodstream isolates were analyzed. Among these, 11 isolates produced extended-spectrum beta-lactamase, while 58 isolates carried an AmpC-encoding gene, including a novel S4 gene with 98% identity to the SRT-1 gene (n = 50), the SRT-2 gene (n = 3), the SST-1 gene (n = 1), and others (n = 4). Isolates carrying S4 exhibited a phenotype of resistance to cefotaxime (CTX) but not ceftazidime (15). In a university hospital in Madrid, Spain, between 2005 and 2020, 141 *Serratia* spp. isolates causing bloodstream infections were identified in 139 patients (16). Studies conducted in Poland in 2003–2004 showed that *S. marcescens* was the fifth most frequently detected clinical isolate (4%) among members of the family Enterobacteriaceae (17). Additionally, a study conducted in Japan in 2008 found that *S. marcescens* accounted for 6.4% of urinary tract infections, making it the fifth most common etiological agent (18).

The prevalence of *Serratia* in farm and wild animals is confirmed by numerous scientific publications. For example, scientists from the Department of Epidemiology at the Indian Veterinary Research Institute demonstrated the prevalence of antimicrobial-resistant Serratia spp. in farm and wild animals, poultry, and reptiles. Isolates were obtained from the cardiac blood of poultry, horses, goats, spotted deer and turtles (19). Additionally, outbreaks of mastitis were reported in cows from 2 different herds on a farm in Finland, where 18 isolates of *S. marcescens* were identified (20).

Researchers in Japan obtained 30 isolates from clinical specimens collected from dogs and cats that exhibited resistance to extended-spectrum cephalosporins. This finding raises concerns for both medical and veterinary practice, as third- and fourth-generation cephalosporins are frequently employed as "last-line" therapies for bacterial infections (21).

Clinical Microbiology Laboratory at the University of Melbourne Veterinary Clinic and Hospital performed 4,536 bacterial identifications in animal patients, with Serratia spp. isolated in 0.7% of cases (11 dogs, 9 cats, 6 horses, 1 rabbit, and 1 bird). Analysis of antibiotic susceptibility testing for 18 *Serratia* spp. isolates showed that 50% were resistant to sulfafurazole/trimethoprim, enrofloxacin, and third-generation cephalosporins. One strain also exhibited resistance to chlorhexidine, which is used for disinfecting tools and work surfaces in the veterinary facility (22). Similar findings have been reported by other authors (7, 23, 24).

Numerous authors have experimentally demonstrated the ability of *Serratia*, particularly *S. marcescens* and *S. liquefaciens*, to produce heat-stable enterotoxins (astA) that act via guanylate cyclase, increasing the level of cGMP in intestinal cells and causing secretory diarrhea. Some strains of *S. marcescens* secrete hemolysins that destroy erythrocytes. These toxins may damage the intestinal epithelium and induce inflammation (16).

Taxonomically, the genus *Serratia* remains ambiguous, and it currently comprises 14 recognized species and 2 subspecies (tab. 1).

Human infections caused by *Serratia*, particularly *S. marcescens*, were not well understood until the latter half of the 20th century. This was likely due to challenges in the taxonomic characterization of the genus and the fact that several species were not identified until the 1970s and 1980s. *S. marcescens* is now recognized as an important human pathogen. Another member of the genus, *S. liquefaciens*, is considered the second most common isolate from human clinical specimens. It is an environmental bacterium associated with disease and was first described in 1931 by Grimes and Hennerty as *Aerobacter liquefaciens*. In 1963, the organism was assigned to the genus *Enterobacter* and was subsequently reclassified as *S. liquefaciens* in 1973 based on phenotypic characteristics (14, 24).

The pathogenicity of *S. liquefaciens* has been established in humans, where it is a recognized pathogen of nosocomial infections. It also causes diseases in productive animals, insects and fish. The ubiquity of *Serratia* spp., together with the risks of disease outbreaks caused by representatives of this species, which do not belong to generally recognized pathogens, justifies the need for further investigation of its circulation and the arsenal of pathogenicity factors. Standard strains are essential for laboratory diagnostics, for evaluating the suitability of nutrient media, and for conducting comparative studies with the characteristics of epizootic isolates.

The aim of this study was to investigate the biological properties of the isolate *S. liquefaciens* obtained from chicken faeces.

Table 1. Current taxonomic map of the genus *Serratia* (14).

Species and subspecies	Year of detection	Habitat	Pathogenicity
S. entomophila	1988 (169)	Insects (*Costelytra zealandica*)	Insects
S. ficaria	1979 (167)	Plants, insects (cycle common fig-fig of wasp)	Humans
S. fonticola	1979 (145)	Water	Humans
S. glossinae	2010 (146)	Insects (*Glossinapalpalis gambiensis*)	Not reported
S. grimesii	1983 (163)	Water, soil	Not reported (isolated from humans)
S. liquefaciens	1931 (158)	Water, soil, animals, insects, plants	Humans, insects
S. marcescens subsp. *marcescens*	1823 (37, 264)	Water, soil, animals, insects, plants	Humans, animals, insects
S. marcescens subsp. *sakuensis*	1998 (109)	Water	Not reported
S. nematodiphila	2009 (425)	Nematodes (*Heterorhabditidoideschong mingensis*)	Not reported
S. odorifera	1978 (165)	Plants	Humans
S. plymuthica	1896 (162)	Water, animals, insects, plants	Humans
S. proteamaculans	1919 (291)	Water, soil, animals, insects, plants	Insects, plants
S. quinivorans	1982 (163)	Water, soil, animals, insects, plants	Humans
S. rubidaea	1940 (363)	Water, plants	Humans
S. ureilytica	2005 (36)	Water	Not reported

MATERIALS AND METHODS

Location of research

Investigations of morphological and biochemical characteristics, evaluation of antibiotic sensitivity, determination of pathogenicity, and establishment of biofilms formation were conducted at Kharkiv Regional State Laboratory of the State Service of Ukraine for Food Safety and Consumer Protection and at the Department of Veterinary Epidemiology and Animal Health, Faculty of Veterinary Medicine, National University of Life and Environmental Sciences of Ukraine, Kyiv, Ukraine. Molecular genetic studies of the isolate were performed at Danylo Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine.

Selection of samples

Research samples were collected from chickens exhibiting diarrhea that were kept under laboratory vivarium conditions and were delivered to the laboratory in accordance with the state standards of Ukraine (DSTU 8703-1:2017, DSTU 8703-2:2017). A total of 23 samples were collected.

Object of research

S. liquefaciens isolate obtained in 2024 from chickens (*S. liquefaciens* 1/2024).

1. *Study of biological properties of Serratia liquefaciens*

1.1. *Isolation of pure culture and determination of morphological properties*

The study was conducted in accordance with current international and national regulatory documents for the detection of enterobacteria (ISO 21528-1:2017, ISO 21528-2:2017).

The conventional bacteriological approach, namely growing on liquid and solid nutrient medium, was employed to ascertain the morphological features.

Bacteriological examination of pathological material was conducted by inoculating cloacal washings onto nutrient broth using a sterile swab. Cultivation was carried out at the optimal temperature for *Serratia* growth, 37°C, for 24 hours.

To obtain a pure culture, the nutrient broth cultures were subcultured using a bacteriological loop with frequent wide strokes onto separate Petri dishes with Endo ta xylose-lysine-deoxycholate agar (XLD-agar), covering the entire agar surface, and thereafter incubated in a thermostat for 24 hours at 37°C. Subsequently, individual representative colonies were extracted from the agar surface using a bacteriological loop, subcultured into meat peptone nutrition broth and meat peptone nutrient slant agar, and incubated at 37°C for 24 hours.

Morphological properties were determined by microscopy of Gram-stained smears, while growth typicality was determined by inoculating *S. liquefaciens* cultures on liquid and solid nutrient media.

To assess bacterial motility, the isolated cultures were grown at 37°C in semi-liquid MPA (0.25 – 0.3%). Inoculation was performed by introducing the sample into a column of semi-liquid agar. Motility was also assessed by microscopy of daily agar cultures using the "crushed drop" technique.

1.2. *Assessment of biochemical properties*

The biochemical characteristics of the isolate were investigated by inoculation on Hiss media supplemented with various sugars (maltose, glucose, mannitol, sucrose, lactose, rhamnose, and raffinose). Additionally, the ability to produce enzymes, ornithine decarboxylase, phenylalanine deaminase, lysine decarboxylase, and arginine dehydrolase, was assessed, along with urea and indole production. The Voges-Proskauer reaction was also performed.

1.3. *Assessment of pathogenicity*

The pathogenicity of the isolate was assessed in 30 white male mice, 4 months old, weighing 21-23 g, by intraperitoneal injection of a 24-hour broth culture of *S. liquefaciens* at doses ranging from 10^1 to 10^9 CFU (3 mice per dose). The control group of mice received sterile MPB injection. The observation period lasted 72 hours. The LD_{100} was defined as the minimum dose of the tested culture that caused 100% mortality in the experimental animals.

Animal experiments were conducted in accordance with the current "General Ethical Principles of Animal Experiments", adopted by the First National Congress of Bioethics and aligned with international bioethical standards (materials of the IV European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Purposes, Strasbourg, 1985) (25). The research programme was reviewed and approved by the Bioethics Commission of the National University of Life and Environmental Sciences of Ukraine (approval No. 022/2024, dated 26.11.2024).

1.4. *Assessment of antibiotic sensitivity*

The sensitivity of the investigated isolate to antibacterial drugs was assessed by the disk fusion method. A specialized nutritional medium, Mueller Hinton Agar, was employed to evaluate sensitivity (26).

Four identical, distinctly separate colonies were picked from a 24-hour agar culture on meat-peptone agar in Petri plates and subsequently transferred to a test tube containing sterile saline using a bacteriological loop to generate a microbial suspension (inoculum). The optical density of the bacterial suspension was adjusted with a densitometer, measuring 0.5 according to McFarland standards.

Standardized antibiotic discs (26 medicines from several pharmacological categories) were used to assess sensitivity. The discs were placed on the agar surface using sterile tweezers, onto which the inoculum (1.0 ml) had been previously deposited. Following disc application, the Petri dishes were positioned in a thermostat and incubated at 37°C for 24 hours. The results (measurement of the growth inhibition zone) were recorded using a specialized ruler. Data were interpreted according to EUCAST guidelines, 2023. The experiments were performed in seven replicates.

1.5. *Evaluation of film formation*

An indirect assessment of bacterial biofilms biomass was performed using the adsorption/resorption of crystal violet, following the established methodology (27). The biofilms were stained using a 0.1% aqueous solution of crystal violet at 30°C for 60 minutes. The study was conducted under two culture temperatures: 23°C and 37°C. Sterile MPB served as the control. To ensure data reliability, the experiments were performed in seven replicates.

2. *Molecular-genetic research*

The molecular genetic methods for studying bacteria, particularly the sequencing of 16S rRNA gene fragments and phylogenetic analysis, were implemented according to the procedures outlined by Tkachuk et al. (28). In summary, genomic DNA was extracted from a pure bacterial culture for 16S rRNA gene sequencing and subsequently amplified using 27F and 1492r primers. PCR products were purified and sequenced in both directions using the ABI 310 (Applied Biosystems). The resulting sequences were compared with those in the GenBank database using BLASTn, and the highest percentage of sequence similarity (>98.7%) was used for species identification. Sequences showing greater than 98.7% similarity were further analyzed for variable and parsimony-informative sections utilizing MEGA6 (29) for identification.

3. *Statistical studies*

The data obtained from the biofilm formation studies under different temperature conditions were analyzed using SPSS Statistics software.

RESULTS

1.1. *Isolation of a pure culture and assessment of morphological characteristics*

After 24 hours of initial inoculation on Endo agar, small, homogenous pink colonies exhibiting an S-shape and measuring 1-2 mm in diameter were observed. On XLD-agar, the isolated culture produced uniform, colorless colonies exhibiting an S-shape, measuring 1-2 mm in diameter. Gram-stained smears were prepared from isolated colonies. Microscopic examination revealed small, homogenous, gram-negative rods with rounded ends, resembling ovoids, measuring 3-5 x 0.8-1.5 microns. In MPB, the pure culture produced consistent turbidity in the medium after 24 hours. In smears from broth cultures, the cells appeared singly; the dimensions of the bacteria cultured on nutritional media measured 3-5 x 0.8-1.5 microns. Small transparent colonies, occasionally exhibiting a faint yellowish hue, were detected on the MPA, characterized by a rounded, convex, S-shaped morphology.

Upon assessment of motility, *S. liquefaciens 1/2024* was identified as motile.

1.2. *Assessment of biochemical characteristics*

Under cultivation conditions in a thermostat at 37°C for 24 hours, *S. liquefaciens* 1/2024 metabolised maltose, glucose, mannitol, and sucrose, while failing to metabolise lactose, rhamnose, or raffinose. It produced ornithine decarboxylase and lysine decarboxylase but did not produce urea, phenylalanine deaminase, arginine dihydrolase, or indole. The Voges-Proskauer test yielded a negative result (tab. 2).

Table 2. Enzymatic properties of the isolate *S. liquefaciens* 1/2024 and *S. marcescens*.

Indicator name	*S. liquefaciens*	*S. marcescens*
Maltose	+	+
Glucose	+	+
Mannitol	+	+
Sucrose	+	+
Lactose	–	–
Rhamnose	–	+/–
Raffinose	–	–
Ornithine decarboxylase	+	+

Indicator name	*S. liquefaciens*	*S. marcescens*
Urea production	–	–
Phenylalanine deaminase	–	–
Lysine decarboxylase	+	+
Arginine dihydrolase	–	–
Indole	–	–
Voges-Proskauer (VP)	–	–

Note: «+» – reaction present, «-» – reaction absent.

1.3. *Assessment of pathogenicity*

No mortality was observed in mice within 72 hours following the administration of *S. liquefaciens 1/2024* culture at doses ranging from 10^1 to 10^7 CFU. However, 100% mortality occurred in groups receiving doses of 10^8 to 10^9 CFU within 12 to 18 hours. These results indicate that the specified isolate is pathogenic to white mice at a dose of 10^8 CFU.

1.4. *Antibiotic sensitivity testing*

The European Committee on Antimicrobial Susceptibility Testing (EUCAST) defines the area of technical uncertainty (ATU) as a situation in which antimicrobial susceptibility testing results do not allow for a definitive classification of an isolate as either susceptible or resistant. This may arise due to discrepancies in testing methodologies or the unique characteristics of the individual microbe. EUCAST does not establish distinct clinical breakpoints for *S. liquefaciens.*

The tested culture exhibited resistance to semisynthetic and inhibitor-protected penicillins (Ticarcillin, Ampicillin/Sulbactam, Amoxicillin/Clavulanicacid, Ticarcillin/Clavulanicacid), with the exception of Piperacillin, for which an inhibition zone of 18 mm was observed (tab. 3).

Table 3. Sensitivity of *S. liquefaciens* 1/2024 to β-lactam antibiotics.

Antibacterial drug	Diameter of inhibition of culture growth around the disk with antibacterial drug, mm	[4]ATU, mm
Ampicillin/sulbactam, [2]20, [3]SAM	6	[5]14
Amoxicillin/clavulanic acid, [2]10, [3]AMC	6	[5]19-20
Ticarcillin, [2]75, [3]TI	6	[5]20-23
Ticarcillin/clavulanicacid, [2]75\10, [3]TCC	7	[5]20-23
Piperacillin, [2]100, [3]PI	18	[5]20
Cefalotin, [1](I), [2]30, [3]CEP	6	[5]15-17
Cephalexin, [1](I), [2]30, [3]CL	12	[5]14
Cefaclor, [1](II), [2]30, [3]CF	10	[5]15-17
Cefuroxime, [1](II), [2]30, [3]CXM	6	[5]19
Cefamandole, [1](II), [2]30, [3]MA	14	[5]15-17
Cefixim, [1](III), [2]5, [3]CFM	13	[5]17
Cefoperazone, [1](III), [2]75, [3]CPZ	18	[5]16-20
Meropenem, [2]10, [3]MEM	44	[5]16-22 ([6]<28)

Notes: «[1]» – AMP generation; «[2]» – AMP concentration (µg); «[3]» – AMP code as indicated on the disc; «[4]» – Area of technical uncertainty for microorganism sensitivity/resistance to AMP; «[5]» – Interpretation of results per EUCAST (Version 13.1, valid from 2023-06-29); «[6]» – in carbapenemase screening, the meropenem screening limits> 0.125 mg/L (zone diameter <28 mm); «*» – indicates uniform growth of resistant colonies in the specified range (mm).

The culture exhibited sensitivity to cephalosporins (Cephalexin, Cefaclor, Cefamandole, Cefixim), with the exception of Cefalotin and Cefuroxime (tab. 3).

The culture exhibited sensitivity to carbapenems (Meropenem), with a growth inhibition zone of 44 mm. (tab. 3).

The culture exhibited sensitivity to aminoglycosides (Gentamicin, Netilmicin). Inhibition zones about 24 mm in diameter were observed surrounding the discs containing Netilmicin (tab. 4).

The tested culture was sensitive to tetracyclines (the culture's sensitivity to Tetracycline, and Doxycycline was manifested with growth inhibition zones of 23 mm and 26 mm, respectively) and to quinolones (a culture inhibition zone with a diameter of 36 mm was recorded around the disc with Nalidixic acid).

A growth inhibition zone measuring 30 mm was observed around the Lomefloxacin disc, while double zones of growth inhibition of 40/32 mm, 30/27 mm, and 30/27 mm were noted around the Ciprofloxacin, Ofloxacin, and Chloramphenicol discs, respectively, indicating an enhanced sensitivity of the cultures. The culture exhibited sensitivity to Polymixin B and Furazolidone, shown by zones of growth inhibition of 15 mm and 14 mm in diameter, respectively. The isolate exhibited moderate sensitivity to Fusidic acid, with a growth inhibition zone of 7 mm, indicating in vitro resistance to this medication at the specified dose.

Table 4. Sensitivity of *S. liquefaciens* 1/2024 to antimicrobial agents.

Antibacterial drug	Diameter of inhibition of culture growth around the disk with antibacterial drug, mm	[4]ATU, mm
Gentamicin, [1](I), [2]10, [3]CN	19	[5]17
Netilmicin, [1](III), [2]30, [3]NET	24	[6]13-14
Tetracycline, [2]30, [3]TE	23	[6]≥19
Doxycycline, [2]30, [3]DO	26	[6]≥19
Nalidixic acid, [1](I), [2]30, [3]NA	36	[6]14-18
Ciprofloxacin, [1](II), [2]5, [3]CIP	*40/32	[5]22-24
Ofloxacin, [1](II), [2]5, [3]OFX	*30/27	[5]22-24
Lomefloxacin, [1](II), [2]10, [3]LOM	30	[6]19-21
Chloramphenicol, [2]30, [3]C	*32/28	[6]13-17
Polymixin, [1]B, [2]300, [3]PB	16	[7]–
Furazolidone, [2]50, [3]FX	14	[7]–
Fusidic acid, [2]10, [3]FC	7	[7]–

Notes: «[1]» – AMP generation; «[2]» – AMP concentration (μg); «[3]» – AMP code as indicated on the disc; «[4]» – Area of technical uncertainty for microorganism sensitivity/resistance to AMP; «[5]» – Interpretation of results per EUCAST (Version 13.1, valid from 2023-06-29); «[6]» – Interpretation per Ministry of Health of Ukraine guidelines (2007); «[7]» – Thresholds not defined; «*» – indicates uniform growth of resistant colonies in the specified range (mm).

1.5. *Biofilm formation study*

When analyzing the biofilm-forming capacity of *S. liquefaciens* 1/2024, it was found (tab. 5) that denser biofilm production occurred during cultivation at 23°C compared to 37°C. Under these conditions, *S. liquefaciens* 1/2024 formed a biofilm that was 92.1% denser at 23°C ($P < 0.05$), which may indicate the bacterium's adaptation to more favorable environmental conditions.

Table 5. Results of studying the capacity of *S. liquefaciens* 1/2024 to form biofilms at 23°C and 37°C (M±m, n=7).

Culture conditions	The optic density of formed biofilm, **λ** 570
+23°C	0.265±0.05*
+37°C	0.138±0.038
Control (sterile MPB)	0.035±0.01

Note: P<0.05 compared to culture at +37°C

To check if the data followed a normal distribution, the Kolmogorov-Smirnov test (with Lilliefors correction) and the Shapiro-Wilk test were applied to two experimental temperature conditions: 23°C and 37°C. In both cases, the p-values were less than 0.05. For t23, the p-values were p = 0.043 (Kolmogorov-Smirnov) and p = 0.039 (Shapiro-Wilk). For t37, the p-values were p = 0.045 (Kolmogorov-Smirnov) and p = 0.030 (Shapiro-Wilk).

Q-Q plots were constructed to visually assess the normality of the distribution. The 23°C graph (fig. 1) shows significant deviations of the points from the diagonal line, especially at the extreme values. This indicates asymmetry or outliers. Similarly, the graph for 37 °C (fig. 2) also deviates from linearity, with the data points not aligning closely with the expected quantiles of a normal distribution. This is consistent with the test results.

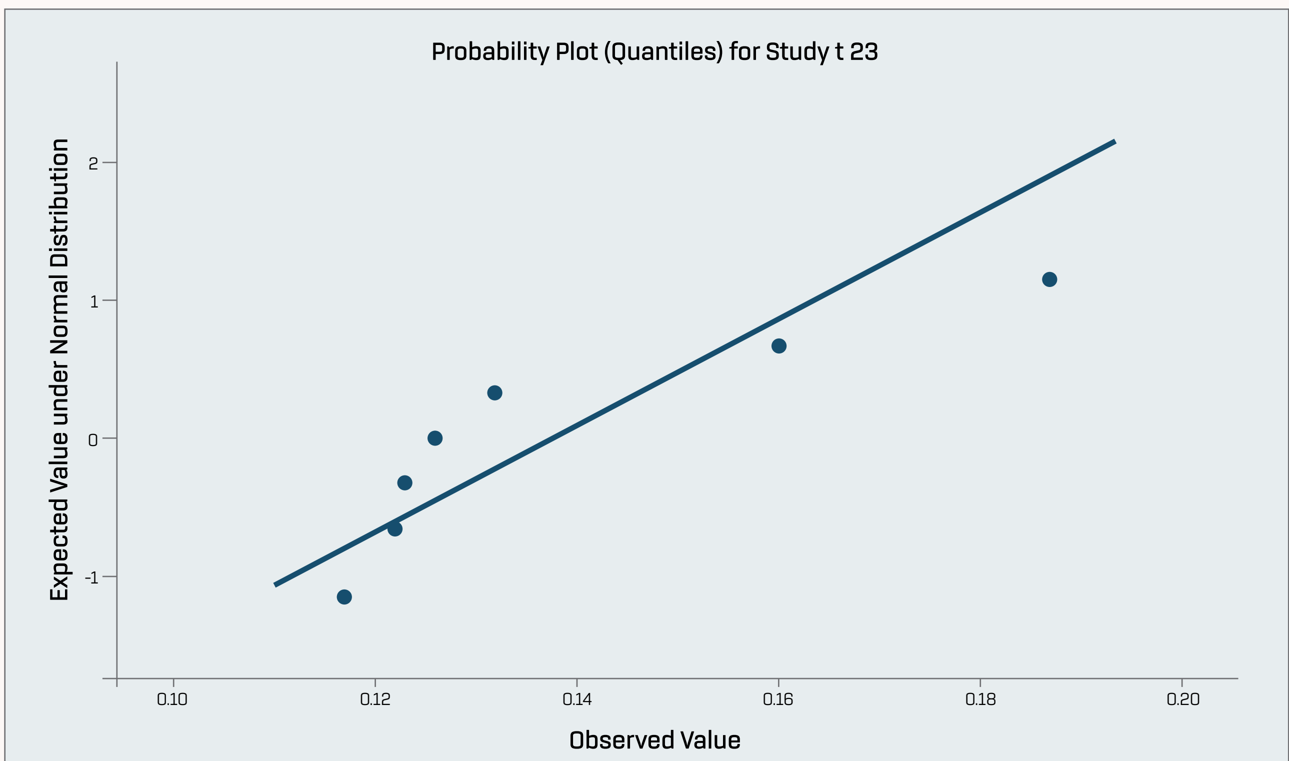

Figure 1. Visual assessment of distribution normality (Q-Q plots) at 23˚C.

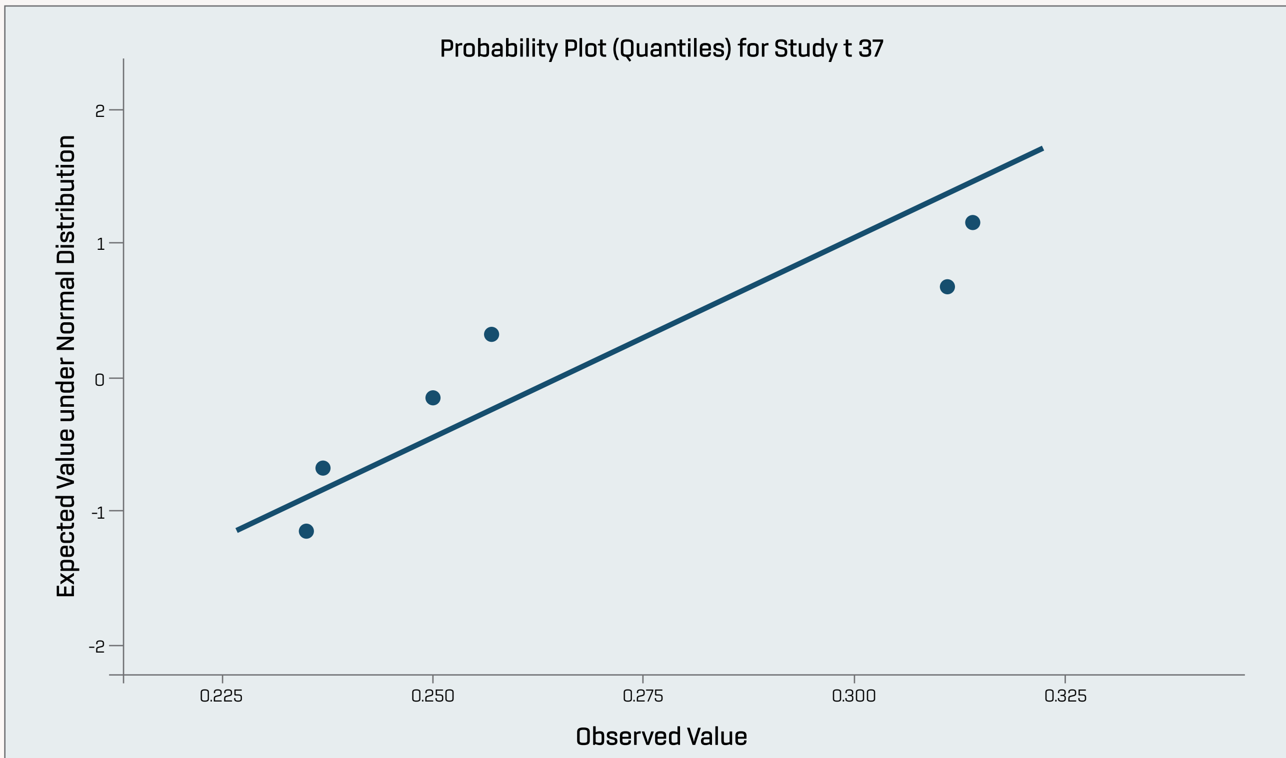

Figure 2. Visual assessment of distribution normality (Q-Q plots) at 37°C.

Thus, the graphical evaluation supports the statistical conclusion that the data distributions under both temperature conditions deviate from normality. This indicates that the empirical distribution is statistically significantly different from the normal distribution, and the null hypothesis of normality was rejected at the 0.05 significance level.

Further statistical analysis required the use of nonparametric methods, which do not assume a normal distribution of data. In such cases, parametric methods like Student's t-test are not appropriate.

Therefore, the nonparametric Mann-Whitney U test was chosen to compare two independent samples. This test does not require the assumption of a normal distribution and is resistant to outliers and skewed data. The obtained p-value of 0.001 is statistically significant ($p<0.05$), allowing us to reject the null hypothesis of equal distributions. This indicates that temperature has a significant effect on the studied indicator.

2. *Molecular genetic studies of the S. liquefaciens 1/2024 strain*

The investigation yielded a nucleotide sequence of 633 nucleotides, which has been registered in the GenBank database under the identifier PQ308601.1.

A preliminary comparison with the genomes in this database revealed 99.21% identity with other strains of the species (fig. 3), including the standard *S. liquefaciens* ATCC 27592. A dendrogram was built to illustrate the evolutionary connections between the examined strain and other species within the genus *Serratia*, placing the *S. liquefaciens* 1/2024 strain in the same clade as other strains of this species. The results of the phylogenetic analysis confirmed the classification of the investigated strain as belonging to the species *S. liquefaciens*.

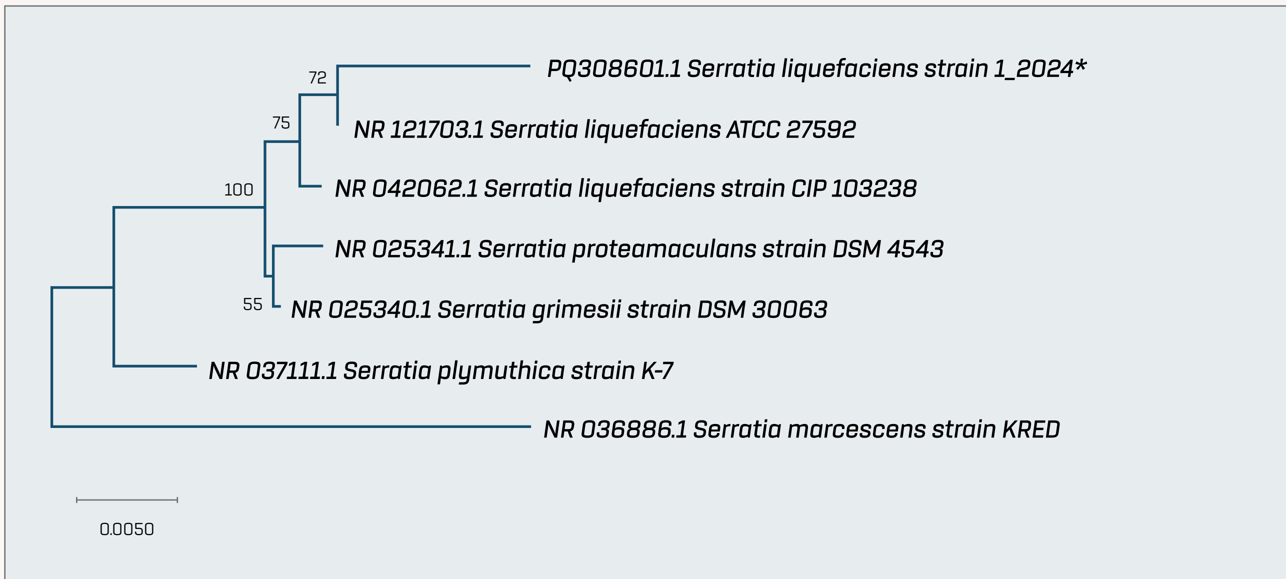

Figure 3. Dendrogram illustrating genetic similarity between *S. liquefaciens strain 1_2024* and other members of the Serratia genus.

DISCUSSION

The ubiquity of *S. liquefaciens* makes it a subject of particular concern within the framework of the "One Health" concept, which recognizes the close interdependence between the health of humans, domestic and wild animals, plants, and the environment, including entire ecosystems (30). *S. liquefaciens* is a notable opportunistic pathogen with zoonotic potential and is responsible for nosocomial infections, including those in veterinary settings. It exhibits resistance to multiple antibiotics and has the ability to form biofilms, thereby posing a threat to poultry, cattle, salmonids, trout, turbot, and various companion animals (16, 21, 31, 32, 33, 34, 35, 36).

S. liquefaciens is a member of the Enterobacteriaceae family and is prevalent in both environmental settings and cattle populations. Infection often occurs through water, feed, or infected ambient items. It is essential to consider the findings of several authors (37), which indicate that the source of histamine in seafood, which may lead to human food poisoning, is histamine-producing bacteria, such as *S. liquefaciens.*

Favorable factors for the penetration, reproduction, and pathogenic activity of *S. liquefaciens* in the animal body include stress of various origins (such as poor housing conditions, inadequate nutrition, and concurrent infections) as well as immunodeficiency states. In poultry, clinical manifestations may include septicaemia, respiratory symptoms, purulent skin and subcutaneous lesions, enteritis, arthritis, and tendovaginitis (18).

The optimal temperature for the proliferation of *S. liquefaciens* is 37°C. Under these conditions, motile cells with elevated proteolytic activity are capable of synthesizing pigment. Research indicates that certain *Serratia* strains can produce thermostable (ST) and thermolabile (LT) enterotoxins, similar to those produced by *Escherichia coli* (16).

The obtained isolate *S. liquefaciens* 1/2024 exhibited resistance to penicillin group antibiotics (Ampicillin/Sulbactam, Amoxicillin-Clavulanic acid, Ticarcillin/Clavulanic acid), cephalosporins (Cephalothin, Cefuroxime), among others, corroborating findings from several authors (14, 38).

It should be noted that the strain remains sensitive to carbapenems, fluoroquinolones, aminoglycosides, and tetracyclines, which may indicate the absence of specific resistance mechanisms to antimicrobial agents.

At present, antimicrobial resistance is considered a key factor contributing to pathogenicity, based on current scientific understanding. As a result, infections caused by antibiotic-resistant organisms in susceptible individuals can lead to prolonged treatment durations, increased mortality rates, and the dissemination of resistant bacterial clones in the environment. The growing prevalence of antibiotic resistance among pathogens poses a significant challenge to the healthcare system (39,40). The global scale of antibiotic resistance among microorganisms necessitates urgent action to prevent the emergence and spread of antibiotic-resistant strains (41, 42, 43).

Our findings demonstrate the ability of *S. liquefaciens 1/2024* strain to produce biofilms. Numerous authors (44, 45, 46) have noted that biofilm formation is a common ecological strategy among various bacteria, providing significant advantages such as increased resistance to antibiotics and the ability to evade host immune responses.

The experimental study investigated the effect of temperature (23 °C and 37 °C) on variables obtained from seven independent replicates in each group. Preliminary statistical analysis revealed that the sample data did not follow a normal distribution, confirming the need for nonparametric method of analysis.

Therefore, the Mann-Whitney U test, one of the most commonly used nonparametric tests for analyzing independent samples, was used to test the hypothesis that there were statistically significant differences between the groups. The analysis revealed a significant difference ($p = 0.001$), indicating that temperature has a substantial effect on the studied parameter. The obtained value of $p = 0.001$ is statistically significant ($p < 0.05$), allowing for the rejection of the null hypothesis of equal distributions. These findings highlight that temperature is a critical factor influencing the studied indicator. Therefore, the temperature regime should be carefully considered when conducting such experiments.

The Mann-Whitney U test is widely used in biomedical research (47), particularly when the data are ordinal or do not meet the assumptions of proportional odds. Additionally, several studies highlight that the Mann-Whitney U test is a reliable tool for analyzing non-normal distribution data and recommend its use in medical research (48). Therefore, the use of the Mann-Whitney U test for statistical analysis in this study is appropriate when the assumption of normal distribution is not met.

Thus, the obtained results allow us to conclude that the temperature regime influences the behavior or properties of the object under study. This indicates the need to consider the temperature factor when designing experiments and interpreting research findings, especially when environmental conditions may affect the outcome. Despite the absence of a normal distribution, which is common in small samples and experimental data in biology, medicine, and related sciences, the use of a nonparametric approach, specifically the Mann-Whitney test, ensured the reliability of the conclusions drawn.

The sequencing results of the 16S rRNA gene fragments, along with subsequent phylogenetic analysis, justified the inclusion of the *S. liquefaciens* 1/2024 strain in the GenBank NCBI database under the accession number PQ308601.1. This strain clustered with other strains of the species, notably the reference strain *S. liquefaciens* ATCC 27592, for which the complete genome sequence has been established (49, 50).

Consequently, the data obtained justify the use of the *S. liquefaciens* 1/2024 strain as a standard, namely for evaluating nutritional media productivity, conducting comparative assessments of field isolates, and testing novel antimicrobial drugs, among other applications.

Infectious diseases hinder sustainable societal development by affecting plant, animal, and human health, while also jeopardizing food security and the biodiversity of natural ecosystems. Contemporary challenges, such as climate change, population displacement due to armed conflicts, environmental and food issues, and the globalization of trade, are closely linked to the emergence of zoonotic diseases and have a detrimental impact on public health (1, 30, 40).

Research aimed at improving diagnostic techniques and studying the biological characteristics of infections is especially relevant in these challenging times.

CONCLUSIONS

1. The *S. liquefaciens* 1/2024 strain, isolated from chicken cloacal washings, exhibits typical characteristics of the *Serratia* genus and is pathogenic to white mice (10^8CFU).
2. It demonstrates biofilm-forming ability and multiple resistance to antimicrobial agents, including resistance to penicillins, cephalosporins, and fusidic acid.
3. Sequencing of the 16S rRNA gene fragment revealed 99.21% similarity to the reference strain *S. liquefaciens* ATCC 27592, and it is listed in the GenBank NCBI database under accession number PQ308601.1.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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ETHICAL APPROVAL

This study was approved by the Bioethics Commission of the National University of Life and Environmental Sciences (NULES) of Ukraine, decision No. 022/2024, dated November 26, 2024.

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Liliia Vygovska, WoS Researcher ID: ADC-0717-2022, SCOPUS ID 57222986117;

Artem Ushkalov, SCOPUS ID 58069384100;

Liubov Zelena, WoS Researcher ID: IDH-7309-2013;

Ushkalov Valerii, WoS Researcher ID: AAS-4217-2020, SCOPUS ID 59473747300;

Yuriy Vishovan, SCOPUS ID 58643511800.