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Research article

Integrated assessment of mucilage impact on human health using the One Health approach: Prevalence and antimicrobial resistance profiles of *Escherichia coli* and *Clostridium perfringens* in the Marmara Sea, Türkiye

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ABSTRACT

This study employed a One Health approach to assess the potential impact of mucilage on human health by characterizing the prevalence and antimicrobial resistance (AMR) profiles of *Escherichia coli* and *Clostridium perfringens* strains isolated during the 2021 mucilage event in the Marmara Sea, Türkiye. Mucilage, a gelatinous organic substance exacerbated by climate change, disrupts marine ecosystems by depleting oxygen, threatening biodiversity, and serving as a reservoir for pathogenic microorganisms. Surface and benthic mucilage samples collected from the Marmara Sea were analysed for AMR profiles using genome analysis, the BD Phoenix™ 100 automated system, and E-test methods. The study identified 13 *E. coli* and one *C. perfringens* strain, harboring 244 and six AMR genes from 21 and eight drug classes, respectively, along with multiple virulence factors (VFs). The *E. coli* strains exhibited four distinct serotypes (O138:H28 [Mu-3], O18:H49 [Mu-4], O128:H12 [Mu-35] and O101:H10 [Mu-125]), reported for the first time from Türkiye and mucilage. Notably, anaerobic microorganisms like *C. perfringens* thrived in mucilage, underscoring their ecological significance. Seasonal and climatic factors influencing mucilage formation amplify its role in transmitting antimicrobial-resistant pathogens, posing significant risks to public and environmental health. The findings highlight the urgent need for continuous monitoring and mitigation strategies for mucilage-related hazards.

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1. Introduction

Mucilage, also known as marine saliva or snow, is rich in organic components. Its prevalence has increased due to climate change, leading to small pellets that grow into larger formations covering the sea surface. While mucilage naturally occurs in small sizes in open oceans, mucilage blooms caused by global warming have led to the complete closure of the sea surface and its intrusion into deeper waters, causing oxygen deficiency and coating fish and other aquatic animals over the past decade [1]. Mucilage is a gelatinous and adhesive substance, rich in dissolved and polymeric organic matter, with hydrogel properties, dense and high viscosity. It comprises excreted, secreted, or leaked polymeric materials produced by various marine organisms, primarily extracellular polysaccharides. Due to its jelly-like and sticky characteristics, mucilage can contain various marine organisms, including viruses, bacteria, phytoplankton, and zooplankton [2].

The mucilage disaster that affected the entire Marmara Sea in May and June 2021 posed a significant threat to the ecosystem, marine life, and biodiversity in Türkiye. Insufficient wastewater treatment, intense industrial activities in the region, and global climate change contribute to this issue. Stagnant weather conditions, rising temperatures, and highly variable nutrient ratios in the sea content exacerbate the mucilage problem in the Marmara Sea, Türkiye. The gelatinous and colloidal exopolymers, secreted into the aquatic environment by some phytoplankton species under stress factors arising from external influences, play a crucial role in mucilage formation [1,3]. Mucilage can spread like a blanket, covering hundreds of kilometers of coastline, inhibiting oxygen transfer and resulting in the mass death of living organisms, fish eggs, and larvae. Moreover, mucilage can host pathogenic bacteria, including coliforms and *Escherichia coli*, as well as viruses that threaten marine flora and fauna. It also leads to clogging marine organisms' gills, negatively affecting fisheries and public health. The microbial community associated with mucilage is influenced by its origin, composition, developmental stage, and seasonal variations in environmental parameters. Mucilage represents a unique microhabitat with significantly higher concentrations of organic and inorganic nutrients than those in the surrounding water. Heterotrophic microorganisms and organisms that thrive on organic matter find mucilage a rich food source, creating a habitat and feeding area that attracts them. Mucilage can also act as a vehicle for transmitting pathogenic prokaryotes, including aerobic, anaerobic, and facultative aerobic bacteria species, which might not be found or might be present in lower concentrations in nearby seawater [1,3,4]. Consequently, the prokaryotic community associated with mucilage significantly differs from that of the surrounding water [1,2]. Additionally, mucilage contributes to the spread of antibiotic-resistant bacteria (ARBs) and their resistance genes, along with the dissemination of human and animal pathogenic species [5].

The Marmara Sea, surrounded by seven densely populated cities in Türkiye with various anthropogenic activities, has experienced more frequent mucilage blooms in the past two decades. These activities include household pollution, heavy industrialization, agricultural pollution, commercial fishing, shipyards, and specialized marine terminals with high maritime traffic density. In Spring 2021, a massive mucilage event in the Marmara Sea impacted the entire ecosystem, drawing government and public attention to detect and monitor this phenomenon. Besides the effects of mucilage on water and aquatic animal health, the potential of mucilage to concentrate high abundances of pathogenic bacteria highlights the associated risks for human contact. The World Health Organization (WHO) has identified *Campylobacter jejuni, C. coli, E. coli, Salmonella* spp., *Legionella* spp., *Shigella* spp., *Yersinia enterocolitica,* and *Vibrio cholerae* as the most significant waterborne pathogens threatening public health. The WHO still considers these "emergent diseases" and demands urgent attention and action. Contaminated water sources serve as reservoirs for these pathogens, and without proper water disinfection and sanitation practices, mortality rates are likely to increase [6].

E. coli, a mesophilic, Gram-negative facultative anaerobic rod, is commonly found in the gastrointestinal tracts of animals and humans, including their feces. The Environmental Protection Agency (EPA) recommends *E. coli* as an indicator of fecal pollution [6]. Although *E. coli* is generally considered commensal and harmless to animal and human health, it can cause illnesses such as swimming-associated gastroenteritis and food poisoning when contaminated marine products are consumed [7,8]. Some strains of *E. coli*, such as Shiga-toxigenic *E. coli* (STEC), can grow within a wide temperature range of 7–50 °C and produce toxins known as Shiga toxins or verotoxins. These toxins can cause severe intestinal infections, potentially leading to renal failure and death in humans [9]. In coastal environments, exceeding a certain threshold of *E. coli* concentration (500 CFU/100 mL^{-1}) restricts recreational activities like swimming and diving due to the potential for severe public health problems [10,11].

Clostridium perfringens, an obligate anaerobic, Gram-positive, rod-shaped, and spore-forming microorganism, is widely distributed in soil and aquatic environments. While *C. perfringens* can cause gas gangrene and two main types of food-borne ailments (food infection and intoxication) in humans, it also leads to severe diseases such as enterotoxemia and lethal necrotic enteritis in various animal species [12,13]. Previous studies have shown a correlation between the presence and concentrations of *C. perfringens* in environmental waters and the risk of infection from recreational water areas, including human pathogenic microorganisms [14,15]. Furthermore, *C. perfringens* has been associated with severe diseases like myositis, enterotoxemia, peritonitis, and septicemia in aquatic animals [16,17]. Due to its intestinal origin, resistance to disinfectants used in water treatment, inability to reproduce in seawater, and the persistence of its spores for months, *C. perfringens* has been proposed as an alternative fecal indicator bacteria (FIB) for monitoring microbial water quality, serving as a co-indicator for viruses and protozoa [18,19]. Given the serious public health threat posed by these bacteria species in seawater and aggregates or mucilage, simultaneous detection of *E. coli* and *C. perfringens* can better estimate the associated health risk.

In our study, we designed within one health concept the possible effect of mucilage on human health by isolating and characterizing *E. coli* and *C. perfringens* isolated from a massive mucilage event in Türkiye in 2021 that affected the whole Marmara Sea. Analyzing *E. coli* and *C. perfringes* regarding AMR and virulence genes sheds light on the zoonotic potency of these agents.

2. Materials and methods

2.1. Sampling

Sampling studies were carried out between November 2021 and July 2022 in Winter, Spring, and Summer Seasons in Northern Marmara (Cable Bay located around Istanbul, Heybeli Ada; 40°52′41.3″N/29°04′56.6″ E) and Southern Marmara (Balikesir, Bandirma; 40°21′53.86″N/27°59′3.52″ E) regions. In the seasonal sampling, the benthic mucilage found in each region was carried out by a diver who has experience in diving with scientific equipment and has mastered the sampling methodology. During the dives, samples were collected from both the depth of mucilage (Group 1) and the surface of the benthic mucilage area (Group 2) with the help of a sterile syringe with 50 mL capacity. Seawater samples (Group 3 and Group 4) were also collected from a region where mucilage was not present, provided that it was close to the station where it was taken and at the same depth. Four different sample groups and at least 5 L of water and mucilage samples were taken from each station [20]. After sampling, temperature, dissolved oxygen concentration, and pH were determined with a multiparameter water measuring device (SI analytics, HandyLab). Chlorophyll-a concentrations of water samples brought to the laboratory were determined according to the method suggested by Butler (1984) [21]. Following the sampling, the samples were transferred to the laboratory of Bursa Uludag University, Faculty of Veterinary Medicine, Aquatic Animal Diseases Department. The samples were transported in ice-cold containers within 6 h to the laboratory.

2.2. Isolation and identification of Escherichia coli and Clostridium perfringens

The standard membrane filtration method was used in the study. The isolation of *E. coli* and *C. perfringens* from seawater samples with and without mucilage was carried out according to ISO 9308–1:2014 [22] and ISO 14189:2013 [23] protocols, respectively. For this purpose, 10-fold serial dilutions were initially prepared for each sample, and 100 mL of the samples and appropriate dilutions were filtered through a 0.45 μm membrane filter. Filter papers were placed onto Tergitol TTC lactose agar. Petri dishes with membrane filters were then incubated aerobically at 44 °C for 24–48 h. All yellow/orange colored colonies were considered suspicious for *E. coli*. Gram stain, motility examination, and biochemical tests (indole, methyl red, Voges-Proskauer, citrate, oxidase, β-glucuronidase, lysine decarboxylase, hydrogen sulfide, and glucose, lactose, and sucrose to confirm suspicious colonies fermentation) were carried out for accurate characterization of the isolates.

For *C.perfringens*, tryptose sulfite cycloserine (TSC, Oxoid) agar supplemented with D-cycloserine (Oxoid) was used. Briefly, after filtration, membranes were placed on the surface of TSC agar plates. Then the membrane was covered with a second layer of melted TSC agar and incubated the plates anaerobically at 44 °C for 24 h. After incubation, one to 10 typical black (faint or distinct) or grey to yellow-brown colonies evaluated for presumptive *C. perfringens* were subcultured for purity on Brain Heart Infusion agar (BHI, Difco). Reduction nitrate to nitrite, motility test, and gelatine liquefaction were performed for biochemical confirmation.

For molecular identification DNA was extracted using a spin column filtration kit according to the manufacturer's instructions (QIAamp DNA mini kit). Identification and sequence analyses were performed based on the 16S rRNA gene region using the universal primers 27F and 1492R [24]. In addition, the multiplex polymerase chain reaction (PCR) amplification of *uidA* and *uspA* genes was used to identify *E. coli* isolates [25].

2.3. Antimicrobial susceptibility testing

The BD Phoenix™ 100 automated system (BD Diagnostic, Sparks, MD, USA) was used to evaluate the antimicrobial susceptibility and detect carbapenemase and extended-spectrum β-lactamases (ESBL)-producing *E. coli* isolates through minimum inhibitor concentration, MIC, by broth microdilution method [26]. Antimicrobial susceptibility testing for amikacin, amoxicillin, amoxicillin-clavulanate, ampicillin, ampicillin-sulbactam, cefazolin, cefepime, cefotaxime, ceftazidime, ceftazidime-avibactam, ceftolozane-tazobactam, ceftriaxone, cefuroxime, ciprofloxacin, ertapenem, gentamicin, imipenem, levofloxacin, meropenem, ofloxacin, piperacillin-tazobactam, tigecycline, tobramycin, and trimethoprim-sulfamethoxazole was performed using the NMIC/ID-433 and the NMIC/ID-505 panels mentioned above (BD Phoenix). The interpretation of the results was achieved by the system equipped with software suitable for the interpretation of AST results using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints [27] (document V13.1, 2023, https://www.eucast.org).

For antimicrobial susceptibility of *C. perfringens* isolates, the MIC for amoxicillin, metronidazole, vancomycin, levofloxacin, teicoplanin, benzylpenicillin, ceftazidime, erythromycin, clindamycin, rifampicin, cefotetan, tetracycline, ciprofloxacin, and cefotetan + cloxacillin determined by E-test method, according to the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI) [28]. Fastidious Anaerobe agar, a suitable medium for antimicrobial susceptibility testing, with 5 % defibrinated horse blood, was used. After anaerobic incubation at 37 °C for 24 h, the interpretation of the MIC values was considered according to the EUCAST guidelines [27] (document V13.1, 2023, https://www.eucast.org).

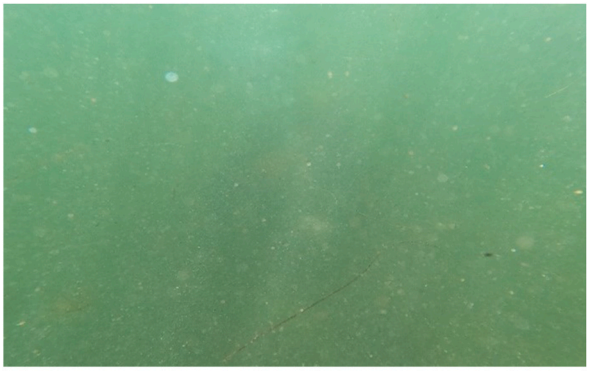
2.4. Genome sequencing

The *E. coli* strains Mu-3, Mu-4, Mu-35, Mu-125 and *C. perfringens* Mu-24 were chosen as the representative strain based on their sampling location. 50 ng of bacterial DNA was used for whole genome analysis. Whole-genome shotgun sequencing of bacterial DNA was performed by outsourcing using the Illumina Miniseq platform in the form of 2x150 bp PE. Fastq files obtained as a result of sequencing were trimmed using the default settings on the BV-BRC (Bacterial and Viral Bioinformatics Resource Center) online server

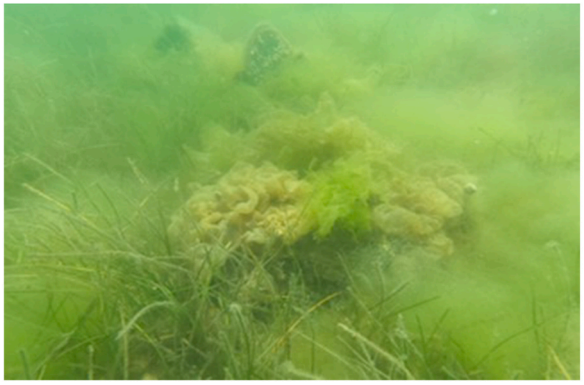
(https://www.bv-brc.org/) and combined using the Unicycler algorithm. Genome assembly/assembly errors were checked with Pilon v1.23 using default parameters [29]. The resulting draft genomes were uploaded to the TYGS (Type Strain Genome Server https://tygs.dsmz.de/) server and identified [30].

2.5. Genome analysis

AMR and virulence genes in the bacterial genome were individually identified by the blastp algorithm using the comprehensive Antimicrobial resistance database (CARD, database, version 3.1.0) and the virulence genes database (VFDB, core database containing 3658 experimentally validated virulence genes) [31–33]. In definition, threshold values; with e-value <1e-20 and identification rate ≥53 % and coverage ≥80 % for AMR genes [34], virulence genes with e-value <1 e−5 and identification rate ≥70 % and coverage ≥80 % [35]. If multiple virulence and AMR genes overlap at the same locus in the genome, only the best-aligned gene was considered [36]. In addition, the Antibiotic Resistant Target Seeker (ARTS) version 2 was employed to determine antimicrobial resistance models, duplicated and horizontally acquired genes [37]. The distribution of genomic islands involving VFs, AMR genes and other sequences with medical and environmental importance was examined on the IslandCompare software (v1.0) available at https://islandcompare.ca/ [38]. The plasmids in the genomes were predicted on the PlasmidFinder 2.1 available at https://cge.food.dtu.dk/services/PlasmidFinder/using default recommendations [39]. The detection of clustered regularly interspaced short palindromic repeats (CRISPR) and associated proteins (Cas) was acquired by the CRISPRCasFinder available at https://crisprcas.i2bc.paris-saclay.fr/ [40]. In addition, the potential to cause infection in humans was determined *in silico* with Pathogenfinder. Molecular serotyping was done using SerotypeFinder 2.0 (https://cge.food.dtu.dk/services/SerotypeFinder/). The annotation of secondary metabolite biosynthetic gene clusters was achieved by the antiSMASH version 7.0.0, available at https://antismash.secondarymetabolites.org/#!/start [41]. The dbCAN2 database [42] provided by the KBase server (https://www.kbase.us/) [43] was used to predict the carbohydrate-active enzymes (CAZymes). The genes related to exopolysaccharide production were annotated on the RAST server (https://rast.nmpdr.org/) [44]. Ecological distribution and habitat preferences of the strains were analysed on the Protologger software [45].



a



b

Fig. 1. Photos taken from the benthic mucilage sampling site.

3. Results

3.1. Sampling

Sampling studies were conducted between November 2021 and July 2022 in Northern Marmara (Cable Bay around Istanbul, Heybeli Ada; 40°52′41.3″N/29°04′56.6″E) and Southern Marmara (Balikesir, Bandirma; 40°23′18.19 It was made seasonally from "N/27°47′44.46″E) regions. During the sampling periods, no mucilage was observed on the sea surface, and mucilage samples were collected from the bottom with the help of a sterile injector (Fig. 1 [a, b]). The results regarding the water parameters are given in Table 1. Due to the natural reduction of mucilage and effective cleaning efforts, the mucilage layer at the bottom was determined to be the highest in the December 2021 period compared to the 2022 sampling groups.

3.2. Isolation and identification of E. coli and C. perfringens

A total of 13 typical *E. coli* colonies (eight from the northern Marmara Sea; five from the southern Marmara Sea) growth on Tergitol TTC lactose agar were obtained, and conventional tests were performed. All colonies (n:13) were Gram-negative, motile, indole (+), methyl red (+), Voges Proskauer (−), citrate (−), oxidase (−), β-glucuronidase (+), lysine decarboxylase (−), hydrogen sulfide (+), glucose, lactose and sucrose fermentation (+). The molecular method was employed by multiplex Polymerase Chain Reaction (mPCR), in which *uidA* and *uspA* genes were amplified. The bands with a length of 164 bp for the *uspA* region and 884 bp for the *uidA* region were considered positive (Data was not shown). Of these, a total of eight strains were isolated from the depth of the mucilage (Group 1), two from the surface of the benthic mucilage area (Group 2), two from the seawater surface without mucilage (Group 4), and one from the seawater of the benthic area without mucilage. Four (from the depth of mucilage; group 1) out of 13 *E. coli* strains isolated from 12 seawater and mucilage samples were selected for further analyses (antimicrobial susceptibility test and genome analyses).

Only one *C. perfringens* colony (from the depth of mucilage of the northern Marmara Sea; Group 1) isolated from a total of 12 samples, non-motile, reduced nitrate to nitrite, and liquefied gelatin in 48 h, was confirmed by the 16S rRNA sequencing analysis using the 27F and 1492R universal primers (Data was not shown). Purification and sequencing of PCR products giving 1465 bp bands as a result of amplification of the 16S rRNA region of the isolates using primers 27F and 1492R were performed with Sanger sequencing, and GenBank accession numbers were obtained after the raw data were trimmed and aligned. Information about the isolates is given in Table 2.

3.3. Antimicrobial susceptibility testing

The susceptibility patterns of *E. coli* and *C. perfringens* strains to antimicrobial agents are shown in Table 3. All *E. coli* strains in this study were susceptible to amikacin, amoxicillin-clavulanate, ceftazidime-avibactam, ceftolozane-tazobactam, ertapenem, gentamicin, imipenem, meropenem, piperacillin-tazobactam, and tobramycin. Resistance (intermediate and resistant) to tigecycline, cefazolin, and cefuroxime was found in 100 % of the strains. No resistance to carbapenems was detected in any of the *E. coli* strains. Three strains (Mu-4, Mu-35 [collected from Istanbul Province in the winter season], and Mu-125 [collected from Bandirma Province in the spring season]) were resistant to amoxicillin, ampicillin, ciprofloxacin, levofloxacin, and ofloxacin, while one isolate (Mu-3; derived from Bandirma Province in the spring season) was susceptible. Mu-3 *E. coli* strain has the least resistance (resistance to tigecycline, intermediate resistance to cefazolin, and cefuroxime) than the others. The Mu-4 strain, which was found ESBL positive, showed the highest AMR patterns (12 out of 24 antimicrobial agents).

In our study, the *C. perfringens* isolate exhibited susceptibility to the antimicrobial agents amoxicillin, metronidazole and vancomycin. However, we were unable to perform an antimicrobial evaluation for the following antimicrobials: levofloxacin, teicoplanin, benzylpenicillin, ceftazidime, erythromycin, clindamycin, rifampicin, cefotetan, tetracycline, ciprofloxacin, and cefotetan + cloxacillin. The reason for this limitation was the absence of published cutoff values for these antimicrobials in the CLSI and EUCAST guidelines. The antimicrobial susceptibility results for *C. perfringens* isolates are summarized in Table 3.

3.4. Genome analysis

Whole genome analysis was performed by selecting representatives from the bacterial pathogens that were detected most in mucilage by cultural analysis. DNA extraction of the molecularly identified isolates was carried out according to the spin column filtration method. According to this method, an average of 30 ng DNA samples were obtained from the isolates from which DNA was extracted. In the new generation sequence analysis made with service procurement, raw data trimming and assembly were done using the BV-BRC server. The main characteristics of the whole-genome sequence of strains are presented in Table S1.

AMR and virulence genes in the genomes were identified using CARD and VFDB databases. The antimicrobial and virulence gene numbers detected in the genomes are given in Fig. 2. These genes were found to encode resistance to many antimicrobial classes such as fluoroquinolones, macrolides, tetracyclines, aminoglycosides, and virulence genes related to pathogenicity features such as adhesion, motility, invasion, and biofilm formation. Detailed results are given in Table 4. Moreover, the duplicated and horizontally acquired genes, as well as the AMR models, were analysed by the ARTS server. The genomes of the *E. coli* strains have 77 duplicated genes (Table S2) within 1196 core genes responsible for the primary metabolism, while the numbers of the genes acquired by horizontal gene transfer were predicted as 409, 428, 534 and 561 for strain Mu-3, Mu-125, Mu-35 and Mu-4, respectively. In addition, the genomes include various AMR genes encodes mechanism, ATP-binding cassette (ABC) antibiotic efflux pump, Class C beta-lactamases,

Table 1
Physicochemical features of the water samples collected from the Marmara Sea.

Sampling Period		Group 1 Benthic Mucilage (4–6 m)			Group 2 Benthic Mucilage-Surface			Group 3 Mucilage-free benthic area (4–6 m)			Group 4 Mucilage-Free Surface		
		Winter 2021	Spring 2022	Summer 2022	Winter 2021	Spring 2022	Summer 2022	Winter 2021	Spring 2022	Summer 2022	Winter 2021	Spring 2022	Summer 2022
Northern Marmara	**Sample Code**	2021-1 IST1	2022-1 IST1	2022-2 IST1	2021-1 IST2	2022-1 IST2	2022-2 IST2	2021-1 IST3	2022-1 IST3	2022-2 IST3	2021-1 IST4	2022-1 IST4	2022-2 IST4
	Temperature (°C)	4.2	7.9	15.4	4.3	8.7	15.5	4.4	6.7	13.3	4.5	8.6	15.2
	Dissolved Oxygen (mg/L)	10.3	12.2	16.0	11.2	11.5	16.1	12.1	10.2	17.2	12.5	10.6	13.2
	pH	7.2	7.6	8.10	7.3	7.6	8.2	7.6	7.8	8.2	7.5	7.9	8.2
	Chlorophyll *a* (mg/l)	3.4	3.8	2.7	3.1	2.6	2.4	1.8	1.9	1.8	0.9	1.6	1.3
Southern Marmara	**Sample Code**	2021-1 BAN1	2022-1 BAN1	2022-2 BAN1	2021-1 BAN2	2022-1 BAN2	2022-2 BAN2	2021-1 BAN3	2022-1 BAN3	2022-2 BAN3	2021-1 BAN4	2022-1 BAN4	2022-2 BAN4
	Temperature (°C)	7.2	10.5	16.1	7.9	10.5	16.1	7.1	10.5	16,1	8.0	10.5	16.1
	Dissolved Oxygen (mg/L)	12.5	11.6	13.1	12.9	11.9	13.5	12.1	11.0	12.7	12.8	11.6	13.4
	pH	7.0	7.2	7.4	7.2	7.3	7.5	7.5	7.8	7.9	7.6	7.7	7.6
	Chlorophyll *a* (mg/l)	3.1	3.3	2.9	2.7	2.9	2.3	2.0	1.5	2.2	1.1	1.8	1.4

Table 2
Location and NCBI GenBank accession number information of the isolates.

Isolate No	Group	Period	Region	16S rRNA and Whole Genome Accession Number
Mu-2	1	December-2021	Istanbul	OP889001 - Nd[a]
Mu-3	1	December-2021	Bandirma	OP889002 - JAPQMH000000000
Mu-4	1	December-2021	Istanbul	OP889003 - JAPQMI000000000
Mu-5	2	December-2021	Bandirma	OP889004 - Nd
Mu-7	1	December-2021	Istanbul	OP889005 - Nd
Mu-10	2	December-2021	Istanbul	OP889006 - Nd
Mu-24	1	December-2021	Istanbul	OR257476 - JAUHQF000000000
Mu-33	4	December-2021	Istanbul	OP889007 - Nd
Mu-35	1	December-2021	Istanbul	OP889008 - JAPQMJ000000000
Mu-38	3	December-2021	Istanbul	OP889009 - Nd
Mu-125	1	March-2022	Bandirma	OP889010 - JAPQMK000000000
Mu-129	1	June-2022	Bandirma	OP889011 - Nd
Mu-131	1	July-2022	Istanbul	OP889012 - Nd
Mu-163	4	December-2021	Bandirma	OP889013 - Nd

[a] Nd: Not Defined

Table 3
Antibiotic susceptibility results of *E. coli* and *C. perfringens* isolates.

Drug	*E.coli*								*C. perfringens*		
	Mu-3		Mu-4[a]		Mu-35		Mu-125		Drug	Mu-24	
	MIC/ Conc	SIR	MIC/ Conc	SIR	MIC/ Conc	SIR	MIC/ Conc	SIR		MIC/ Conc	SIR
Amikacin	8	S	8	S	4	S	8	S	Amoxicillin	0.016	S
Amoxicillin	4	S	>32	R	>32	R	>32	R	Benzylpenicillin	0.064	ND
Amoxicillin-clavulanate	8/2	S	8/2	S	8/2	S	8/2	S	Cefotetan	0.50	ND
Ampicillin	4	S	>16	R	>16	R	>16	R	Cefotetan + Cloxacillin	0.50	ND
Ampicillin-sulbactam	2/8	S	4/8	S	2/8	S	4/8	R	Clindamycin	3	ND
Cefazolin	>32	R	>32	R	>32	R	>32	R	Ceftazidime	0.19	ND
Cefepime	1	S	8	R	1	S	1	S	Ciprofloxacin	0.50	ND
Cefotaxime	1	S	>4	R	1	S	1	S	Erythromycin	6	ND
Ceftazidime	1	S	8	R	1	S	1	S	Levofloxacin	0.25	ND
Ceftazidime-avibactam	0.25/4	S	0.25/4	S	0.25/4	S	0.25/4	S	Metronidazole	1	S
Ceftolozane-tazobactam	0.5/4	S	0.5/4	S	0.5/4	S	0.5/4	S	Rifampicin	0.016	ND
Ceftriaxone	1	S	>4	R	1	S	1	S	Teicoplanin	0.50	ND
Cefuroxime	>16	R	>16	R	>16	R	>16	R	Tetracycline	1	ND
Ciprofloxacin	0.125	S	>1	R	1	R	>1	R	Vancomycin	1	S
Ertapenem	0.25	S	0.25	S	0.25	S	0.25	S			
Gentamicin	2	S	1	S	0.5	S	1	S			
Imipenem	0.5	S	0.25	S	0.25	S	0.25	S			
Levofloxacin	0.5	S	1	R	2	R	>8	R			
Meropenem	0.125	S	0.125	S	0.125	S	0.125	S			
Ofloxacin	0.5	S	2	R	2	R	>2	R			
Piperacillin-tazobactam	4/4	S	4/4	S	4/4	S	4/4	S			
Tigecycline	1	R	1	R	1	R	1	R			
Tobramycin	2	S	2	S	1	S	2	S			
Trimethoprim-sulfamethoxazole	1/19	S	2/38	S	1/19	S	>8/152	R			

[a] Extended spectrum beta-lactamase.

resistance-nodulation-cell division (RND) antibiotic efflux pump and another efflux pump conferring antibiotic resistance proteins. The strains were also analysed for the distribution of virulence genes, and other genetic features, such as mobile genetic elements, organized into genomic islands (GIs) by comparing with the genomes of the notorious strain O157:H7 and non-pathogenic strain K-12. The GI prediction and comparison conducted on the IslandCompare software revealed that seven GIs harboured by the genome of strain O157:H7 are shared by at least one *E. coli* strain in the present study (Fig. S1). In addition, the phylogenetic tree constructed by Parsnp v1.2 [46] based on single nucleotide polymorphisms (SNPs) in the core genome of the *E. coli* strains implies a close relationship between strain O157:H7 and strain Mu-3. Consistent with all these findings revealing the virulence potential of the strains, the PathogenFinder analysis indicated that strains Mu-3, Mu-4, Mu-35 and Mu-125 were predicted as potential human pathogens with the probability of 0.936, 0.938, 0.940 and 0.943, respectively. The number of pathogenic protein families ranged between 489 and 681 across the *E. coli* strains. The PlasmidFinder detected two plasmid replicons in the genomes of strains Mu-3, Mu-4 and Mu-35, while three plasmid replicons were revealed in the genome of Mu-125 (Table S3). Strains Mu-35 and Mu-125 have IncX1 plasmid replicon with a relatively high identity ratio of 98.93 % and 98.86 %. This conjugative plasmid was reported to be associated with livestock

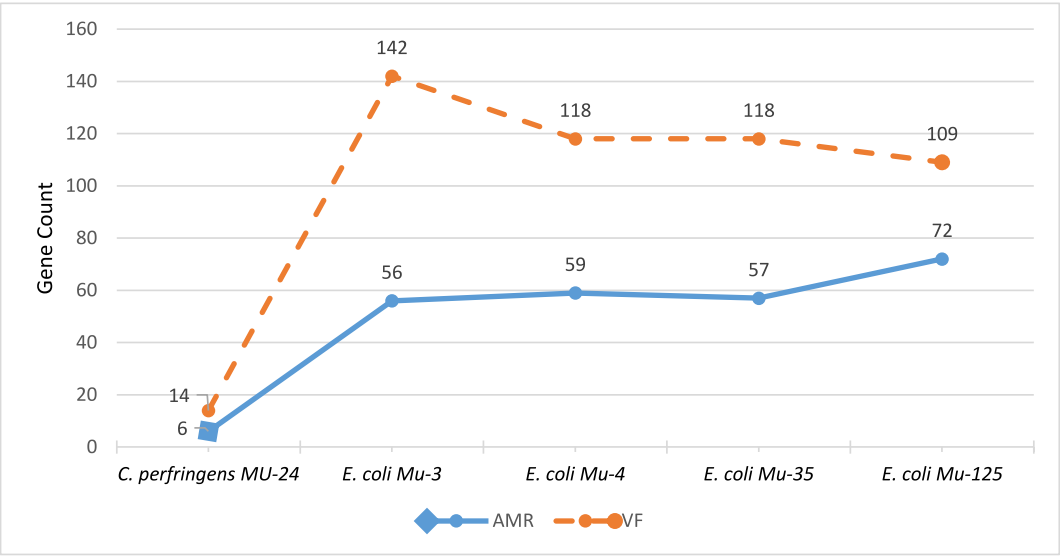


Fig. 2. Antimicrobial resistance (AMR) gene and virulence gene numbers of *E. coli* and *C. perfringens* strains used in the study.

animals, and it might be involved in biofilm formation and multidrug efflux [47,48]. All *E. coli* strains, except Mu-35, harbour IncFII plasmid, a narrow-host range plasmid frequently detected among *E. coli* strains [49]. In previous studies, the presence of an IncFII-type plasmid was implicated in increased invasiveness and virulence of *E. coli* strains [50]. The presence of IncII plasmid replicon in the genome of strain Mu-125 was confirmed by a high level of identity and confidence. The genomes were also analysed for the presence of CRISPR arrays and their associated proteins, i.e. Cas proteins. In the genome of strain Mu-3, a CRISPR array involving 11 spacers was identified with high evidence level. The strain has a CAS-TypeIE cluster containing eight genes, namely cas2_TypeIE, cas1_TypeIE, cas6_TypeIE, cas5_TypeIE, cas7_TypeIE, cse2_TypeIE, cse1_TypeIE, cas3_TypeI. Strain Mu-3 also has five more CRISPR arrays confirmed by low evidence levels. Similarly, strain Mu-4 encodes the CAS-TypeIE cluster with eight genes as well as another CAS cluster including the genes cas3_TypeI and cas3_TypeI. The genome of strain Mu-4 has two CRISPR arrays confirmed by high evidence levels and two arrays with low evidence levels. The annotation for the secondary metabolite biosynthetic gene clusters revealed that four *E. coli* strains have gene clusters for enterobactin and a thiopeptide. In addition to these metabolites, strain Mu-3 has an aryl-polyene gene cluster, while strain Mu-35 encodes a RiPP-like metabolite.

The whole-genome-based *in silico* serotyping of *E. coli* strains revealed that all strains encode O-antigen and H-antigen genes. Two O-antigen processing genes, *wzm* and *wzt,* encoding a transporter protein and an ATP-binding component, respectively, were detected in the genome of strain Mu-125 with a high confidence level. The other three strains harboured the genes encoding for O-antigen flippase (*wzx*) and O-antigen polymerase (*wzy*). In terms of H-antigens, the flagellin gene *fliC* was detected in the genomes of all four strains (Table S4).

Exopolysaccharides found either as the encapsulating material that closely surrounds a bacterial cell or as mucus dispersed throughout the environment play an important role in cooperation and competition within and between bacterial species to enhance their survival in natural environments [51]. In our study, the genomes of all four *E. coli* strains encode genes mainly responsible for the biosynthesis of capsular polysaccharides such as capsular polysaccharide transport protein YegH, polysaccharide export protein YccZ precursor and colanic acid protein WcaK. The strains also harbour genes for putative periplasmic protein YibQ having distant homology with nucleoside diphosphatase and polysaccharide deacetylase. A gene coding for an inner membrane protein YghQ, which may involved in polysaccharide biosynthesis, was detected in the genomes of strains Mu-3 and Mu-4. In addition, strain Mu-3 has genes coding for capsular polysaccharide synthesis enzymes CspC and CspD, required for polysaccharide export and exopolysaccharide synthesis, respectively, while the genome of strain Mu-4 encodes a polysaccharide deacetylase gene.

The carbohydrate-active enzymes (CAZymes) encoded by the genomes of strains *E. coli* Mu-3, Mu-4, Mu-35 and Mu-125 were predicted by the KBase server. The strains have around 300 CAZymes belonging to glycoside hydrolase (GH), glycosyl transferase (GT) and carbohydrate esterase (CE) families, as well as several enzymes with auxiliary activities (AA). Strain Mu-35 also encodes a carbohydrate-binding module (CBM) (Fig. 3).

The genome-based analysis of ecological distribution and habitat preferences revealed that the *E. coli* strains in our study have matched 1332 metagenome-assembled genomes (MAGs) mainly originating from human hosts (Fig. S2). The 16S rRNA gene-based amplicon comparison showed that the *E. coli* strains occurred in all habitats curated by the Protologger software with the highest percentages in the human gut, chicken gut, pig gut, human vagina and activated sludge (Table S5).

The in-depth annotation of the genome of *C. perfringens* Mu-24 indicated that the strain has 51 duplicated genes and 99 genes acquired by horizontal gene transfer events. A total of 25 known resistance models were detected in the genome of strain Mu-24, including genes coding for ATP-binding cassette (ABC) antibiotic efflux pumps, Class A beta-lactamase, major facilitator superfamily (MFS) antibiotic efflux pump, tetracycline resistance ribosomal protection protein that protects RNA-polymerase from tetracycline

Table 4
Information on the AMR and virulence genes detected in the genomes the strains.

***Escherichia coli* Mu-3**	
Antimicrobial Resistance Genes	
Genes	**Drug Class**
gadW, mdtG, H-NS, evgA, cpxA, acrB, Ecol_acrA, kdpE, msbA, mdtH, ugd, mdtA, mdtB, mdtC, YojI, PmrF, evgS, emrR, emrB, bacA, AcrS, AcrE, AcrF, mdtF, mdtM, marA, baeS, baeR, acrD, emrA, gadX, mdtE, CRP, mdtP, mdtO, mdtN, eptA, TolC, Ecol_mdfA, emrK, emrY, EC-14, LptD, Kpne_KpnF, eptB, Kpne_OmpK37, ArnT, OmpA, Paer_soxR, arnA, MexB, rsmA, sdiA, MdtK, rosB, rosA	Penam, fluoroquinolone, macrolide, phosphonic acid, cephalosporin, tetracycline, cefamycin, aminoglycoside, nitroimidazole, peptide, aminocoumarin, glycylcycline, rifamycin, nucleoside, lincosamide, penem, carbapenem, monobactam, sulfonamide, and diaminopyrimidin
Virulence Genes	
Genes	**Mechanism**
vgrG/tssI, csgB, cgsD, cgsE, shuV, ykgK/ecpR, yagY/ecpB, fimD, chuA, chuU, entB, yagX/ecpC, cgsG, fepA, fimB, fimE, entC, yagZ/ecpA, yagW/ecpD, fimA, hcp2/tssD2, fepD, rpoS, chuX, fepB, gspG, fur, espR1, cgsF, fepC, tssF, tssG, ibeB, csgC, fha, entS, chuT, ibeC, huS, fimG, tssB, fimC, yagV/ecpE, csgA, gspD, entE, TraJ, chuW, entA, fimH, fimF, tssJ, chuY, tssL, gspE, espL4, fimI, gspK, gspH, espX2, fepG, espL1, fdeC, espX5, aslA, rcsB, gspL, tssA, entF, gspJ, fepE, fes, gspF, espY5, espY1, espY3, gspI, gndA, gspC, entD, espX1, espY2, phoP, espX4, allB, espR4, cheY, rhs/PAAR, espX6, galF, gmd, flgG, allR, cheB, phoQ, flhA, cheW, acrA, fliM, tufA, fliA, fliG, flgC, fliI, allD, flhC, kdsA, espY4, flgH, motA, fliP, allC, flgI, rfaD, cheZ, fliQ, flmH, lpxC, csrA, rfbK1, htpB, flgD, fliN, gmhA/lpcA, allA, lpfA, galU, allS, cheA, luxS, vipB, cheR, flhD, fimZ, fimD, flgB, rfaE, fimH, lpfB ve spaP	Adhesion, Type III-VI Secretion system effector, Iron uptake, Nutrition/ Metabolic factor, Invasion, Capsule, Mobility, Immune modulation, Biofilm formation
***Escherichia coli* Mu-4**	
Antimicrobial Resistance Genes	
Genes	**Drug Class**
gadW, mdtG, H-NS, evgA, cpxA, acrB, Ecol_acrA, kdpE, msbA,mdtH, ugd, mdtA, mdtB, mdtC, YojI, PmrF, evgS, emrR, emrB, bacA, AcrS, AcrE, AcrF, mdtF, mdtM, marA, baeS, baeR, acrD, emrA, gadX, mdtE, CRP, mdtP, mdtO, mdtN, eptA, CTX-M-15, TolC, Ecol_mdfA, emrK, emrY, Ecol_emrE, EC-18, LptD, Kpne_KpnE, Kpne_KpnF, eptB, Kpne_OmpK37, ArnT, OmpA, Paer_soxR, arnA, rsmA, QnrS1, sdiA, MdtK, rosB, rosA	Penam, fluoroquinolone, macrolide, phosphonic acid, cephalosporin, tetracycline, cefamycin, aminoglycoside, glycylcycline, rifamycin, phenicol, nitroimidazole, peptide, aminocoumarin, nucleoside, lincosamide, penem, carbapenem, monobactam, and diaminopyrimidine
Virulence Genes	
Genes	**Mechanism**
tssB, hcp1/tssD1, csgB, cgsD, cgsE cfaD/cfaE, cfaC, cfaB, fimH, fepB, cgsG, tssL, fepAş, cfaA, fha, entS, yagZ/ecpA, tssJ, hcp2/tssD2, fepD, rpoS, tssA, fur, yagX/ecpC, cgsF, fepC, entC, tssF, ompA, entB, entE, ibeB, gspD, yagY/ecpB, ibeC, tssG, yagW/ecpD, gspK, kgK/ecpR, csgC, vgrG/tssI, gspG, entA, gspE, gspJ, gspF, espL4, yagV/ecpE, csgA, rhs/PAAR, fepG, gspH, rcsB, entF, fes, aslA, gndA, gspL, entD, espL1, gspC, phoP, fepE, espX5, gspI, gtrB, fdeC, acrB, cheY, allB, espX1, galF, gmd, espX4, flgG, allR, cheB, phoQ, flhA, cheW, acrA, hcp/tssD, fliM, tufA, fliA, fliG, fliI, allD, ugd, flhC, flgC, kdsA, 2 flgH, motA, fliP, allC, flgI, rfaD, vipB/tssC, KP1_RS17305, cheZ, fliQ, flmH, lpxC, csrA, rfbK1, htpB, flgD, gmhA/lpcA, allA, galU, fliN, allS, luxS, cheA, lpfA, lpfC, vipB, vipA/tssB, clpV/tssH, cheR, KP1_RS17340, flhD fimZ, flgB, fimD, rfaE, nueA, fimH	Adhesion, Type III-VI Secretion system effector, Iron uptake, Nutrition/ Metabolic factor, Invasion, Immune modulation, Mobility, Biofilm formation
***C.perfringens* Mu-24**	
Antimicrobial Resistance Genes	
Genes	**Drug Class**
Cper_mprF, tetA(P), tetB(P), efrA, TaeA, arlR	Tetracycline, peptide, fluoroquinolone, macrolide, rifamycin, pleuromutilin, disinfecting agents and antiseptics
Virulence Genes	
Genes	**Mechanism**
cloSI, colA, groEL, nagH, nagI, nagJ, nagK, nagL, nanH, nanI, nanJ, pfoA, plc, tufA	Adherence, Alpha-toxin, Alpha-clostripain, Kappa-toxin, Mu-toxin, Sialidase, Theta-toxin
***Escherichia coli* Mu-35**	
Antimicrobial Resistance Genes	
Genes	**Drug Class**
gadW, mdtG, H-NS, evgA, cpxA, acrB, Ecol_acrA, kdpE, msbA, mdtH, ugd, mdtA, mdtB, mdtC, YojI, PmrF, evgS, emrR, emrB, bacA, AcrS, AcrE, AcrF, mdtF, mdtM, marA, baeS, baeR, acrD, emrA, gadX, mdtE, CRP, mdtP, mdtO, mdtN, eptA, TolC, Ecol_mdfA, emrK, emrY, Ecol_emrE, EC-14, LptD, Kpne_KpnE, Kpne_KpnF, eptB, Kpne_OmpK37, ArnT, OmpA, Paer_soxR, arnA, rsmA, sdiA, MdtK, rosB, rosA	Penam, fluoroquinolone, macrolide, phosphonic acid, cephalosporin, tetracycline, cefamycin, aminoglycoside, glycylcycline, rifamycin, phenicol, nitroimidazole, peptide, aminocoumarin, nucleoside, lincosamide, penem, carbapenem, monobactam, and diaminopyrimidine
Virulence Genes	

(*continued on next page*)

Table 4 (*continued*)

***Escherichia coli* Mu-3**	
Genes	**Mechanism**
tssF, hcp1/tssD1, csgB, cgsE, mrkB, fimE, fimE, mrkC, fepB, cgsG, cgsD, mrkF, entC, fimD, hcp2/tssD2, fepD, tssB, rpoS, fepA, fur, entB, entS, cgsF, fepC, vgrG/tssI, csgC, gspD, ibeC, espL1, fimF, fimH, entE, ibeB, tssA, entA, fimG, fimC, gspK, gspG, gspJ, mrkD, vgrG/tssI, gspE, fimG, vgrG/tssI, fimI, csgA, fepG, gspF, entF, rcsB, espX5, gspH, aslA, entD, vgrG/tssI, fes, ompA, gndA, fepE, gspL, gspI, fdeC, gspC, espX5, phoP, vgrG/tssI, rhs/PAAR, gmd, mrkA, cheY, acrB, allB, gtrB, fimA, gmd, gmd, galF, espX4, flgG, cheB, allR, flhA, phoQ, cheW, acrA, rfbK1, fliM, fliA, fliG, flgC, KP1_RS17305, KP1_RS17305, fliI, espL4, allD, ugd, flhC, tufA, gmd, kdsA, motA, flgH, fliP, allC, tufA, rhs/PAAR, espX4, rfaD, flgI, acrB, cheZ, fliQ, rfbK1, flmH, rfbK1, lpxC, csrA, htpB, Hsp60, flgD, fliN, KP1_RS17305, gmhA/lpcA, allA, galU, allS, cheA, vipB, luxS, cheR, KP1_RS17340, flhD, fimZ, flgB, fimD, rfaE, acrB, fimH,	Adhesion, Type III-VI Secretion system effector, Iron uptake, Nutrition/ Metabolic factor, Invasion, Immune modulation, Mobility, Biofilm formation
***Escherichia coli* Mu-125**	
Antimicrobial Resistance Genes	
Genes	**Drug Class**
gadW, mdtG, H-NS, evgA, cpxA, acrB, Ecol_acrA, kdpE, msbA, mdtH, ugd, mdtA, mdtB, mdtC, YojI, PmrF, evgS, emrR, emrB, bacA, AcrS, AcrE, AcrF, mdtF, Ecol_ampC_BLA, mdtM, marA, baeS, baeR, acrD, emrA, gadX, mdtE, CRP, mdtP, mdtO, mdtN, eptA, aadA5, floR, TolC, Ecol_mdfA, mphA, emrK, emrY, catI, Ecol_emrE, tet(B), LptD, Kpne_KpnE, Kpne_KpnF, eptB, aadA2, Kpne_OmpK37, ArnT, OmpA, Paer_soxR, arnA, rsmA, APH(6)-Id, qacEdelta1, dfrA17, APH(3″)-Ib, linG, TEM-1, sdiA, MdtK, mphE, sul2, sul1, rosB, rosA	Penam, fluoroquinolone, macrolide, phosphonic acid, cephalosporin, tetracycline, cefamycin, aminoglycoside, glycylcycline, rifamycin, phenicol, nitroimidazole, peptide, aminocoumarin, nucleoside, lincosamide, carbapenem, monobactam, disulaminopyrimidine, and sulfonamide
Virulence Genes	
Genes	**Mechanism**
csgB, cgsE, yagZ/ecpA, fepB, fimE, gndA, fimD, ugd, galF, entB, cgsG, yagX/ ecpC, fimB, entC, fimF, fepD, rpoS, fepA, fur, entS, cgsF, fepC, fimC, ibeC, yagW/ecpD, cgsD, entE, KP1_RS17280, ibeB, ompA, yagY/ecpB, entA, fimH, csgC, fimI, rfbK1, fepG, yagV/ecpE, espL1, csgA, ykgK/ecpR, entF, espL4, rcsB, espX5, fimG, aslA, fes, KP1_RS17355, entD, fepE, KP1_RS17345, fdeC, phoP, espR1, espX1, allB, acrB, KP1_RS17340, cheY, galF, espX4, fimA, flgG, allR, cheB, phoQ, flhA, cheW, acrA, fliM, fliA, fliG, flgC, fliI, rhs/PAAR, allD, flhC, tufA, kdsA, motA, flgH, fliP, allC, flgI, rfaD, cheZ, espY1, fliQ, flmH, lpxC, csrA, htpB, fimZ, flgD, fliN, gmhA/lpcA, allA, galU, allS, cheA, luxS, espX4, cheR, flhD, flgB, rfaE, rcsA, fliR, exeG, nueA	Adhesion, Type III-VI Secretion system effector, Iron uptake, Nutrition/ Metabolic factor, Invasion, Immune modulation, Mobility, Biofilm formation

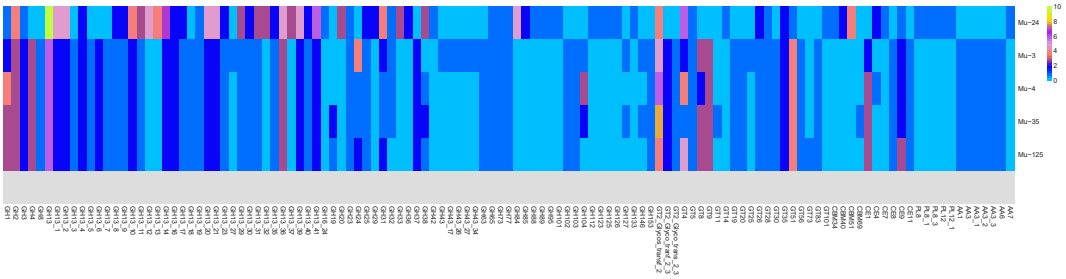


Fig. 3. Carbohydrate-active enzyme classes detected within the genomes of *C. perfringens* and *E. coli* strains.

inhibition and VanR, which is a transcriptional activator regulating VanA, VanH and VanX. Moreover, the PathogenFinder detected 355 pathogenic protein families encoded by the genome of *C. perfringens* Mu-24 and calculated the probability of strain Mu-24 being a human pathogen as 0.849. None of the plasmid replicons common in Gram-positive bacteria was found in the genome of strain Mu-24. On the other hand, strain Mu-24 has two CRISPR arrays verified by high evidence level as well as three Cas clusters. The biosynthetic gene cluster (BGC) detection performed on the antiSMASH server revealed that the genome of strain Mu-24 encodes four BGCs, including the genes coding for two cyclic-lactone-autoinducers, a ranthipeptide and a RiPP recognition element-containing cluster.

The genome of *C. perfringens* Mu-24 encodes 240 CAZymes belonging to glycoside hydrolase (GH), glycosyltransferase (GT), carbohydrate-binding module (CBM), carbohydrate esterase (CE), polysaccharide lyase (PL) and an enzyme with auxiliary activities (Fig. 3).

The ecological distribution and habitat preference analysis of *C. perfringens* Mu-24 showed that 61 MAGs mainly obtained from human hosts were matched with the genome of the strain (Fig. S3). The 16S rRNA gene-based analysis revealed that strain Mu-24

might be detected in pig gut, wastewater and activated sludge at higher ratios. However, the mean relative abundance of the strain was higher in plant metagenome, bovine gut and pig gut (Table S6).

4. Discussion

In the present study, we conducted an integrated assessment using the One Health approach to investigate the potential impact of mucilage on human health. The isolation and characterization of *E. coli* and *C. perfringens* strains revealed the hazards associated with a significant mucilage event that occurred in Türkiye in 2021, leading to widespread effects on the entire Marmara Sea ecosystem. The isolation of 13 *E. coli* and one *C. perfringens* strain harboring 244 AMR genes spanning from 21 drug classes and six genes from eight drug classes, respectively, in addition to virulence genes, underscored the paramount importance of mucilage for human and aquatic animal health, as it provides essential nutrients for microorganisms within aquatic ecosystems. Seasonal changes and global warming significantly influence the biological structure of aquatic ecosystems. The warming of surface water and increased water column stability can favor the formation of mucilage [1]. In recent decades, the occurrences of mucilage formation in marine environments have attracted interest due to socioeconomic and public health concerns. Mucilage formation in the Marmara Sea has been reported in several studies to investigate the environmental factors and causative organisms during mucilage formation.

During our sampling for this project, the mucilage event in 2021 expanded extensively, covering hundreds of kilometers of coastline in the study area. While the majority of mucilage clustered near the sea surface, some emerged during decomposition, forming a blanket-like cover across the water. Mucilage formation was detected as deep as 30 m below the surface in the Marmara Sea, with the highest density observed at depths ranging from 5 to 25 m during dives. The extent of coverage varied across locations and was influenced by weather conditions, including wind, currents, and waves. Unfortunately, after the "mucilage disaster," global warming has become one of the main problems in 2023, leading to sea temperatures reaching approximately 30 °C in the Mediterranean Sea and around 25 °C in the Marmara Sea by the end of July 2023 (data not shown).

Various phytoplanktonic algae, as well as eutrophication and increased temperature, were reported to have played a role in mucilage formation in the previous studies [52–54]. It is known that mainly diatom species producing extracellular polysaccharide substances are effective on mucilage formation, while some bacteria were also reported to contribute to the process [52,55,56]. In natural habitats, exopolysaccharides provide a matrix for bacterial communities to maintain their structural and functional integrities. However, in disturbed conditions like mucilage aggregates, they have become a hub for the dissemination of pathogens as well as VFs via horizontal gene transfer events. Notably, the increasing prevalence of multidrug-resistant *Enterobacteriaceae* has been of considerable concern in recent years due to a dramatic increase in nearly untreatable infections. The spread of multidrug resistance is highly dependent on mobile genetic elements (MGEs) such as conjugative plasmids, integrins and transposons, which allow for the accumulation and horizontal transfer of resistance genes or entire gene arrays. Thus, close monitoring of the spread and evolution of MGEs in general, and conjugative plasmids in particular, is an important means of tackling the dissemination of multidrug-resistant bacteria [47]. The present study revealed that the mucilage aggregates harbour pathogenic bacteria with a high potential to transfer their AMR genes and other VFs of importance for public health. In particular, the plasmid replicons and multidrug resistance elements encoded by *E. coli* strains might pose a threat to human and animal health. Moreover, the CRISPR arrays, as well as evidence of horizontal gene transfer in the genomes of the strains, imply their resilient nature to changing environmental conditions. The determination of a high amount of horizontally acquired AMR genes in *E. coli* strains in our study presented that mucilage is a suitable environment to transfer pathogenic microorganisms besides genetic elements causing the transfer of other microorganisms.

The gold standard for intraspecies classification of *E. coli* is phenotypic serotyping using antibodies targeting specific somatic (O) and flagellar (H) surface antigens and occasionally the capsular (K) antigens as well. Rapid, accurate, and inexpensive serotyping has long played an important role in surveillance and outbreak detection activities because it provides critical subtyping information quickly [57]. In recent decades, whole genome-based diagnostic tools have become very useful in replacing laborious laboratory practices such as DNA-DNA hybridization and serotyping analysis. In the present study, the *E. coli* strains were determined as O138:H28 (Mu-3), O18:H49 (Mu-4), O128:H12 (Mu-35) and O101:H10 (Mu-125). To the best of our knowledge, this is the first report of four serotypes from the mucilage event in Türkiye. Because mucilage is a formation seen on the sea surface or deep, we need to ask where these *E. coli* serotypes came from or where they go.

The whole-genome data can also be utilized to evaluate the functional and ecological characteristics of the bacteria. The ecological distribution and habitat preferences analyses revealed that the *E. coli* strains have distributed across different ranges of habitats but mainly dominated in gut habitats. The other strain, *C. perfringens* Mu-24, has appeared to be less encountered in different ecosystems while it matched mainly with the amplicons originated from wastewater and activated sludge as well as pig gut.

C. perfringens is a pathogenic bacterium within the *Clostridium* genus. This anaerobic microorganism exhibits spore-forming capabilities and is widely distributed throughout nature, being found in decaying vegetation, marine sediment, the intestinal tracts of humans and other vertebrates, insects, and soil [58]. Remarkably, it boasts the shortest reported generation time among all known organisms, requiring just 6.3 min for growth in a thioglycolate medium. In the context of the United States, *C. perfringens* ranks among the leading causes of food poisoning, alongside norovirus, *Salmonella*, *Campylobacter*, and *Staphylococcus aureus* [59]. It is important to note, however, that the ingestion of this bacterium may sometimes occur without causing harm. Our study focused on the isolation of *C. perfringens*, an anaerobic bacterium, which clearly demonstrated that mucilage not only serves as a favorable growth environment for facultative aerobes but also supports the growth of anaerobic microorganisms. The information regarding anaerobes in mucilages has been limited until now, and our results shed light on this overlooked aspect of mucilages in the context of the One Health concept. By understanding the significance of anaerobic microorganisms in mucilage, we contribute valuable insights to the broader understanding of mucilage's implications for human and environmental health.

Compared to the previous studies performed on the Eastern Mediterranean coasts [60–62] our findings revealed that *E. coli* was the most prevalent species in the northern Marmara Sea, consistent with previous studies by Cardak and Altug (2010) [60] and Sivri and Seker (2010) [61]. However, an intriguing contrast was observed when comparing our results to those of Celik et al. (2021) [63], who reported no detection of *E. coli* in mucilage samples collected from the Istanbul coastline. The discrepancy between our findings and those of Celik et al. (2021) [63] suggests potential variations in fecal contamination levels between the northern and southern regions of the Marmara Sea. This discrepancy is further supported by Sivri et al. (2018) [64], who found no presence of *E. coli* during winter months in the Marmara Sea. Our study highlights the importance of considering regional differences when assessing the prevalence of *E. coli* in marine environments. Furthermore, our investigation revealed that the Istanbul Province exhibited a higher abundance of *E. coli* compared to the Bandirma Province. This finding aligns with previous research on the high prevalence of *E. coli* strains in seawater samples and aquatic environments [65,66]. In another study conducted in Mexico, *C. perfringens* was detected in seawater, emphasizing the potential presence of various pathogens in marine environments [67]. To gain further insights into the distribution of *E. coli* and other pathogens in marine environments, Danovaro et al. (2009*)* [1] utilized microscopic and molecular techniques. Their investigation demonstrated higher concentrations of *E. coli* and other pathogens in mucilage compared to the surrounding seawater. In our study, we also found a higher abundance of *E. coli* strains in addition to *C. perfringens*, which is consistent with the previous findings [4,68]. The abundance of *E. coli* and *C. perfringens* in marine environments raises concerns about potential public health risks. Therefore, understanding the distribution and prevalence of these bacteria is crucial for ensuring the safety of coastal waters and safeguarding public health.

Our study sheds light on the prevalence and distribution of *E. coli* in different regions of Türkiye's marine environment. The observed variations in *E. coli* abundance between the northern Marmara Sea and the southern region, as well as between different provinces, underscore the need for region-specific monitoring and management strategies to mitigate potential health risks associated with fecal contamination in marine ecosystems. Considering relatively wide ecological distribution patterns and high potential for AMR and VFs as well as mobile genetic elements, the strains obtained from mucilage samples pose a serious threat to the environment as well as human and animal health.

In recent years, the global increase in AMR, hardened by the dissemination of antimicrobial-resistant bacteria and resistance genes, has emerged as a significant public health concern [69,70]. In marine environments, humans, marine sediments, and animals (e.g., seagulls) are key contributors to the dissemination of antimicrobial-resistant bacteria and related genes. In the current study, we investigated the presence and variability of antimicrobial-resistant bacteria and resistance genes in *E. coli* and *C. perfringens* strains from the Marmara Sea, Türkiye. The current study revealed the presence and variation of antimicrobial-resistant bacteria and resistance genes in *E. coli* and *C. perfringens* from the Marmara Sea. Antimicrobial-resistant *E. coli* strains were isolated from seawater [71,72] and marine sediments [66]. The present results showed that the AMR was not widespread (29.2 %) on both sites of the Marmara Sea, Türkiye, with resistance observed to at least 1 of the 24 antimicrobials tested. Our findings are compatible with previously published results on AMR genes research [73–75]. However, the levels of AMR observed in our study differed significantly from findings reported in Kuwait [76], Brazil [77], and China [78], revealing resistance levels above 67 % in *E. coli* isolated from seawater.

Notably, while ESBL-producing bacteria are generally resistant to carbapenem antibiotics [69,79], our study identified no carbapenem resistance in the *E. coli* O18:H49 strain (Mu-4). This observation aligns with the results of Kiffer et al. (2006) [80], who similarly found no resistance to carbapenems in *E. coli* isolates. The AMR detected in both *E. coli* and *C. perfringens* strains isolated from the Marmara Sea coastline indicates a high level of bacteriological pollution in the seawater. Anthropogenic activities, wastewater discharges, aquaculture, and animal excrement contribute to this pollution, posing an increased risk to aquatic ecosystems and public health.

Regarding the distribution of AMR genes, our study revealed that *E. coli* O101:H10 (Mu-125) strain had the highest frequency of AMR genes, followed by *E. coli* O18:H49 (Mu-4), *E. coli* O128:H12 (Mu-35) and *E. coli* O138:H28 (Mu-3) strains. The strains had AMR genes associated with resistance to aminoglycosides (*aph(6)-Id*, *aph(3″)-Ib*, *aadA2*, *aadA5*), beta-lactams (*bla*, *CTX-M-15*, and *TEM-1*), erythromycin (*mphA* and *mphB*), quaternary ammonium compounds (*qacEdelta1*), sulphonamides (*sul1* and *sul2*), tetracycline (*tet*(A), *tet*(B)), or trimethoprim (*dfrA17*). The *tet(B)*, *bla, aadA2* and *aadA5* genes were detected in *E. coli* O101:H10 (Mu-125) strain. Among the *E. coli* strains, *E. coli* O101:H10 (Mu-125) had ampC-type beta-lactamase encoding *Ecol_ampC_BLA,* whereas the others did not. This gene confers resistance to penicillin-like and cephalosporin-class antibiotics in *E. coli*. The $bla_{CTX\text{-}M\text{-}15}$ is a CTX-M group 1 variant, most commonly associated with animal and human infection. CTX-M-15-encoding *E. coli* have been detected in seawater [81], fish [82], various food samples [83], animals [84], and urinary tract-infected patients [85]. Similarly, *E. coli* O18:H49 [Mu-4] strain harboured the *CTX-M-15* gene and was phenotypically ESBL-positive.

The mucilage crisis in the Marmara Sea has highlighted the intricate interplay between environmental and public health factors, necessitating a comprehensive One Health perspective. The excessive input of nutrients—primarily nitrogen and phosphorus—into the Marmara Sea from untreated or poorly treated wastewater has acted as a catalyst for algal blooms and other phytoplankton proliferation, ultimately leading to mucilage formation. This phenomenon aligns with global observations, where eutrophication has been identified as a major driver of harmful algal blooms in marine systems [86].

From an environmental standpoint, the primary sources of nutrient influx into the Marmara Sea include agricultural runoff, industrial discharge, and domestic wastewater. These pollutants disrupt marine ecosystems and indirectly threaten human health by fostering conditions that promote the growth and dissemination of antimicrobial-resistant bacteria [87]. As demonstrated in this study, mucilage serves as a reservoir for pathogenic microorganisms, including antimicrobial-resistant bacteria, compounding public and environmental health risks. This dual impact underscores the urgent need for integrated mitigation strategies that address environmental degradation and public health risks. Recent research has consistently shown that urbanization and inadequate wastewater management significantly contribute to nutrient pollution in the Marmara Sea [88].

Improved wastewater treatment infrastructure and strict enforcement of environmental regulations are essential to reducing nutrient loads. Rising sea temperatures, driven by global climate change, exacerbate the effects of nutrient pollution by extending the growth periods for algae and phytoplankton [89]. This phenomenon underscores the importance of addressing climate change alongside local environmental management efforts. Introducing advanced treatment technologies, such as tertiary treatment and nutrient recovery systems, can significantly reduce nutrient discharge into the Marmara Sea [90]. Encouraging farmers to adopt precision fertilization and other nutrient management techniques can mitigate agricultural runoff. Educating communities about improper waste disposal's environmental and health consequences can foster collective action [91].

5. Conclusion

This study underscores the critical interplay between environmental degradation and public health, emphasizing the Marmara Sea's mucilage crisis as a pressing One Health challenge. The mucilage provides a conducive environment for antimicrobial-resistant pathogens, including *E. coli* serotypes and *C. perfringens*, posing significant risks to human, animal, and environmental health. To address this issue, it is vital to implement advanced wastewater treatment systems with nutrient recovery technologies, enforce strict controls on agricultural runoff, and enhance public awareness of sustainable waste management practices. Tackling these sources of eutrophication, combined with measures to monitor and mitigate the spread of AMR, will help protect the Marmara Sea ecosystem. These efforts must be integrated into a comprehensive One Health framework to ensure long-term environmental and public health resilience.

CRediT authorship contribution statement

Artun Yibar: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation. **Hilal Ay:** Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Fuat Aydin:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation. **Secil Abay:** Writing – review & editing, Validation, Software, Methodology, Formal analysis, Data curation. **Emre Karakaya:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Formal analysis, Data curation. **Tuba Kayman:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Conceptualization. **Cem Dalyan:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Fatih Dogan Koca:** Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Duygu Aydogdu:** Writing – review & editing, Writing – original draft, Validation, Software, Methodology, Investigation, Data curation, Conceptualization. **Nihed Ajmi:** Writing – review & editing, Writing – original draft, Methodology, Investigation. **Muhammed Duman:** Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Izzet Burcin Saticioglu:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Data availability

Not applicable.

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Declaration of competing interest

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Appendix A. Supplementary data

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