

Characterization of Antibiotic Resistance and Biofilm Formation in *Enterobacteriaceae* Isolated from Milk-Based Desserts

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ABSTRACT

This study investigated the antibiotic susceptibility patterns and biofilm-forming capacities of *Enterobacteriaceae* isolated from 30 commercially produced dairy-based dessert samples. *Enterobacteriaceae* were detected in 37% of the samples, yielding a total of 26 isolates. The isolates were obtained from supangle (n=1), trileçe (n=2), magnolia dessert (n=3), profiterole (n=7), and éclairs (n=13). Half of the isolates (50%) were susceptible to all antibiotics tested. The highest resistance was to ampicillin, with 23% of the isolates resistant and 8% showing intermediate resistance. Only one isolate (4%) was resistant to gentamicin, kanamycin, and streptomycin, while 8% exhibited intermediate resistance to ciprofloxacin. All isolates were susceptible to chloramphenicol, trimethoprim-sulfamethoxazole, tetracycline, cefoxitin, and ceftriaxone. It was determined that 96% of the isolates had the capacity to form biofilms. Among these, 58% produced weak biofilms, 27% strong biofilms, and 12% moderate biofilms. Overall, the findings highlight the presence of antibiotic-resistant and biofilm-forming *Enterobacteriaceae* in ready-to-eat dairy desserts, indicating potential risks related to microbial persistence and emphasizing the importance of effective hygiene practices in food production environments.

Keywords: *Enterobacteriaceae*; milk-based dessert; antibiotic resistance; biofilm

Sütlü Tatlılardan İzole Edilen *Enterobacteriaceae* İzolatlarında Antibiyotik Direnci ve Biyofilm Oluşumunun Karakterizasyonu

ÖZ

Bu çalışmada, satışa sunulan 30 sütlü tatlı örneğinden izole edilen *Enterobacteriaceae* izolatlarının antibiyotik duyarlılık paternleri ve biyofilm oluşturma kapasiteleri araştırılmıştır. Örneklerin %37'sinde *Enterobacteriaceae* tespit edilmiş ve toplam 26 izolat elde edilmiştir. İzolatlar supangle (n=1), trileçe (n=2), magnolia tatlısı (n=3), profiterol (n=7) ve ekler (n=13) örneklerinden izole edilmiştir. İzolatların %50'si test edilen tüm antibiyotiklere duyarlı bulunmuştur. En yüksek direnç ampisiline karşı belirlenmiş; izolatların %23'ü dirençli, %8'i ise orta düzey dirençli olarak saptanmıştır. Sadece bir izolatın (%4) gentamisin, kanamisin ve streptomisine dirençli olduğu, ayrıca izolatların %8'inin siprofloksasine karşı orta düzey direnç gösterdiği belirlenmiştir. Tüm izolatlar kloramfenikol, trimetoprim-sülfametoksazol, tetrasiklin, sefoksitin ve seftriaksona duyarlı bulunmuştur. İzolatların %96'sının biyofilm oluşturma kapasitesine sahip olduğu belirlenmiştir. Bu izolatların %58'i zayıf, %27'si güçlü ve %12'si orta düzeyde biyofilm üretmiştir. Genel olarak, bulgular tüketime hazır sütlü tatlılarda antibiyotik dirençli ve biyofilm oluşturan *Enterobacteriaceae*'nin varlığını göstermekte ve mikrobiyal kalıcılıkla ilişkili potansiyel risklere işaret etmekte ve gıda üretim ortamlarında etkili hijyen uygulamalarının önemini vurgulamaktadır.

Anahtar Kelimeler: *Enterobacteriaceae*; sütlü tatlı; antibiyotik direnci; biyofilm

1. INTRODUCTION

Dairy products, including milk-based desserts, serve as key nutritional sources, providing substantial energy in the human diet. Due to their rich nutrient composition, they provide a favorable environment for microbial growth, including pathogenic microorganisms. The *Enterobacteriaceae* family, commonly used as fecal indicator organisms in foods, plays a crucial role in this context. Moreover, it serves as an effective parameter for assessing inadequate hygiene conditions and deficiencies in food production processes. The *Enterobacteriaceae* family represents a heterogeneous group of widely distributed, Gram-negative, facultative anaerob, non-spore-forming, rod-shaped bacteria. While certain strains of these species exist as non-pathogenic commensals, certain members of this family, such as *Salmonella* spp., *Shigella* spp., and *Escherichia coli*, are important foodborne pathogens that pose a threat to public health (Mladenović et al., 2021).

Antimicrobial resistance (AMR) has emerged as an emerging global concern, threatening food and environmental safety, veterinary practice, and human treatment due to the potential transfer of resistance genes from commensal bacteria to pathogens or among pathogenic strains. AMR has been recognized by the World Health Organization as one of the top ten global public health threats facing humanity (Friedman, 2015; WHO, 2021).

Biofilms represent a significant challenge in the food processing industry. Bacteria can form biofilms on a variety of surfaces, including wood, polypropylene, glass, plastic, stainless steel, and even food, within a few hours to days. Biofilms provide microorganisms with protection against environmental stresses and enhance their resistance to antimicrobial agents, chemical disinfectants, and sanitizers, thereby contributing to microbial persistence and potential toxicity. Furthermore, biofilms formed by foodborne pathogens can substantially increase the risk of foodborne infections, posing serious public health threats and resulting in adverse economic consequences (Carrascosa et al., 2021; Liu et al., 2023).

The present study aimed to determine the antibiotic resistance profiles and biofilm-forming capacities of *Enterobacteriaceae* isolates obtained from commercially available milk-based dessert samples.

2. MATERIAL AND METHODS

2.1. Sample collection

A total of 30 dairy-based dessert products were randomly purchased in Edirne Province, Türkiye, between April and June 2025. The samples consisted of 4 supangles, 7 profiteroles, 10 éclairs, 4 magnolia desserts, and 5 trileçes. All samples were stored in refrigeration conditions during transportation and microbiological analysis was performed in a maximum period of 24 h from purchase.

2.2. Isolation of Enterobacteriaceae

10 g of each food sample was aseptically taken and added in 90 ml of phosphate buffer saline (PBS) as a diluent (Invitrogene, USA) and was homogenized in a stomacher (Isolab, Turkey) for 2 min and inoculated in Violet Red Bile Glucose (VRBG) Agar (Sigma - Aldrich, USA) using standard pour plate techniques and the plates were incubated for 24 h at 37°C. The oxidase test was performed, for confirmation, using oxidase strips (Sigma - Aldrich, USA) (ISO 21528-2). One to three presumptive colonies showing typical aspects of *Enterobacteriaceae* with dark purple colonies surrounded by reddish halos, were included in further studies.

2.3. Determination of Antibiotic Resistance

Disk diffusion method according to Clinical Laboratory Standards Institute guidelines (CLSI, 2020) on Mueller-Hinton agar (Merck, Germany) plates was used to determine the antibiotic resistance of *Enterobacteriaceae* isolates. A total of 10 antibiotics from six different classes were included in the study: β -lactams [ampicillin (AM, 10 μ g), cefoxitin (CTX, 30 μ g), ceftriaxone (CRO, 30 μ g)], aminoglycosides [gentamicin (CN, 10 μ g), kanamycin (KAN, 30 μ g), streptomycin (S, 10 μ g)], phenicols [chloramphenicol (C, 30 μ g)], tetracyclines [tetracycline (TE, 30 μ g)], fluoroquinolones [ciprofloxacin (CIP, 2 μ g)], and folate pathway inhibitors [trimethoprim-sulfamethoxazole (SXT, 1.25/23.75 μ g)]. Thus, the aim was to evaluate the broad resistance profiles of the isolates.

2.4. Determination of Biofilm Forming Ability

Biofilm forming abilities of *Enterobacteriaceae* strains were determined using crystal violet staining method with using 96-well flat-bottom polystyrene microplates (Greiner Bio-One), following a previously described method. (Stepanovic et al., 2004). Overnight (18 h) bacterial cultures were diluted in Luria Bertani (LB) without NaCl to an OD₅₇₀ of 0.2. The negative control wells contained only sterile broth. The experiments were run in triplicates. A total of 230 μ L of (LB) without NaCl was added to each well of a flat-bottom 96-well polystyrene microplate, followed by the addition of 20 μ L of the standardized bacterial suspension. The plates were incubated for 24 hours at 37°C. After incubation, the contents of the wells were discarded, and the wells were washed three times with 300 μ L of sterile distilled water. Then, 250 μ L of methanol (\geq 99.9%) was added to each well and incubated at room temperature for 15 minutes. After removing the methanol, the plates were air-dried. Each well was then stained with 250 μ L of 0.1% crystal violet and incubated at room temperature for 5 minutes. Excess stain was removed by rinsing the plates under tap water, followed by air drying. Bound dye was solubilized by adding 250 μ L of 33% (v/v) glacial acetic acid per well. The optical density of each well was measured at 590 nm using a Multiskan EX microplate reader (Bio-Rad). Biofilm forming abilities of isolates were determined for 24 h at 37°C.

Absorbance was measured by using a Multiskan EX reader (Bio-Rad) at 570 nm. Based on the OD results, isolates were categorized as follows: $OD \leq OD_c$ = non-biofilm producer, $OD_c < OD \leq 2 \times OD_c$ = weak biofilm producer, $2 \times OD_c < OD \leq 4 \times OD_c$ = moderate biofilm producer, $4 \times OD_c < OD$ = strong biofilm producer as previously described (Stepanovic et al., 2000). The OD value for each isolate was calculated as the mean of three replicates. OD_c (cut-off OD) was defined as the average OD of the negative control wells.

3. RESULTS AND DISCUSSIONS

Members of the *Enterobacteriaceae* family are widely distributed in nature and possess a high capacity to adapt to diverse environmental conditions, making their contamination of foods difficult to prevent. Their ability to grow over a wide pH range allows them to survive for extended periods in food products. In addition, many *Enterobacteriaceae* species of foodborne relevance are mesophilic, with optimal growth at approximately 37 °C, a characteristic that facilitates their persistence during food handling, processing, and storage. Several foodborne pathogens can survive harsh conditions in foods without losing pathogenicity, posing an ongoing risk of foodborne illness (Mladenović et al., 2021; He et al., 2021). This ecological versatility contributes to the long-term survival of *Enterobacteriaceae* in foods and highlights their importance as indicator organisms and potential reservoirs of antibiotic resistance and biofilm-forming strains.

In this study, *Enterobacteriaceae* was detected in 37% (11 out of 30) of dairy dessert samples. 26 *Enterobacteriaceae* strains were isolated. One isolate was recovered from supangle, two from trileçe (a milk-based sponge cake soaked in three types of milk), three from magnolia dessert (a creamy layered pudding dessert popular in Türkiye), seven from profiterole, and thirteen from éclairs (Table 1).

In our previous study on ready-to-eat foods, street-vended foods, salads, and dessert samples were microbiologically evaluated (Aksoy, 2021). The detection rate in dessert samples was determined to be 54.5%. In a study conducted in Egypt, *Enterobacteriaceae* were detected in 85% of ready-to-eat foods. In ice cream and yogurt samples included in the study, this rate was determined to be 87% and 80%, respectively (Mohammed et al., 2025). In contrast, a study conducted in China reported an *Enterobacteriaceae* detection rate of 12% in ready-to-eat foods (Liu et al., 2023). These findings indicate that *Enterobacteriaceae* detection rates in ready-to-eat foods, including desserts, vary considerably, which may be attributed to differences in production practices, hygiene standards, storage conditions, and regional food safety regulations. The detection of *Enterobacteriaceae* in food is not necessarily a direct sign of a public health threat; rather, it often reflects inadequate sanitation, suboptimal hygiene conditions, or improper handling procedures (Schwan et al., 2022). Ingesting contaminated foods is known to result in a wide range of foodborne diseases, exceeding 200 in number, and can occasionally cause persistent health

effects, especially in high-risk groups including the elderly, pregnant women, and infants (Mengistu et al., 2022). In this context, monitoring the antibiotic resistance profiles of foodborne pathogens, together with their biofilm-forming capacities that enhance persistence within food systems, is increasingly important.

Table 1: Source, antibiotic resistance profile and biofilm forming abilities of *Enterobacteriaceae* isolates

Strain code	Source	Antibiotic resistance profile	Biofilm forming ability (OD590)	Nature of biofilm
1	supangle	S (I)	0.264±0,041	moderate
2	profiterole	-	0.134±0,006	weak
3	éclair	-	0.132±0,007	weak
4	éclair	-	0.128±0,002	weak
5	éclair	CN	0.140±0,002	weak
6	éclair	-	0.127±0,004	weak
7	profiterole	CIP (I)	1.215±0,034	strong
8	profiterole	-	1.188±0,042	strong
9	profiterole	-	1.178±0,055	strong
10	éclair	CIP (I)	0.564±0,093	strong
11	éclair	K (I)	0.379±0,035	moderate
12	éclair	AM (I)	1.634±0,049	strong
13	éclair	-	0.143±0,007	weak
14	éclair	AM	0.135±0,002	weak
15	éclair	-	0.143±0,006	weak
16	profiterole	AM (I)	1.148±0,218	strong
17	profiterole	AM	0.241±0,013	weak
18	profiterole	AM	0.209±0,014	weak
19	éclair	-	0.162±0,011	weak
20	éclair	-	0.140±0,016	weak
21	éclair	AM	0.141±0,003	weak
22	magnolia dessert	-	0.139±0,008	weak
23	magnolia dessert	-	0.142±0,007	weak
24	magnolia dessert	-	0.121±0,002	no biofilm
25	trileçe	AM	0.726±0,051	strong
26	trileçe	AM	0.277±0,102	moderate

Among the *Enterobacteriaceae* isolates, 50% (13 strains) were susceptible to all 10 antibiotics tested in this study. The highest level of resistance was seen against ampicillin. Of the isolates, 23% (6 strains) were resistant and 8% (2 strains) were intermediately resistant to ampicillin. Only 4% (1 strain) of the isolates were resistant to gentamicin, kanamycin and streptomycin. In addition, 8% (2 strains) of the isolates exhibited intermediate resistance to ciprofloxacin. All isolates were susceptible to chloramphenicol, trimethoprim-sulfamethoxazole, tetracycline, cefoxitin, and ceftriaxone (Figure 1).

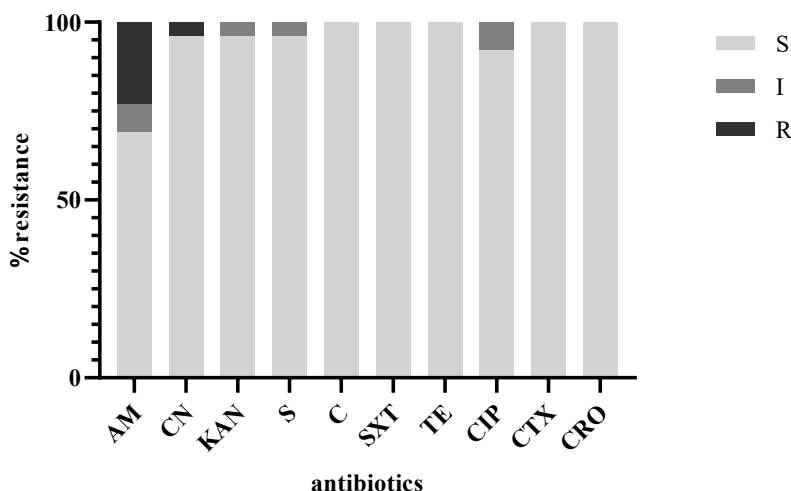


Figure 1: Antibiotic resistance percentage of *Enterobacteriaceae* isolates

None of the isolates showed multidrug resistance. A total of six different resistance profiles were identified among the isolates. The resistance profiles of the isolates are presented in Table 2.

Table 2: Resistance profiles of *Enterobacteriaceae* isolates

Strain code	Number of strain	Resistance profile
1	1	S (I)
11	1	K (I)
7, 10	2	CIP (I)
12, 16	2	AM (I)
5	1	CN
14, 17, 18, 21, 25, 26	6	AM

In this study, 50% of the *Enterobacteriaceae* isolates were found to be susceptible to the 10 antibiotics tested. The highest resistance rate (23%) was observed against ampicillin. β -lactams constitute one of the most essential classes of antimicrobial agents used in veterinary medicine and food animal production. Ampicillin, which belongs to the aminopenicillin subgroup of β -lactams and also is applied extensively in both veterinary and human medicine (Smet et al., 2010). Similarly, in a study conducted in Poland, the *Enterobacteriaceae* isolates recovered from ready-to-eat foods showed the second highest resistance rate (19%) to ampicillin (Zarzecka et al., 2022). In their study investigating the resistance mechanisms of ampicillin-resistant *Enterobacteriaceae* isolates recovered from cheese, Hammad et al. (2009) reported that the most frequently detected resistance mechanism (28.3%) was the production of TEM-1-type β -lactamase.

β -lactamase activity refers to the enzymatic breakdown of β -lactam antibiotics, and within *Enterobacteriaceae*, this mechanism plays a central role in mediating resistance and they are spread among bacterial species by plasmids. The ability to produce β -lactam inactivating enzymes, particularly extended-spectrum β -lactamases and carbapenemases, leads to multidrug resistance profiles that significantly limit available treatment strategies (De Angelis et al., 2020).

Quinolones constitute a class of fully synthetic antimicrobial agents widely employed in human medicine. Among them, ciprofloxacin is particularly prominent and is commonly prescribed for infections associated with Gram-negative pathogens, including members of the *Enterobacteriaceae* family (Azargun et al., 2020). In the present study, moderate resistance to ciprofloxacin was detected in 8% of the *Enterobacteriaceae* isolates, indicating that although ciprofloxacin remains an important therapeutic option, emerging resistance within this group should be carefully monitored.

Biofilms are structured microbial communities that develop when microorganisms adhere to biotic or abiotic surfaces and initiate the production of an extracellular matrix. These complex architectures enable microorganisms to withstand environmental challenges and significantly increase their tolerance to antimicrobial agents, thereby promoting their long-term survival and pathogenic potential. In natural and man-made environments, biofilms represent a predominant mode of microbial growth. By forming these protective layers, microorganisms can endure a wide range of stressors including UV exposure, extreme pH and temperature conditions, nutrient deprivation, osmotic stress, and antimicrobial treatments. In this sense, biofilms function as a resilient barrier that safeguards cells from hostile surroundings (Yin et al., 2019). Within food processing and healthcare settings, the biofilm-forming ability of spoilage and pathogenic bacteria poses a major concern. Once established, these microbial communities are difficult to eliminate through routine sanitation practices, allowing bacteria to persist on equipment and surfaces. Such persistence can lead to increased contamination in processing environments and final food products, ultimately causing reduced shelf life, economic losses, and a heightened risk of foodborne disease outbreaks (Schwan et al., 2022).

Among the *Enterobacteriaceae* isolates, 96% (25 out of 26) exhibited biofilm-forming capacity. Of these biofilm-positive isolates, 58% (15 strains) produced weak biofilms, 27% (7 strains) produced strong biofilms, and 12% (3 strains) produced moderate biofilms (Figure 2).

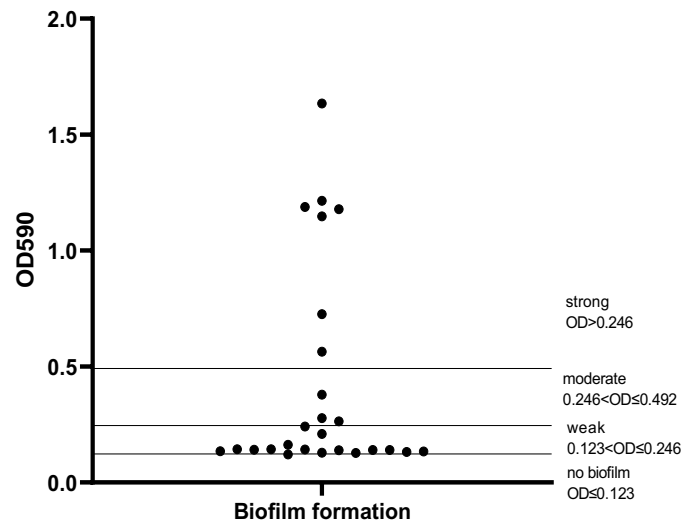


Figure 2: Biofilm formation abilities of *Enterobacteriaceae* isolates

When the *Enterobacteriaceae* isolates are classified based on their biofilm-forming capacities and antibiotic resistance profiles, they are found to segregate into 13 distinct groups. (Figure 3).

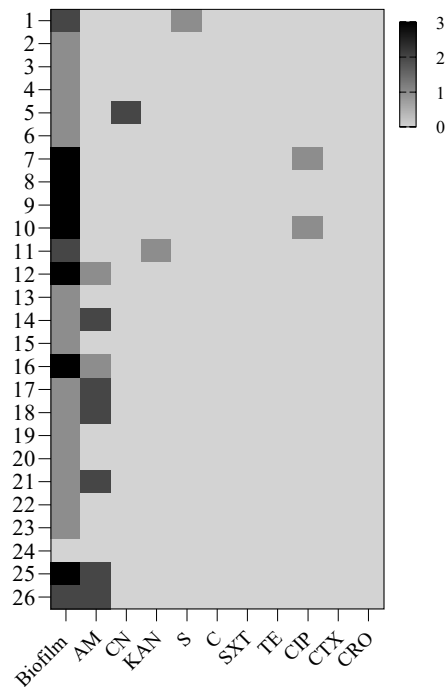


Figure 3: Heatmap of *Enterobacteriaceae* isolates

In the present study, a high prevalence of biofilm-forming ability was observed among the *Enterobacteriaceae* isolates, with 96% (25/26) demonstrating this phenotype. Notably, weak biofilm formation was the most common pattern (58%), followed by strong (27%) and moderate (12%) biofilm producers. This distribution indicates that although most isolates possess the ability to form biofilms, the intensity of biofilm production varies considerably. The predominance of weak biofilm producers is noteworthy; however, even weak biofilms are known to confer substantial protection under industrial and clinical conditions. The presence of strong biofilm-forming isolates further highlights the potential risk, as these strains may contribute disproportionately to environmental persistence and contamination events. These findings underscore the necessity for targeted hygiene strategies and continuous monitoring to mitigate biofilm-associated risks in food production environments.

4. CONCLUSION

This study provides information on the persistence and antibiotic resistance of *Enterobacteriaceae* found in ready-to-eat dairy desserts, underscoring the importance of effective sanitation and regular monitoring to protect public health. The findings also indicate that further research is needed to better understand the mechanisms that enable foodborne pathogens to persist and develop resistance within food systems. While the results highlight the importance of effective hygiene and sanitation practices and regular microbiological monitoring, the lack of a pre-enrichment step may have led to an underestimate of the *Enterobacteriaceae* presence due to the inability to detect sublethal damaged cells. Furthermore, the lack of species-level identification of the isolates and the limited sample size in the study are significant limitations that restrict the scope of the findings.

CONFLICT OF INTEREST STATEMENT

There is no conflict of interest among the authors.

CONTRIBUTIONS OF AUTHORS

D.Y.Y., conceptualization, methodology, formal analysis, investigation, validation, writing—original draft, writing—review & editing

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