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Isolation of *Staphylococcus* Species from Some Clinical and Food Samples and Investigation of Their Biofilm Formation Abilities

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Abstract: This study aimed to identify *Staphylococcus* species from clinical and food samples and investigate the biofilm formation ability of the isolates using various methods. Thirty clinical samples were brought to the diagnostic laboratory of the Microbiology Department at the Veterinary Faculty of Harran University, and 100 food products obtained from food outlets operating in Şanlıurfa province were designated for examination. Isolations from clinical samples were performed using bacterial culture techniques. ISO standards were followed for the analysis of food samples. All isolates were identified at the species level through verification with a VITEK-2 device. The biofilm formation ability of the isolates was explored using three different methods: Congo red agar, tube, and microplate. Fisher's Exact Test was employed for statistical analyses. As a result, 32 *Staphylococci* belonging to seven different species were isolated, with 11 from clinical samples and 21 from food samples. *Staphylococcus aureus* and *Staphylococcus pseudintermedius* were the most frequently identified species. Most of the isolates (81.25%) could form biofilms at varying levels, and the results from the methods used to detect biofilm formation were consistent. Statistical evaluation of the relationship between the biofilm-forming abilities of the isolates revealed no significant relationship between clinical and food isolates. However, a substantial relationship was found between coagulase-positive and coagulase-negative isolates. This study highlighted the ongoing potential threat of *Staphylococcus* species to human and animal health and concluded that rational control methods should be implemented to guard against bacterial contamination in products, particularly to prevent biofilm formation by taking necessary precautions during the production and marketing of foods.

Keywords: *Biofilm, Food, Infection, Isolation, Staphylococcus.*

Bazı klinik örneklerden ve gıda numunelerinden *Staphylococcus* türlerinin izolasyonu ve biyofilm oluşturma yeteneklerinin araştırılması

Özet: Bu çalışmayla klinik ve gıda örneklerinden *Staphylococcus* türlerinin tanımlanması ve bu etkenlerin biyofilm oluşturma yeteneklerinin farklı yöntemlerle araştırılması amaçlandı. Harran Üniversitesi Veteriner Fakültesi Mikrobiyoloji Ana Bilim Dalı Tanı Laboratuvarına getirilen 30 adet klinik örnek ile Şanlıurfa'da faaliyet gösteren gıda satış yerlerinden temin edilen 100 adet gıda numunesi çalışmada inceleme örneği olarak kullanıldı. Klinik örneklerden izolasyonlar bakteriyel izolasyon yöntemleriyle gerçekleştirilirken gıda numunelerinden izolasyonlar için ISO standartları kullanıldı. Tüm izolatlar VİTEK-2 cihazıyla doğrulanarak tür seviyesinde tanımlandı. İzolatların biyofilm oluşturma yetenekleri, kongo kırmızısı agar, tüp ve mikropleyt olmak üzere üç farklı yöntemle araştırıldı. İstatisiksel analizler için Fisherin Exact Testi uygulandı. Sonuç olarak klinik örneklerden 11, gıda numunelerinden 21, toplamda 32 adet Stafilokok izolasyonu gerçekleştirildi. Bu etkenlerin yedi farklı türe ait olduğu tespit edildi. *Staphylococcus aureus* ve *Staphylococcus pseudintermedius* en çok tanımlanan türler oldu. İzolatların büyük bir bölümünün çeşitli seviyelerde olmak üzere biyofilm oluşturabildikleri görüldü. Biyofilm oluşumunun tespiti için kullanılan yöntem sonuçlarının birbirleriyle uyumlu olduğu görüldü. İzolatların biyofilm oluşturma yetenekleri arasındaki ilişkinin araştırıldığı istatistiksel değerlendirmede, klinik ve gıda izolatlarının arasında anlamlı bir ilişki tespit edilemezken, koagulaz pozitif ve negatif izolatlar arasında anlamlı bir ilişkinin var olduğu belirlendi. Bu çalışma ile *Staphylococcus* türlerinin insan ve hayvan sağlığına yönelik potaniyel tehditlerinin devam ettiği görüldü. Özellikle gıdaların üretiminden pazarlanmasına geçen sürede gerekli önlemlerin alınarak ürünlerin bakteri kontaminasyonlarından uzak tutulması ve özellikle biyofilm oluşumlarının engellenmesi için akılcı mücadele yöntemlerinin uygulanması gerektiği kanaatine varıldı.

Anahtar Kelimeler: *Biyofilm, enfeksiyon, gıda, izolasyon, Stafilokok.*

Introduction

The genus *Staphylococcus*, which is widespread in the environment (Heo et al., 2020), is taxonomically classified within the *Eubacteria* kingdom, *Firmicutes* phylum, *Bacilli* class, *Bacillales* order, and *Staphylococcaceae* family (Yüksekdağ and Baltacı, 2013). As of 2019, *Staphylococci*, encompassing 53 species and 27 subspecies (Heo et al., 2020), are divided into two main groups: coagulase-positive and coagulase-negative *Staphylococci*, based on their ability to produce coagulase. While coagulase production was once linked to pathogenicity, this understanding has evolved with the realization that many coagulase-negative *Staphylococci* are responsible for infections. Consequently, coagulase production is no longer considered a reliable marker for pathogenicity (Songer and Post, 2012). However, coagulase production can indicate high virulence (Quinne et al., 2011). It has been reported that 40 species and 24 subspecies of *Staphylococci* include coagulase-negative members. Except for opportunistic-pathogenic species, coagulase-negative *Staphylococci* are harmless and do not cause disease. They can be found in fermented foods and utilized as starter cultures. Coagulase-positive *Staphylococci* exhibit the highest virulence within the genus (Heo et al., 2020).

In 1881, *Staphylococci* were identified as a causative agent of infection and developed resistance to penicillin and later to methicillin over time. *Staphylococcus aureus* has become the most significant health issue as a nosocomial pathogen worldwide (Kireçci, 2009). *Staphylococci* can be found as commensals in the normal skin and mucosal flora of living organisms. Therefore, human intervention has been the primary cause of food contamination. In addition to many food products, particularly ready-to-eat foods, raw meat and meat products, raw milk, and dairy products pose risks concerning *Staphylococcus aureus* enterotoxins (Muratoğlu et al., 2015). Besides local purulent infections, they can also cause mastitis in cattle, pyemia in sheep, and botryomycosis in horses. *Staphylococci* lead to exudative epidermitis in pigs, as well as ear infections, conjunctivitis, skin inflammations, urinary tract infections, bone infections, and wound infections in dogs and cats. They can also cause joint, tendon sheath, bursa disorders, endocarditis, and yolk sac infections in poultry (Songer and Post, 2012; Quinn et al., 2011). In humans, *Staphylococci* induce similar infections and sepsis. In particular, *Staphylococcus aureus* is often responsible for infections and poisoning due to the enterotoxins it produces in foods (Aygen et al., 1997; Mubarak, 2021; Xiying et al., 2024). Enterotoxigenic *Staphylococci* can especially be isolated from protein-rich foods of animal origin (Erol and İseri, 2004).

Biofilm formation is recognized as one of the most important virulence factors of microorganisms (Temel and Eraç, 2018). A biofilm is a complex structure of bacterial colonies embedded in an exopolysaccharide matrix that adheres to foreign surfaces in living organisms (Sharma et al., 2023). Its structure includes intricate molecules such as proteins, polysaccharides, extracellular DNA, water, and ions. Bacteria can adhere to both living and non-living surfaces and colonize them. These colonies may also contain mixed species (Temel and Eraç, 2018). Biofilms increase antimicrobial resistance in the host and can trigger inflammatory responses, potentially leading to chronic inflammation (Aydemir, 2018). In a comprehensive review study on this subject, researchers have reported that biofilms cause approximately 70% of all human microbial infections and lead to various diseases, including non-healing chronic wounds, endocarditis, periodontitis, cystic rhinosinusitis, fibrosis, meningitis, osteomyelitis, kidney infections, and infections related to prostheses and implantable devices (Sharma et al., 2023). In food contamination, microorganisms may also produce biofilms (Öksüztepe and Demir, 2019). Deficiencies in sanitation procedures at food establishments can lead to biofilm formation on various surfaces (Sharma et al., 2023). Biofilms formed in production facilities promote bacterial colonization and protect these bacteria from many unfavorable conditions (Temel and Eraç, 2018).

This study aimed to investigate the presence of *Staphylococcus* species in samples from various clinical cases and food materials offered for sale in Şanlıurfa region, as well as to explore the biofilm formation ability of isolated *Staphylococci* using different methods.

Material and Methods

Samples

Thirty clinical samples from various clinical cases were brought to the diagnostic laboratory of Harran University, Faculty of Veterinary Medicine, Department of Microbiology between June 2023 and July 2024. Additionally, 100 food samples obtained from Şanlıurfa province during the same period were used as examination samples for agent isolation (Table 1 and 2).

Table 1. Clinical samples and sample numbers.

Origin of the clinical samples (From..)	Sample numbers according to the species of the animals				Total
	Cat	Dog	Cattle	Chicken	
Wound infection	4	4	-	-	8
Eye infection	3	1	-	-	4
Ear infection	2	3	-	-	5
Mastitis	-	-	12	-	12
Beak infection	-	-	-	1	1
Total	**9**	**8**	**12**	**1**	**30**

Isolation of *Staphylococcus* species from clinical and food samples

Clinical specimens were inoculated directly onto mannitol salt phenol red agar (MSA) (Merck, Germany), and the Petri dishes were incubated under aerobic conditions at 37 °C for 24 hours (Quinn et al., 2004; Quinn et al., 2011).

Table 2. Food samples and sample numbers.

Food samples	Numbers of food materials
Adana kebab	3
Cake	12
Frozen cake	9
Rice pilaf with vermicelli	3
Melt cheese	4
Cheese	12
Doner is made of chicken meat	13
Butter	2
Salad	13
Chocolate cake	8
Stuffed meatballs	2
Rice pilaf	14
Kebab	5
Total	**100**

Twenty-five grams of the food samples were weighed and added to 225 ml of Buffered Peptone Water (BPW) (Merck, Germany) using a sterile spatula. After homogenizing with a stomacher (Smasher-Biomerieux, France) for 20 seconds, the medium was incubated in an incubator (Memmert, Germany) under aerobic conditions at 37 °C for 24 hours. Following this pre-enrichment, subcultures were made by inoculating 100 µl of the incubated and non-diluted BPW onto Baird-Parker Agar (BPA), a selective-differential medium prepared by adding egg yolk-tellurite emulsion (Merck, Germany) to the agar base of Baird-Parker (Merck, Germany). Petri dishes were incubated under aerobic conditions at 37 °C for 24 hours, and the incubation period was extended to 48 hours for suspected colonies. Typical colonies on BPA were evaluated based on their black color and halo formation (ISO 6888-1:2021).

Colonies from clinical and food sources were subcultured onto tryptic soy agar (TSA) (Merck, Germany) for purification and incubated under aerobic conditions at 37 °C for 24 hours. The pure colonies were examined using Gram staining (Merck, Germany), and Gram-positive cocci underwent a catalase test (Bactident, Merck, Germany). Subsequently, suspected *Staphylococcus* species were identified, and a coagulase test was performed (ISO 6888-1:2021; Quinn et al., 2004; Quinn et al., 2011).

Identification of colonies at the species level

Confirmation and species-level identification of the isolated colonies suspected to be *Staphylococci* were performed using a VITEK-2 device (Biomerieux, France).

Biofilm determination by the CRA method

To evaluate the phenotypic biofilm-forming properties of the isolates, staphylococcal strains, along with positive and negative control strains, were obtained from the TSA using a quarter of a loop and transferred into sterile glass tubes containing 10 ml of tryptic soy broth (TSB) (Merck, Germany). The tubes were incubated for 24 hours at 37 °C under aerobic conditions without shaking. After incubation, cultures were inoculated onto CRA, which was prepared by adding 50 g of sucrose, 37 g of brain heart infusion agar, and 0.8 g of Congo red agar (Merck, Germany) per liter. Biofilm formation was assessed using two different methods: the single colony method (smear plate technique) and the dropping method. A loopful of culture was inoculated onto CRA plates for the single colony method. In the dropping method, 0.1 ml of liquid culture was pipetted onto five different points of the CRA plates using an automatic pipette. CRA plates were incubated at 37 °C for 24 hours under aerobic conditions. After incubation, isolates forming dry, crystalline black colonies were categorized as strong or moderate biofilm producers based on the intensity of color formation. Isolates forming red or pink colonies were considered weak or no-biofilm producers. The CRA method was performed in triplicate for each isolate (Gündoğ et al., 2023).

Determination of biofilm by tube method

In the Tube Adherence Method, staphylococcal isolates were transferred from TSA petri dishes into sterile glass tubes containing 10 ml TSB and incubated at 37 °C under aerobic conditions for 24 hours. After incubation, *Staphylococcus* strains that formed or did not form biofilms on the walls of the glass tubes were washed twice with phosphate-buffered saline (PBS) (Merck) (pH: 7.3), and the tubes were stained with 0.1% crystal violet for 1 hour. Following the staining, the tubes were washed twice with PBS to remove excess dye and air-dried. All tubes were evaluated by comparing the staining results to the reference strains used as controls. Biofilm formation was considered positive if a visible film was observed on the walls or bottom of the tubes. Biofilm production was graded as follows: biofilm negative (-), weak biofilm (+), moderate biofilm (++), and strong biofilm (+++) formation. The studies were repeated three times for each isolate (Christensen et al., 1982).

Determination of biofilm by microplate method

The isolates were transferred to a TSB liquid medium and incubated at 37 °C under aerobic conditions for 24 hours. Twenty microliters of each culture were added to microplate test wells (Greiner BioOne, Austria), which contained 230 µl of TSB in triplicate, and then incubated in an aerobic incubator at 37 °C for 24 hours. After incubation, the wells were discarded and washed three times with 350 µl of sterile distilled water. To fix the cells, 250 µl of methanol was added to each well and held for 15 minutes. The microplates were subsequently discarded and allowed to dry in an inverted position at room temperature for approximately 12 hours. The biofilm layer was stained by adding 250 µl of crystalline violet solution to each well for 5 minutes at room temperature. The wells were then rewashed under running water. After thoroughly removing the excess dye, the microplates were dried at room temperature. The dye bound to the cells was solubilized by adding 33% glacial acetic acid (Merck, Germany) to each well, and the optical density (OD) was measured at 570 nm using a microplate reader (VersaMax, USA). The cut-off OD (ODC) value was determined based on the wells containing only medium and served as a negative control. The results were evaluated

according to Table 3, based on average OD values from three replicates (Stepanović et al., 2004).

Table 3. Criteria for evaluation of the biofilm-forming abilities level.

Biofilm-forming abilities level	Calculation of ODC
No Biofilm	OD ≤ ODC
Weak	ODC
Medium	2× ODC < OD ≤ 4× ODC
Strong	OD > 4X ODC

Statistical analysis: The effect of the clinical or food origin of *Staphylococcus* isolates on their ability to form biofilm, specifically, whether the relationship between the origin of the isolates and their biofilm-forming ability was statistically significant, was analyzed using Fisher's Exact Test method, creating a 2x2 contingency table. The significance level was set at $\alpha = 0.05$. Additionally, in a separate statistical study, the effect of the isolates being coagulase positive or negative on their biofilm-forming ability, specifically, whether the relationship between coagulase enzyme production and biofilm formation was significant, was also analyzed using Fisher's Exact Test method, creating a 2x2 contingency table. The significance level was again accepted as $\alpha = 0.05$.

Reference strains: In all tests, *Staphylococcus aureus* ATCC 25923 served as the reference control strain for coagulase-positive *Staphylococci*, while *Staphylococcus epidermidis* ATCC 35984 was utilized for coagulase-negative *Staphylococci*.

Results

Regarding the isolation and identification of staphylococcal species revealed that thirty-two isolates were identified as suspected *Staphylococci* based on their Gram staining and biochemical characteristics, with 11 isolates derived from 30 clinical samples and 21 from 100 food samples. The isolates were confirmed and identified at the species level using VITEK 2. All suspected isolates were verified (see Tables 4 and 5). The isolation rates of the species-level identified isolates are detailed in Table 6.

Table 4. Test results of isolates from clinical samples.

No	Orgine	Mannitol fermentation in MSA	Gram staining	Catalase	Coagulase	VITEK
1	A wound infection of a dog	+	+	+	+	*Staphylococcus aureus*
2	An eye infection of a dog	+	+	+	-	*Staphylococcus sciuri*
3	A wound infection of a cat	-	+	+	+	*Staphylococcus pseudintermedius*
4	An ear infection of a dog	-	+	+	+	*Staphylococcus pseudintermedius*
5	A skin infection of a cat	-	+	+	+	*Staphylococcus pseudintermedius*
6	An ear infection of a cat	-	+	+	+	*Staphylococcus pseudintemedius*
7	An eye infection of a cat	-	+	+	+	*Staphylococcus pseudintemedius*
8	A case of mastitis in a cow	+	+	+	+	*Staphylococcus aureus*
9	A case of mastitis in a cow	+	+	+	+	*Staphylococcus aureus*
10	The beak infection of a chicken	-	+	+	-	*Staphylococcus epidermidis*
11	An ear infection of a dog	-	+	+	-	*Staphylococcus epidermidis*

Table 5. Test results of isolates from food samples.

No	Origin of the food samples	Growth in BPA	Gram stain	Catalase	Coagulase	VITEK
1	Adana kebab	black	+	+	-	*Staphylococcus lentus*
2	Cake	black	+	+	-	*Staphylococcus lentus*
3	Rice pilaf with vermicelli	black with a halo	+	+	+	*Staphylococcus pseudintermedius*
4	Melt cheese	black with a halo	+	+	+	*Staphylococcus pseudintermedius*
5	The meat of the chicken	black with a halo	+	+	+	*Staphylococcus aureus*
6	Cake	black with a halo	+	+	+	*Staphylococcus aureus*
7	Cake	black	+	+	-	*Staphylococcus xylosus*
8	Frozen cake	black	+	+	-	*Staphylococcus warneri*
9	Butter	black	+	+	-	*Staphylococcus sciuri*
10	Cake	black	+	+	-	*Staphylococcus xylosus*
11	Salad	black	+	+	-	*Staphylococcus xylosus*
12	Chocolate cake	black	+	+	-	*Staphylococcus xylosus*
13	Doner is made of chicken meat	black	+	+	-	*Staphylococcus xylosus*
14	Cheese	black with a halo	+	+	+	*Staphylococcus aureus*
15	Stuffed meatballs	black	+	+	-	*Staphylococcus warneri*
16	Doner is made of chicken meat	black with a halo	+	+	+	*Staphylococcus aureus*
17	Rice pilaf	black with a halo	+	+	+	*Staphylococcus pseudintermedius*
18	Kebab	black	+	+	-	*Staphylococcus lentus*
19	Chocolate cake	black	+	+	-	*Staphylococcus xylosus*
20	Cheese	black	+	+	-	*Staphylococcus warneri*
21	Cake	black with a halo	+	+	+	*Staphylococcus aureus*

Table 6. Isolation rates of *Staphylococcus* species.

Species	Clinical samples (n=30)	Food samples (n=100)	Total (n=130)
Staphylococcus pseudintermedius	5 (16.6%)	3 (3%)	8 (6.15%)
Staphylococcus aureus	3 (10%)	5 (5%)	8 (6.15%)
Staphylococcus xylosus	0 (0%)	6 (6%)	6 (4.61%)
Staphylococcus lentus	0 (0%)	3 (3%)	3 (2.3%)
Staphylococcus warneri	0 (0%)	3 (3%)	3 (2.3%)
Staphylococcus sciuri	1 (3.33%)	1 (1%)	2 (1.53%)
Staphylococcus epidermidis	2 (6.66%)	0 (0%)	2 (1.53%)
Total	11 (36.6%)	21 (21%)	32 (24.6%)

Table 7. Comparative results of biofilm-forming abilities of the isolates.

No	Origin of the samples (From..)	Isolated species	Biofilm-forming abilities of the isolates		
			CRA method	Tube method	Micropleyt method
1	A wound infection of a dog	*Staphylococcus aureus*	Strong	Strong	Strong
2	An eye infection of a dog	*Staphylococcus sciuri*	Moderate	Moderate	Medium
3	A wound infection of a cat	*Staphylococcus pseudintermedius*	Moderate	Weak	Weak
4	An ear infection of a dog	*Staphylococcus pseudintermedius*	Low	Weak	Weak
5	A skin infection of a cat	*Staphylococcus pseudintermedius*	Low	Moderate	Medium
6	An ear infection of a cat	*Staphylococcus pseudintermedius*	Moderate	Moderate	Medium
7	An eye infection of a cat	*Staphylococcus pseudintermedius*	Low	Weak	Medium
8	A mastitis case of a cattle	*Staphylococcus aureus*	Strong	Strong	Strong
9	A mastitis case of a cattle	*Staphylococcus aureus*	Strong	Strong	Strong
10	A beak of a chicken	*Staphylococcus epidermidis*	Strong	Moderate	Strong
11	An ear infection of a dog	*Staphylococcus epidermidis*	Strong	Weak	Strong
12	An Adana kebab	*Staphylococcus lentus*	No biofilm	Negative	No biofilm
13	A cake	*Staphylococcus lentus*	No biofilm	Negative	No biofilm
14	A rice pilaf with vermicelli	*Staphylococcus Pseudintermedius*	Low	Moderate	Weak
15	A melt cheese	*Staphylococcus pseudintermedius*	Low	Moderate	Weak
16	A meat of chicken	*Staphylcoccus aureus*	Strong	Strong	Strong
17	A cake	*Staphylococcus aureus*	Strong	Strong	Strong
18	A cake	*Staphylococcus xylosus*	Moderate	Strong	Weak
19	A frozen cake	*Staphylococcus warneri*	No biofilm	Negative	No biofilm
20	A butter	*Staphylococcus sciuri*	Moderate	Moderate	Medium
21	A cake	*Staphylococcus xylosus*	Moderate	Weak	Medium
22	A salad	*Staphylococcus xylosus*	Moderate	Weak	Weak
23	A chocolate cake	*Staphylococcus xylosus*	Moderate	Moderate	Weak
24	A doner made of chicken meat	*Staphylococcus xylosus*	Moderate	Moderate	Weak
25	A cheese	*Staphylococcus aureus*	Strong	Strong	Strong
26	A stuffed meatball	*Staphylococcus warneri*	No biofilm	Negative	No biofilm
27	A doner made of chicken meat	*Staphylococcus aureus*	Strong	Strong	Strong
28	A rice pilaf	*Staphylococcus pseudintermedius*	Moderate	Weak	Weak
29	A kebab	*Staphylococcus lentus*	No biofilm	Negative	No biofilm
30	A chocolate cake	*Staphylococcus xylosus*	Moderate	Weak	Weak
31	A cheese	*Staphylococcus warneri*	No biofilm	Negative	No biofilm
32	A cake	*Staphylococcus aureus*	Strong	Moderate	Strong
R1	*Staphylococcus aureus* ATCC 25923		Strong	Strong	Strong
R2	*Staphylococcus epidermidis ATCC* 35984		Strong	Strong	Strong

R: Reference

Biofilm test results: The comparative test results of the methods used to determine the biofilm-forming abilities of the isolates are presented in Table 7.

Statistical analysis findings

There was no statistically significant relationship between the food or clinical origin of the isolates and their ability to form biofilms (P>0.05). A statistically significant relationship was found between the production of the coagulase enzyme by the isolates and their ability to form biofilms (P<0.05).

Discussion

Many studies have been conducted to isolate *Staphylococcus* spp. from clinical samples over time. Uysal and Kırkan (2012) isolated 42 staphylococcal agents from 60 wound swab samples, 30 of which were coagulase-positive and 12 were coagulase-negative. Among the coagulase-positive isolates, 22 were *S. aureus*, eight *S. intermedius*, and among the coagulase-negative isolates, seven were *S. hyicus*, two *S. sciuri*, two S. *haemolyticus*, and one *S. cohnii* subsp.

cohnii. Molnar et al. (1994) reported that some species, such as *S. hominis* and *S. epidermidis*, can adapt to and establish significant dominance on human skin and mucosa. Öcal et al. (2022) reported that *S. hominis* and *S. epidermidis* were the most frequent isolates among *Staphylococci* in their study. In another study, *S. aureus* was isolated from 28, *Streptococcus uberis* from 21, and *Streptococcus dysgalactiae* from 8 of 100 milk samples obtained from cattle with subclinical mastitis. No bacterial growth was detected in 43 samples (Genç and Kaya, 2015). In another study, 75 *S. aureus* strains were isolated from 512 samples of horses with skin infections (Chiers et al., 2003). In a study examining 158 milk samples from 7 dairy cow herds in East and West Azerbaijan regions of Iran using bacteriological and molecular methods, the isolation of many *Staphylococcus* species from 113 samples was reported. The researchers identified five of the 113 isolates as *S. aureus* and 108 as coagulase-negative *Staphylococci* (CoNS). They identified 44 of the 108 CoNS species as *S. haemolyticus*, 17 as *S. chromogenes*, 11 as *S. epidermidis*, *S. arneri*, and *S. cohnii*, six as *S. simulans*, four as *S. hominis*, three as *S. capitis*, and one as *S. xylosus*. They reported that only *S. haemolyticus*, *S. warneri*, and *S. chromogenes* species were isolated from clinical mastitis cases (Hosseinzadeh and Saei, 2014). In another study by Göçmen et al. (2018), researchers used 7% sheep blood agar and BPA to isolate staphylococcal species from various clinical materials of 67 animals. They applied catalase and coagulase tests for the Gram-positive cocci derived from pure bacterial colonies and performed species-level identification using the VITEK 2 device.

In this study, the MSA medium was utilized as a selective medium for isolating agents from clinical specimens, similar to approaches taken by other researchers (Tanış and Gülseren, 2020). The MSA medium, which provides a high-density salt environment, also displayed high selectivity. However, mannitol fermentation was positive only for *S. aureus* and varied for other coagulase-positive isolates. This result indicates that mannitol fermentation is not exclusive to the presence of coagulase. In veterinary medicine, *S. aureus* and *S. pseudintermedius*, which are the main coagulase-positive *Staphylococci* species, and *S. chromogenes* and *S. epidermidis*, which are coagulase-negative, are reported to cause significant diseases (Göçmen et al., 2018). In this study, four different species were isolated from clinical samples: *S. pseudintermedius* 5 (16.6%), *S. aureus* 3 (10%), *S. sciuri* 1 (3.33%), and *S. epidermidis* 2 (6.66%) (Table 4). The differences among the isolation rates of staphylococcal species reported in similar studies may have resulted from factors such as the location where the examination samples were collected, the number of samples, geographical diversity, the type of examination samples, storage conditions, processing methods, and methodological variations in the analyses (Akyol et al., 2023). In this study, *S. pseudintermedius* and *S. aureus* exhibited the highest isolation rates of 6.15%. When the characteristics of these species were analyzed, they differed from other species by being coagulase-positive. Although this suggests that coagulase is an important factor that increases the presence of these microorganisms as dominant species compared to other coagulase-negative species, some researchers reported that biofilm-producing CoNS strains can frequently be isolated from infections (Keskin et al., 2003).

Numerous studies have been conducted over the years to isolate *Staphylococci* from food samples. In these studies, a wide variety of *Staphylococci* types were isolated and identified from both animal and non-animal origin food samples. These isolates were classified according to their coagulase properties (Akyol et al., 2023; Güngören et al., 2022; Resch et al., 2008; Tanış and Gülseren, 2020). In this study, the BPA medium, which provides selectivity and discrimination, was used to isolate *Staphylococci* from food samples. *Staphylococci* were isolated from 21 out of 100 food samples. Compared to previous studies, this isolation rate is lower than that from cheese samples (Güngören et al., 2022; Tanış and Gülseren, 2020). This situation may be attributed to several factors, including the active role of human elements in cheese production and marketing processes, as well as non-compliance with cheese storage and transportation conditions. Furthermore, it is believed that these differences in staphylococcal species isolation rates may be analogous to the previously mentioned reasons for the disparities in isolation rates in clinical materials.

In this study, black-colored colonies grown on the BPA medium, which is utilized to isolate staphylococcal species from food samples, were classified based on the presence or absence of white-bright halos. Additional tests confirmed that colonies with white-bright halos were coagulase-positive, while those without halos were coagulase-negative staphylococcal species (Table 5). Although the manufacturer did not highlight the white-bright halo as a distinguishing feature for detecting coagulase in staphylococcal species, a previous study (Tanış and Gülseren, 2020) suggests that this halo may offer preliminary information about the coagulase activity of the isolates. This implies a possible correlation between lipase (forming a bright ring) and lecithinase (forming turbidity) activities of staphylococcal isolates detectable in the BPA medium and the coagulase activities of these isolates. In a study conducted in Istanbul, this feature of BPA medium was utilized to investigate the microbiological quality of cooked chicken doners. Colonies grown on the medium were classified into black-colored typical colonies with a transparent halo and atypical colonies. The presence of coagulase-positive *Staphylococci* was confirmed by the coagulase test (Alçay, 2019).

In several studies, researchers reported varying rates of isolation for different *Staphylococci* species. Resch et al. (2008) isolated 330 coagulase-negative *Staphylococci* from foods including fermented fish, meat, cheese, and sausages. It was reported that 137 isolates were *S. xylosus*, 106 were *S. carnosus*, 64 were *S. equorum*, 11 were *S. piscifermantans*, 10 were *S. succinus*, and two were *S. condimenti*. In another study of minced meat samples, it was noted that six of the 56 isolates were identified as *S. aureus*, while 50 were classified as CoNS. Of the CoNS species, 36 were *S. xylosus*, seven were *S. hominis*, three were *S. capitis*, two were *S. epidermidis*, and two were *S. cohnii*. The same researchers obtained a total of 41 isolates from chicken meat samples,

all classified as CoNS species: 13 of these were *S. simulans*, 10 were *S. cohnii*, nine were *S. capitis*, six were *S. hominis*, two were *S. auricularis*, and one was *S. haemolyticus* (Gündoğan and Ataol, 2012). In this study, six different species were isolated from food samples and identified as follows: *S. xylosus* 6 (6%), *S. aureus* 5 (5%), *S. pseudintermedius* 3 (3%), *S. lentus* 3 (3%), S. *warneri* 3 (3%), and *S. sciuri* 1 (1%) (Table 5). Although staphylococcal agents were obtained from various species in these studies, the isolation rates varied significantly.

Since staphylococcal species can be found in nearly every environment that negatively impacts human and animal health, researchers have frequently investigated the virulence properties of these agents. The ability of staphylococcal species to form biofilms has also been a focus of many studies, as this enables the agent to survive and maintain its activity. Öcal et al. (2022) examined the ability of staphylococcal isolates to form biofilms on CRA media. They reported no difference in effectiveness between smear and drip methods for detecting biofilm formation; however, the drip method made the results easier to interpret. They also compared the methods used to detect biofilm formation and determined that the microplate method detected significantly more biofilm than the CRA method. A study conducted at Erciyes University researchers reported that 35% of *S. aureus* isolates could form biofilm in CRA, 36% in microplate, and 94.4% in both methods (Gündoğ et al., 2023). Similarly, a study conducted in India found that 79% of 84 *S. aureus* isolates analyzed in studies comparing biofilm diagnostic results were able to produce biofilms using the microplate method and 75% with CRA methods (Jain and Agarwal, 2009). Mathur et al. (2006) investigated the biofilm formation properties of 152 CoNS isolates using CRA and microplate methods. They observed biofilm formation in 8 (5.2%) with CRA and in 82 (53.9%) with the microplate method, stating it was more sensitive. They also isolated *Staphylococci* from blood, infected vehicles, and skin surfaces, reporting high rates of biofilm-forming ability.

Manandhar et al. (2021) stated they could detect biofilm at a higher rate with the microplate method (42.1%) compared to the tube method (31.8%) and CRA method (20.1%). Kord et al. (2018) found biofilm formation in 53.6% of 41 *S. epidermidis* isolates by tube and microplate and 24.4% by CRA. Cafiso et al. (2004) also explored the biofilm formation ability of coagulase-negative staphylococcal isolates isolated from infections by the CRA method and showed that 83% could form biofilm. Demir and Battaloğlu İnanç (2015) evaluated 65 coagulase-negative *Staphylococci* and 127 *S. aureus* isolates from clinical samples using three detection methods concurrently and reported that the results were comparable concerning biofilm detection, with no statistically significant difference between the methods. Some studies in the literature include similar comparisons, along with studies indicating that the microplate method has a sensitivity comparable to that of other methods (Demir and Battaloğlu İnanç, 2015; Gündoğ et al., 2023; Jain and Agarwal, 2009); there are also studies suggesting it may be more sensitive (Manandhar et al., 2021; Mathur et al., 2006). This study investigated the biofilm formation abilities of 32 staphylococcal isolates from various species derived from food and clinical samples using three different methods. The levels of biofilm formation determined by the methods employed were quite similar across all isolates (Table 7). Considering the laboratory infrastructure, it was concluded that any of these methods could be preferred. Researchers attribute differences in biofilm formation to several factors. It has been reported that various factors, such as medium composition (Dhanawade et al., 2010), glucose availability and concentration, hydrogen ion concentration, and the presence of H_2O_2, may influence biofilm formation (Nostro et al., 2014). In this study, no statistically significant relationship was found between clinical and foodborne isolates regarding their ability to form biofilms. However, a statistically significant relationship was identified between the production of the coagulase enzyme by staphylococcal isolates and their ability to form biofilms. The literature does not establish a definite causal relationship between coagulase production and biofilm formation. Nonetheless, there is strong belief that coagulase-positive species generally possess a greater capacity for biofilm production. There are instances where coagulase-negative species also exhibit significant biofilm production. In both groups, biofilm production is determined by a wide range of phenotypic and genotypic factors (Nostro et al., 2014), making it difficult to interpret the dynamics of biofilm formation.

Conclusion

As a result, it was observed that the isolation of *Staphylococcus* spp. from food and clinical samples can be performed easily and frequently. This finding reinforces the importance of maintaining strict hygiene practices throughout all stages of producing and marketing food products from farm to table to prevent microbial contamination. Biofilms can play a significant role in the persistence, chronicity, and recurrence of infections. One of the important challenges in treating such infections is the increasing resistance of biofilm-forming microorganisms to host immune defenses and antimicrobial agents. Regardless of their origins, the biofilm-producing potential of most food and clinical staphylococcal isolates emphasizes the necessity of rational practices in the fight against these agents. Although the general characteristics of biofilm formation mechanisms are similar across many microorganisms, species-specific traits necessitate tailored evaluation and intervention approaches. The ability of these agents to survive in diverse environments contributes to the risk of food contamination and results in significant economic consequences due to antibiotic resistance, ultimately posing a broad threat to public health.

Ethical Approval

This study was approved by the Harran University Animal Experiments Local Ethics Committee (21.12.2023, 2023/008/06 Number Ethics Committee Decision). In addition, the authors declared that Research and Publication Ethical rules were followed.

Conflict of Interest

The authors stated they had no real, potential, or perceived conflict of interest.

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