https://doi.org/10.46389/rjd-2024-0278
www.jrdiabet.ro

Rom J Diabetes Nutr Metab Dis
2024; Volume 31, Issue 1, pages 278-298

DETECTION OF SOME VIRULENCE FACTORS IN *STAPHYLOCOCCUS EPIDERMIDIS* ISOLATED FROM CLINICAL SPECIMENS IN IRAQ.

Sara Amer Naser [1*], Wafaa Fadhil [2], Taghreed Kheder Mohammed [3]

[1] Medical technologist / Medical city / Baghdad teaching hospital

[2] College of the Health and Medical Techniques- Baghdad; Middle Technical University

[3] Al-Mansour Medical Technical Institute, Baghdad, Iraq.

[*] Corresponding author E-mail: saraamernaser@gmail.com

Abstract

Staphylococcus epidermidis is a member of the coagulase-negative staphylococci, and considered the most commensal bacterium that is abundant in the human skin and mucous membrane. It is also the most nosocomial prevalent pathogen. *Staphylococcus epidermidis* can cause different clinical infection when the skin is breaching. The most common clinical infection in hospitals belongs to *Staphylococcus epidermidis*, which associated with life-threatening infections. This is due to the antibiotic-resistant phenotype and the formation of biofilm, which is considered one of their virulence factors, especially on indwelling medical devices and at surgical sites. This study was conducted to determine the virulence factors of *Staphylococcus epidermidis* (*S. epidermidis*) that were isolated from different clinical samples. The clinical samples were collected from blood, wound, nasal, and acne swabs, CSF and medical devices (heart valves and dialysis catheters), during the period from December 2022 to August 2023. 120 clinical samples were collected from Baghdad Teaching Hospital and Ghazi Al-hariri Hospital. The isolated samples were cultured in specific and differential culture media, and the Vitek- 2 system was used to confirm the presence of *Staphylococcus epidermidis*. The results confirmed that only 73 isolates were positive for *Staphylococcus epidermidis*. All isolates were screened for biofilm formation, antibiotic sensitivity tests (AST), molecular studies, and tests for detection of virulence factors such as lipase, gelatinase, protease, and DNase. The lethal dose (LD_{50}) showed the effect of commensal *Staphylococcus epidermidis* on mice, and the bacterial virulence appeared with highly sensitive quantification. The present study showed that *Staphylococcus epidermidis* was a strong biofilm producer in about 36 isolates (49.32), 27 (36.99%) of isolates were considered moderate for biofilm production, and 10 (13.70%) of isolates were weak or non-biofilm producers. The isolates appeared to be resistance to methicillin and other antibiotics, so they were considered multi-drug-resistance pathogens. The molecular study confirmed that all isolates possessed *mecA* genes and lacked *ermA* genes. The mice that were injected with the bacterial suspension showed pathological changes in the internal organs, with picture including skin dermatitis. These results proved that *Staphylococcus epidermidis* is a life threatening pathogen to the humans and animals.

Keywords: differential culture media; AST; virulence factors; molecular study; LD_{50}.

1.Introduction

Coagulase-negative staphylococci (CoNS) inhabit mucosal membranes and human epidermis, and as a result of this, they were considered harmless commensals for wide period of time. Nowadays, they are recognized as etiologic agents of hospital-acquired infections (HAIs), which include infections at the surgical site (SSIs) and bloodstream infections linked to central lines [1]. *Staphylococcus epidermidis* is a widespread bacteria and symbiont of human beings [2]. and has become an important opportunistic pathogen, especially in immunocompromised patients [3]. JOHNS, S. L., SCHUCK, P., and HERR *said that "S. epidermidis* has been regarded as a major accidental pathogen due to its high frequency on the epithelium and mucous membrane and its capacity to grow on healthcare equipment. *S. epidermidis* is specifically considered the cause of nosocomial infections, device-related infections, and bacteremia" [4]. The ability of this pathogen to formation of biofilm on biotic and abiotic surfaces increased the prevalence of *S. epidermidis* and increased antibiotic resistance [5]. SICILIANO and PASSEROTTO said, "Multidrug-resistant *Staphylococcus epidermidis* (MDRSE) is responsible for infections that were considered difficult to treat infections in humans and hospital acquired infections, and more than 70% of healthcare-associated *S. epidermidis* was considered methicillin-resistant. These species were considered reservoirs for genes that were responsible for antibiotic resistance and that can be transferred via horizontal gene transfer to other gram-positive organisms. Resistance to Methicillin was mediated by the *mecA* gene that was included on *Staphylococcal cassette chromosome mec* (*SCC mec*) strains "[6]. There was a large increase in the antibiotic resistance of CoNS strains, specifically in ciprofloxacin, ampicillin, erythromycin, methicillin, macrolide, and aminoglycoside. Sixty to eighty percent of human *Staphylococcus* strains that generate beta-lactamases are resistant to methicillin when isolated in hospital settings. *Staphylococcus epidermidis* strains include resistance to the macrolides class, such as roxithromycin and clarithromycin, and they may be carriers of the *ermA* gene [7]. *Staphylococcal cassette chromosome mec* (*SCC mec*) has been reported as the only vector for the *mec A* gene [8]. The CoNS have the ability to produce a variety of extracellular enzymes as virulence factors, such as lipases, proteases, DNase and gelatinases [9]. Biofilm formation is considered one of the most important virulence factors [10]. Mice were shown to be capable of colonizing with *Staphylococcus* species after inoculation. Mice injected with *Staphylococcus epidermidis* showed signs of inflammation in their livers along with a high concentration of bacterial tissue. [11]. Studies demonstrate that this pathogen can induce atopic dermatitis (AD) and control inflammation and anti-inflammatory cytokines. [12]. The present study was supported by several local investigations, which comprised the following: rheem Saad *et al*., 2022 revealed the pathogenicity of *Staphylococcus epidermidis* and confirmed that *Staphylococcus epidermidis* considered an opportunistic pathogen in her local study in Baghdad Teaching Hospital [13]. Aubaid *et al*., 2022 used the coagulase test as a biochemical test to differentiate coagulase positive *Staphylococcus aureus* from other coagulase negative staphylococci in her local study in Clinic Baghdad Teaching Hospital in Medical City. [14]. The present study agreed with the study of Yaseen *et al.,* 2023 in Baghdad Teaching Hospital, Gazzi Al-Harrii Hospital, and AL-Zafranya Hospital that showed the resistance of *Staphylococcus epidermidis* to pencillin and other antibiotics [15].

Nomenclature and Symbols

CoNS	Coagulase Negative Staphylococci	H_2S	Hydrogen sulphide
LD_{50}	Lethal Dose 50	O.Dc	Optical density control
HAIs	Healthcare-associated infections	PBS	Phosphate buffer saline
		BHI	Brain Heart Infusion
MDRSE	Multidrug-resistant Staphylococcus epidermidis	VP	Voges-Proskauer
SCC mec	Staphylococcal cassette chromosome mec	PCR	Polymerase Chain Reaction
AD	Atopic dermatitis	TBE Buffer	Tris-borate- EDTA buffer
CSF	CerebroSpinal Fluid	H&E stain	Haematoxylin & Eosin stain
MSA	Mannitol Salt Agar		
AST	Antibiotic sensitivity test		

2. Supplies and techniques

2.1 Collection of samples

120 isolates were obtained from different sexes and age groups. These clinical samples were collected from blood, wound swabs, nasal swabs, acne swabs, cerebroSpinal fluid (CSF), and medical devices (heart valves and dialysis catheters), during the period from December 2022 to August 2023. The clinical samples were collected from Baghdad Teaching Hospital and Ghazi Al-hariri Hospital.

2.2 Bacterial isolation and identification

The clinical samples were first cultured on nutrient agar and blood agar at 37° C for 24 hours, then cultured on specific and differential culture media that were used to confirm *Staphylococcus epidermidis*. The brain-heart infusion broth was used to cultivate the bacteria overnight at 37° C, then add 20% glycerol and freeze at -8 ° C to preserve the positive samples. The colonies of bacteria appear as white, cohesive, non-hemolytic colonies on blood agar [16]. The colonies showed a circular, creamy- colored to white- pigmented colony on nutrient agar [17]. While appearing pink in color on Mannitol Salt Agar (MSA) and blue in small colonies on chrome agar, because *Staphylococcus epidermidis* does not have the ability to ferment mannitol [18]. The isolated bacteria from different clinical specimens were confirmed by vitek-2 system. The

probability of positive isolates ranged from (92-99%) [19].

3. Virulence factors

The selected samples that were confirmed by the Vitek-2 system were screened for the production of virulence factors that were performed in specific culture media such as egg yolk agar for lipase enzymatic hydrolysis of lipid [20], gelatinase agar for detection of gelatinase activity, skim milk agar for protease, and DNase agar for detection if the bacteria produced the enzyme deoxyribonuclease [21].

4. Biochemical tests

Voges-Proskauer test, urease test, gram staining, slide coagulase [22]. Catalase, hemolysis, motility, and hydrogen sulfide production were performed on the isolated samples [23].

5. Antibiotic sensitivity test (AST)

Antibiotics susceptibility tests were assessed by the disk diffusion method, which was performed on Mullar-Hinton agar, using 8 antibiotic disks that included methicillin, gentamicin, novobiocin, ciprofloxacin, erythromycin, pencillin, tetracycline, and trimethoprim [24].

6. Biofilm assay

The biofilm was detected by the assay of micro-titer plates with 96 wells, and U-Bottom. 2ml of Brain Heart Infusion (BHI) supplemented with 1% glucose was used to culture *Staphylococcus epidermidis*. The turbidity of the overnight culture of bacteria was adapted to the 0.5 McFadden standard by comparing the bacterial suspension with the 0.5 McFarland standard. 200µL of the bacterial suspension were incorporated into the holes, and then incubated at 37 °C overnight. The holes were drained three times, and the holes were drained by the phosphate-buffered saline, then air dried. 200µL of absolute methanol was used to fix the biofilm for 15 minutes. After draining, the holes were air dried and 200µL of (0.05%) crystal violet stained the holes for five minutes. After draining the holes, washed with PBS 3 times, then air dried. 200µL of ethanol (absolute) was added to the holes. The optical density was determined at 630 nm using an enzyme-linked immunosorbent assay reader.

Isolates were then classified into four categories based on O.D.c and the average O.D. of the strain:

Negative (-) = O.D. ≤ optical density control(O.Dc)

Weekly positive = O.D.c. < O.D. ≤ 2×O.D.c.

Moderate (+) = 2×O.D.c. < O.D ≤ 4×O.D.c.

Strong positive (++) = 4×O.D.c. < O.D.

7. Pathogenicity of Staphylococcus epidermidis in laboratory animals

In this study, the male mice were used as a model. The mice were taken from the College of Veterinary Medicine, and sheltered in the animal house in the college. The intraperitoneal injection of mice was performed in the animal house, and these mice were monitored by the animal house supervisor. The mice were chosen with an age range of 2 to 3 months and 22-30 g in weight. The mice (15 total mice) were divided into three separate sets at random. (control, intraperitoneal injection, and skin scratching set), and there are 5 mice in each group. Phosphate-buffered saline was given to the control set, and the second set was intraperitoneal injected with bacterial suspension, while in the third set, the skin of mice was scratched and contaminated with bacterial suspension, and the mice were daily monitored. The scarified skin, liver, intestine, and heart were isolated from the dead mice and sacrificed mice, and the organs from the control group were also isolated for histopathology, for determination of bacterial pathogenicity, and histopathological studies.

8. DNA extraction

The overnight bacterial culture on chrome agar was used for DNA extraction by using a DNA extraction kit, followed the manufacturer's protocol. The purity was measured by Nanodrop (Nabi /korea), and then stored at −20 °C for further investigation.

9. PCR amplification

The PCR was performed after DNA extraction to amplify two genes (*mecA* and *ermA*) by using specific primers, as shown in Table 1. Gel electrophoresis on agarose was performed by using the DNA ladder marker 100 pb (intron). The PCR and gel electrophoresis were achieved according to the manufacturer's protocol. The optimum condition of detection is shown in Table 2.

Table 1: Specific primers for *mecA* and *ermA* genes [25]

Genes	Primer	Sequence	Tm °C	GC %	size of product	Reference
mecA	**forward**	**5'-AAAATCGATGGTAAAGGTTGGC-3'**	**53.8**	**40.9**	**532base pair**	**(Chabi and Momtaz, 2019).**
mecA	**reverse**	**5'-AGTTCTGCAGTACCGGATTTGC- 3'**	**57.6**	**50**	**532base pair**	**(Chabi and Momtaz,**

						2019).
ermA	**forward**	**5'-3'**	**AAGCGGTAAACCCCTCTGA**	**56**	**52.6**	**190base pair**	**(Chabi and Momtaz, 2019).**
ermA	**reverse**	**5'-3'**	**TTCGCAAATCCCTTCTCAAC-**	**53.2**	**45**	**190base pair**	**(Chabi and Momtaz, 2019).**

Table 2: Thermocycle condition

No.	Phase	Tm °C	Time	No. of the cycle
1	**Initial - Denaturation**	94 °C	5 min.	1 cycle
2	**Denaturation - 2**	94 °C	40 sec.	30 cycle
3	**Annealing**	60 °C	40 sec.	30 cycle
4	**Extension-1**	72 °C	40 sec.	30 cycle
5	**Extension -2**	72 °C	7 min.	1 cycle

10. Results and discussion

This study was conducted on a total of 120 specimens in different sexes and age groups. Only 73 (41.66 %) diagnosed positive for *Staphylococcus epidermidis* (26 males and 47 females) as shown in Table 3. Age ranged of the study population was from 10 to 65 years. The majority of the respondent cases and the higher ratio of infection significantly appears at the age group of 36 to 50 years followed by age group of 25 to 35 and 51 to 65 years and then age group of 10 to 24 years as shown in Table 4.

Table 3: Distribution the type of samples among sex

Type of sample	Sex No.	Male	%	Female	%
Blood	29	8	30.77	21	44.68
Wound swab	8	3	11.54	5	10.64
Nasal swab	7	3	11.54	4	8.51
Acne swab	7	3	11.54	4	8.51
C.S.F.	7	2	7.69	5	10.64
Heart valve	8	5	19.23	3	6.38
Dialysis Catheter	7	2	7.69	5	10.64
Total	73	26	35	47	64

Table 4: Distribution the type of samples among age group

Type of sample	Age groups									
	(10-24)	%	(25-35)	%	(36-50)	%	(51-65)	%	Total	%
Blood	2	18.18	10	45.45	15	53.57	2	16.67	29	40.85
Wound swabs	1	9.09	3	13.64	2	7.14	2	16.67	8	11.27
Nasal swabs	2	18.18	3	13.64	2	7.14			5	7.04
Acne swabs	4	36.36	3	13.64					7	9.86
C.S.F.	2	18.18			3	10.71	2	16.67	7	9.86
Heart valve			3	13.64	3	10.	2	16.67	8	11.27

Dialysis catheter			**3**	**71 10. 71**	**4**	**33.33**	**7**	**9.86**
Total	**11**	**22**	**28**		**12**			
%	**15.0 7**	**30.1 4**	**38.3 6**		**16.4 4**			

In the present study, the positive cases obtained from blood seem to occur much higher than the patient's samples collected from other cases; these patients have blood stream infection.

10.1 Antibiotic resistance

Staphylococcus epidermidis showed different patterns of susceptibility to antibiotics, as shown in Table 5. The erythromycin, pencillin, tetracycline, and trimethoprim showed the highest rate of resistance (14.43%), while the methicillin resistant rate was (13.83%), and novobiocin had (12.25%) followed by the lowest rate of resistance with ciprofloxacin (10.28%), and gentamicin (5.93%), and there was intermediate resistance to gentamycin (24%). All *S. epidermidis* isolates were multiple drugs-resistant as they showed the highest resistance against at least five structurally different antimicrobial agents. Some of the *S. epidermidis* isolates showed sensitivity to gentamycin (36.36 %) and ciprofloxacin (38.18 %), novobiocin (20%), methicillin (5.45%), as shown in Table 5. The 70 (13.83%) of *Staphylococcus epidermidis* isolated showed resistance to methicillin, and the remaining 3 (5.45%) isolates were considered susceptible to methicillin.

Table 5: Antimicrobial susceptibility test

NO.	Antibiotics	Resistance	%	Intermediate	%	Sensitive	%
1	**Novobiocin**	**62**	**12.25**	**-**		**11**	**20**
2	**Methicillin**	**70**	**13.83**	**-**		**3**	**5.45**
3	**Erythromyn**	**73**	**14.43**	**-**		**-**	
4	**Gentamicin**	**30**	**5.93**	**18**	**24**	**20**	**36.36**

5	**Pencillin**	**73**	**14.43**	**-**	**-**	
6	**Tetracycline**	**73**	**14.43**	**-**	**-**	
7	**Ciprofloxacin**	**52**	**10.28**	**-**	**21**	**38.18**
9	**Trimethoprim**	**73**	**14.43**	**-**		

10.2 Tests for some virulence factors

The present study revealed some virulence factors of *Staphylococcus epidermidis* that showed positive results for lipase and protease enzymes and negative results for DNase and gelatinase enzymes.

10.3 Biochemical test

All isolates of *Staphylococcus epidermidis* were non-motile, coagulase-negative, and showed positive results for hydrogen sulfide (H_2S), urease, and Voges-Proskauer VP, while showing negative results for hemolysis (gamma hemolysis).

10.4 Biofilm formation

The present study demonstrated that 36 (49.32%) of *S. epidermidis* isolates were strong biofilm producers, 27 (36.99%) of isolates were considered moderate for biofilm production, and 10 (13.70%) of isolates were weak or non-biofilm producers, as shown in Table 6, according to the microtiter plate assay, as shown in Fig. 1.

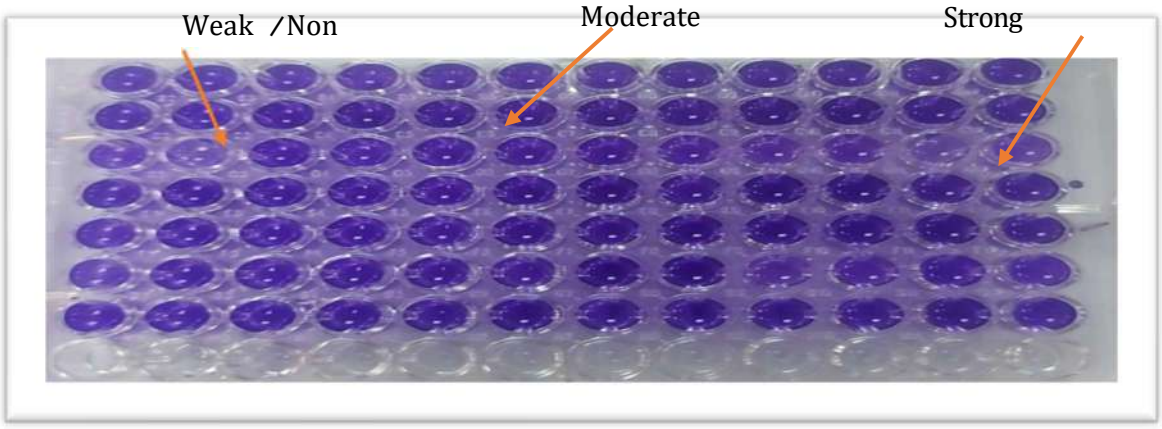

Fig. 1: A microtiter plate showed biofilm stained with crystal violet.

Table 6: the percentage of biofilm

Biofilm	Strong	Moderate	Weak/ Non
Type of sample	36	27	10
Total %	49.32%	36.99%	13.70%

10.5 Identification of S. epidermidis by Molecular method (Convential PCR)

This research confirmed that one gene was predominant and one gene was non-predominant. The isolates of *S. epidermidis* (10) were selected for DNA extraction and PCR amplification to identify and confirm the presence of two genes *mecA* and *ermA* genes, using specific primers. Electrophoresis has been done on agarose in comparison with the DNA ladder marker 100 pb to determine DNA fragments after the extraction to detect the result of PCR in the presence of the standard DNA to distinguish the bundle size of the outcome of the interaction of PCR on the agarose gel, as shown in Fig. 2.

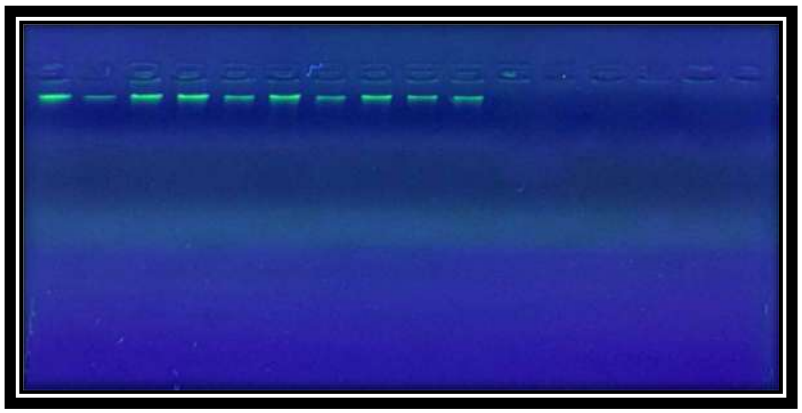

Fig. 2: Gel electrophoresis of genomic DNA extraction from *S. epidermidis* (10) isolates, 1% agarose gel at 5vol /cm for 30 min.

The result showed that all isolates (10 samples) have the *mecA* gene, as shown in Fig. 3.

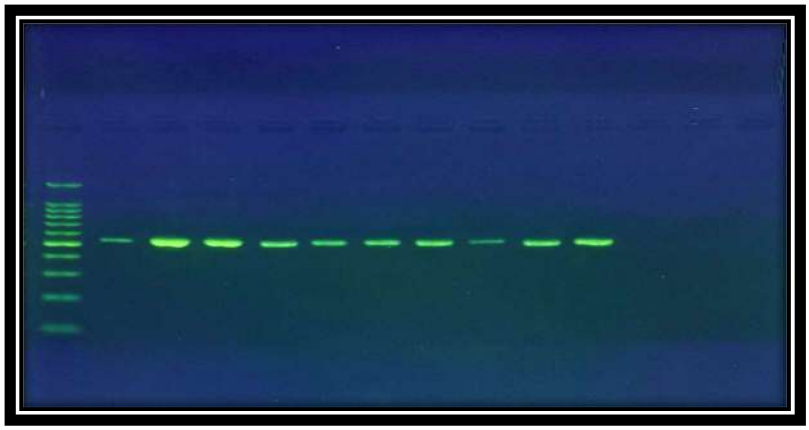

Fig. 3: PCR product, *mecA* gene, the band size 532 bp. The product was electrophoresis on 2% agarose at 5 volt/cm^2. 1x TBE buffer for 1hr. N: DNA ladder (100).

The PCR technique confirmed that *S. epidermidis* isolates were lacked the *ermA* genes, as shown in Fig. 4.

Fig. 4: PCR product, *ermA* gene, the band size *ermA* gene, the band size of *remA* gene was 190 bp, no band appear on the gel. The product was electrophoresis on 2% agarose at 5 volt/cm^2. 1x TBE buffer for 1hr. N: DNA ladder (100).

The present study showed that the selected 10 isolates of the total 73 isolates of *S. epidermidis* possessed the *mecA* gene and lacked the *ermA* gene in their genome, depending on the amplification results of PCR. The possession of *mecA* genes was explained by the resistance of 70 (13.83%) isolates of *S. epidermidis* to methicillin, which was confirmed by an antibiotic sensitivity test.

10.6 Histopathological examination

The present study showed that these animals suffered from acute infection with temporary behavioral changes such as abdominal stretching and restlessness. The mice displayed intense grooming, lethargy, reduced locomotion, and were sedated when compared with the control. The histopathological results included the control group, injury group, and skin group. The injury group was subdivided into liver, heart, and intestine, according to the site of infection.

The result of histopathology revealed that the control group showed normal appearances with normal cytoarchitecture in the liver, skin, heart, and small intestine, as shown in Fig. 5,6,7, and 8.

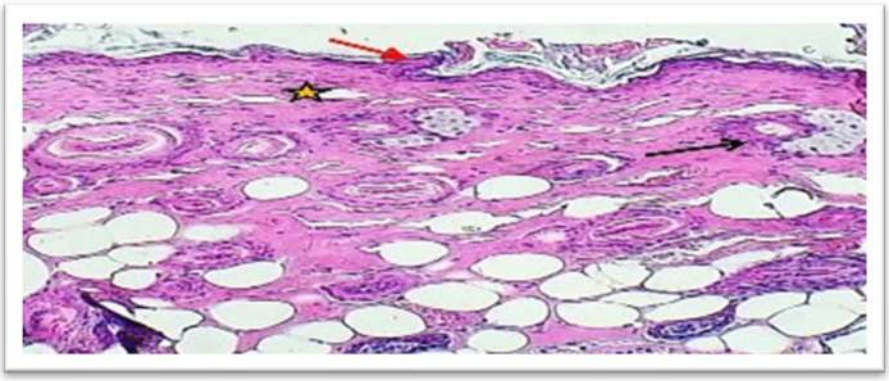

Fig. 5: **Section of skin (control) shows: normal epidermis (Red arrow), normal dermis fibrous tissue (Asterisk) & hair follicle with sebaceous gland (Black arrow). H&E stain.100x**

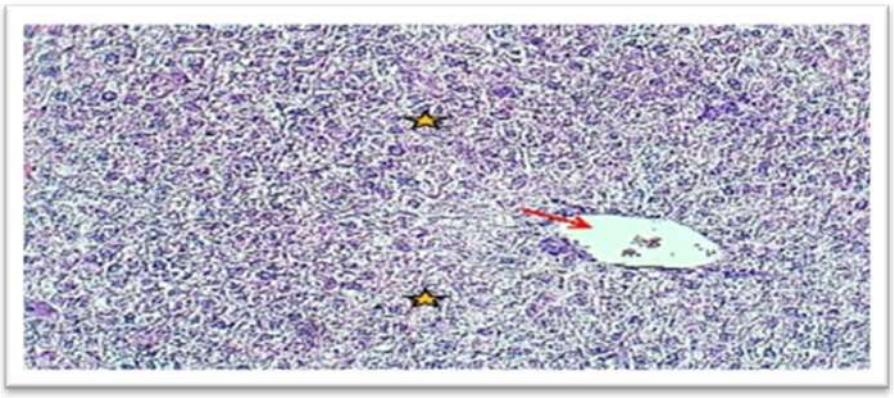

Fig. 6: section of liver (Control) shows: Central vein (arrow) & hepatic cords appearance with arrangement (Asterisks). H&E stain.100x.

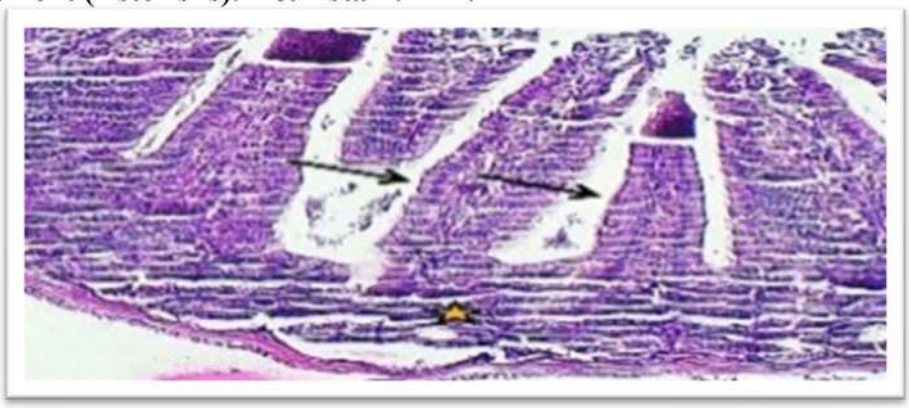

Fig. 7: Section of small intestine (Control) shows: normal appearance of villi (Arrows) & intestinal glands (asterisk) .H&E stain.400x.

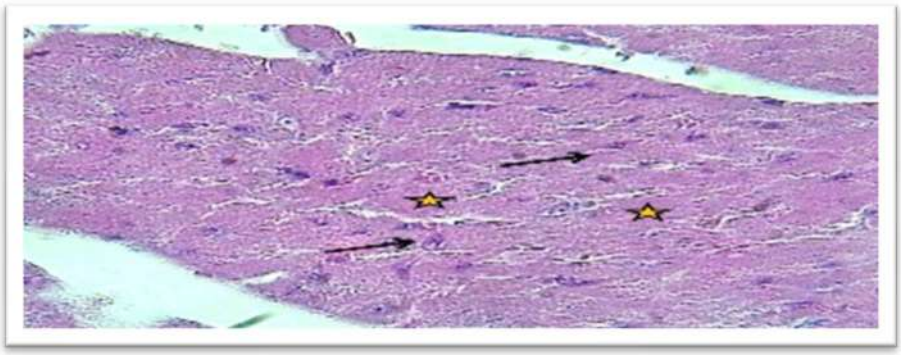

Fig. 8: Section of myocardium (Control) shows: normal appearance of myofibers (Asterisks) & nuclei (Arrows).H&E stain.400x.

While in the injury group, the histopathological figures showed marked vascular congestion at the portal triad and sinusoids with marked disarrangement and damage of the hepatic cords and necrosis, severe hepatitis, and marked atrophy of the hepatocytes. There was marked infiltration of mono-nuclear and polymorphic nuclear leukocytes with giant cell formation and damage to the central vein cord, as shown in Fig. 9,10, and 11.

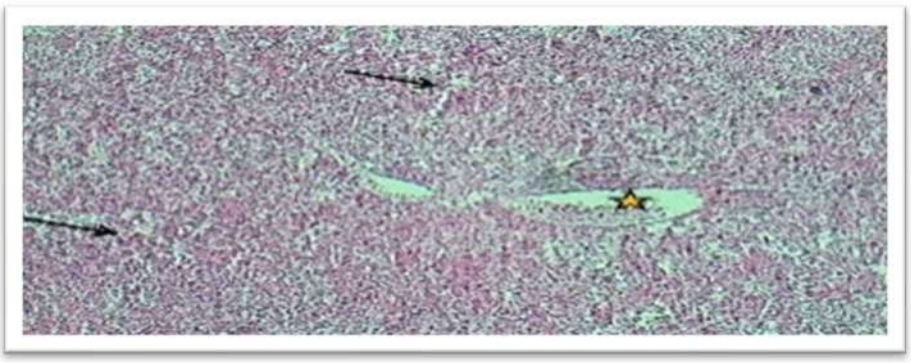

Fig. 9: Section of liver shows: marked vascular congestion at portal triad (Asterisk) with marked damage of hepatic cords by necrosis (Arrows). H&E stain. 100x.

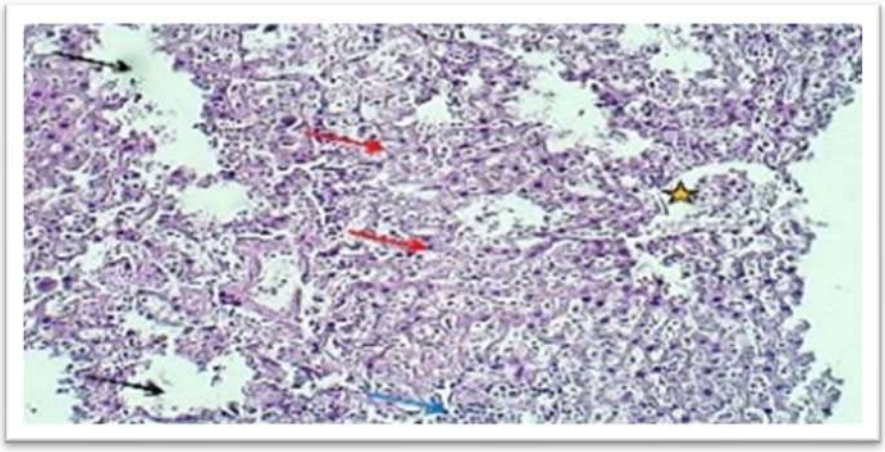

Fig. 10: Section of liver shows: marked hepatitis that characterized by disarrangement with damage of hepatic cods and sinusoid with marked atrophy of hepatocytes (Red arrows), infiltration of leukocytes (Blue arrow) with damage of central vein cords(Asterisk). H&E stain. 100x.

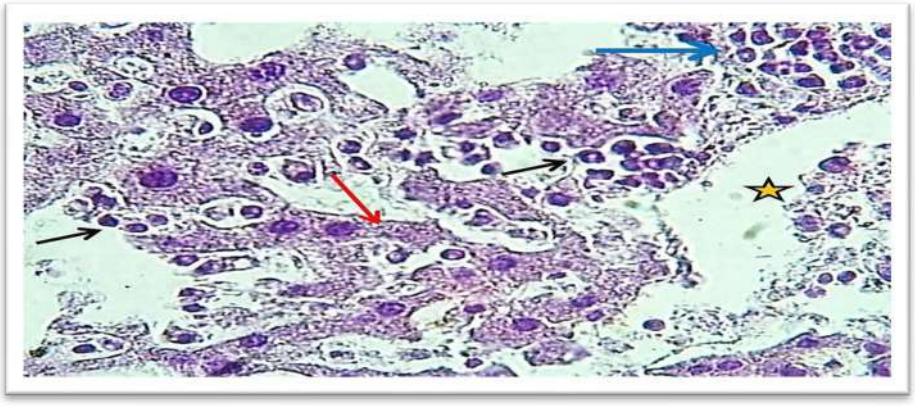

Fig. 11: Section of liver shows: disarrangement with damage of hepatic cods and sinusoid with marked atrophy of hepatocytes (Red arrows), infiltration of mono nuclear and polymorphic nuclear leukocytes (Black arrows) with damage of central vein cords filed with debris (Asterisk). H&E stain. 400x.

The histopathological figures of the heart showed severe myocardial infarction with a markedly wavy appearance and atrophy of the affected myofibers and edema, as well as ventricular thrombus formation, as in Fig. 12, and 13.

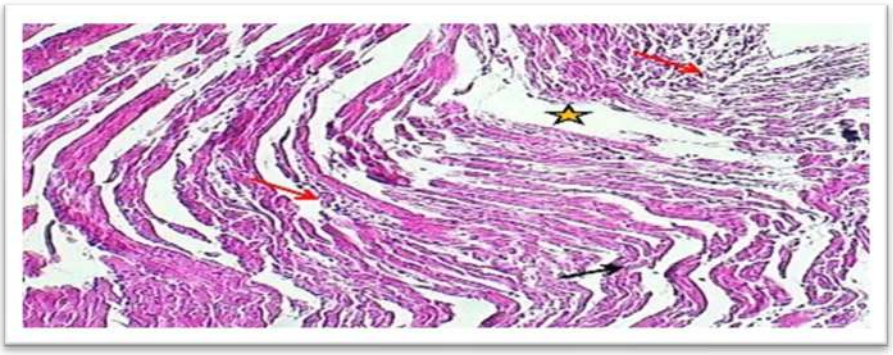

Fig. 12: Section of heart shows: sever myocardial infarction with marked wavy appearance of affected myofibers (Black arrows), atrophy of myofibers (Red arrows) & edema (Asterisk). H&E stain. 400x.

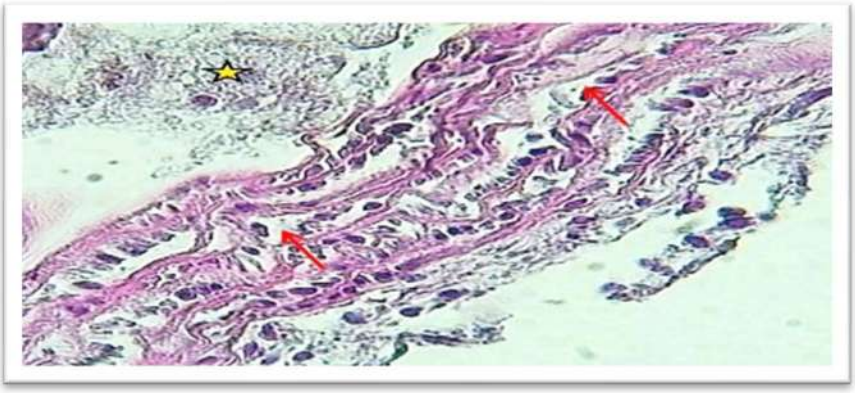

Fig. 13: Section of coronary artery shows: sever medial degeneration with necrosis of coronary artery (Arrows) with marked thrombus formation (Asterisk) (Asterisk). H&E stain. 400x

The histopathological figures of the small intestine showed severe enteritis characterized by marked damage to mucosal villi with necrosis of intestinal glands and severe destructive enteritis characterized by marked loss of mucosal villi and complete loss of their cytoarchitecture with marked luminal tissue debris, as shown in Fig. 14, and 15.

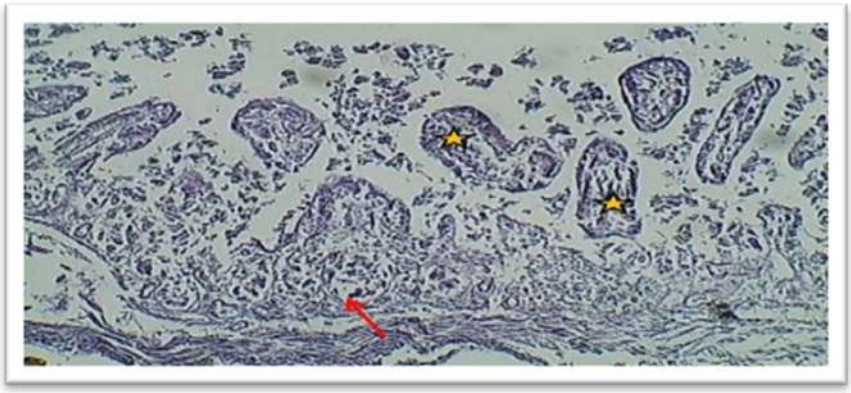

Fig. 14: Section of small intestine (Injury group) shows: sever enteritis that characterized with marked damaged of mucosal villi (asterisks) with necrosis of intestinal glands (arrow). 400x.

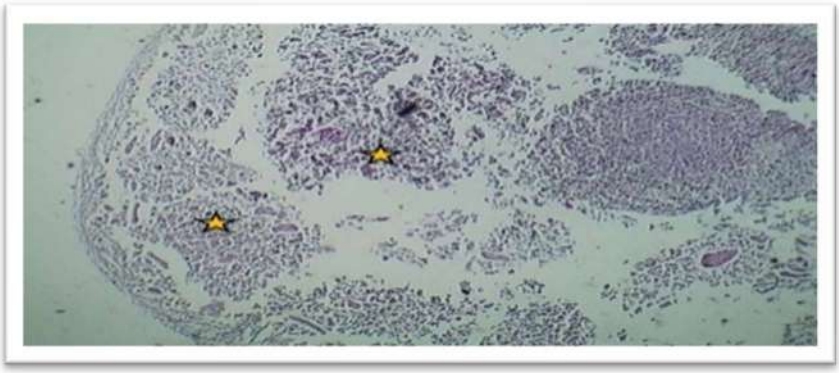

Fig. 15: Section of small intestine (Injury group) shows: sever destructive enteritis that characterized with marked loss of mucosal villi and with complete lost their cytoarchetecture with marked luminal tissue debris (asterisks). 400x.

The histopathological figures of the skin revealed severe dermatitis that was characterized by marked damage, loss of epidermis, and ulcer formation that was covered by necrotic tissue. The dermis revealed marked degeneration with necrosis of dermal fibrous tissue and infiltration of mononuclear leukocytes, as shown in Fig. 16, and 17.

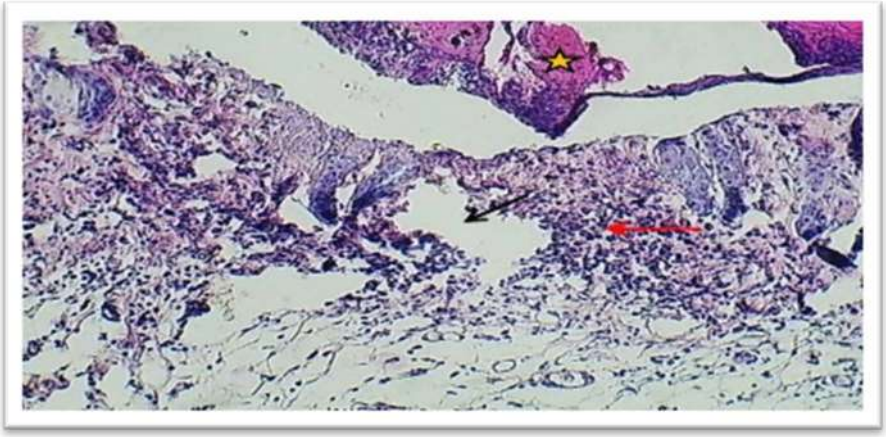

Fig. 16: Section of skin shows necrotic tissue (Asterisk), dermatitis with tissue depletion (Black arrow), necrosis of dermal fibrous tissue with infiltration of MNCs (Red arrow) (Asterisk). 100x.

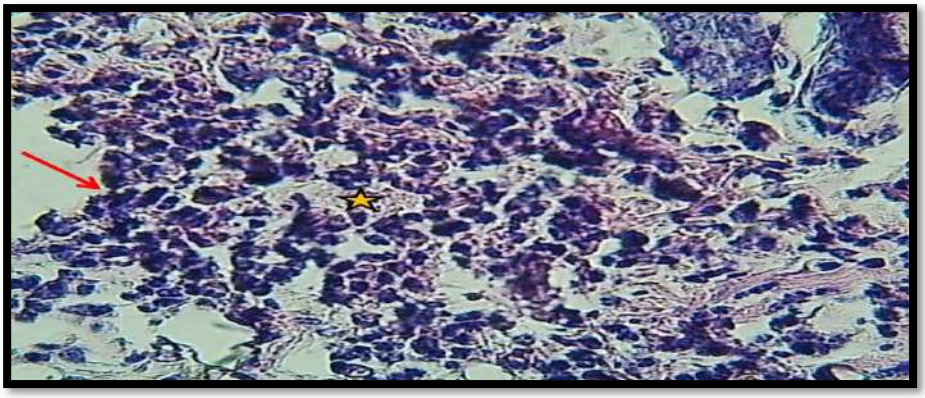

Fig. 17: Section of skin shows dermatitis with necrosis of dermal fibrous tissue (Asterisk) with infiltration of MNCs (arrow). 400x.

Conclusion

The study shows that the majority of the respondents are in the cases the age group of 38.36%(36 to 50) years, followed by the age group of 30.14%(25 to 35) and 16.44%(51-65) years, and then the age group of 15.07%(10-24) years, indicating that all isolates of *S. epidermidis* have the *mecA* gene and lack ermAgene in their genome. Antibiotic sensitivity tests revealed all *S. epidermidis* isolates were multiple drug resistant, and most isolates were methicillin resistant due to the acquisition of the *mecA* gene. The histopathological results indicated that *S. epidermidis* colonized the skin and some internal organs of mice and can cause serious

pathological changes on certain organs such as the skin, liver, intestine, and heart, which, depending on their severity, could be fatal.

Acknowledgement

I acknowledge the medical city-Baghdad Teaching Hospital and Ghazi Al-hariri Hospital in particular the bacteriology department, and everyone who helped me in my research.

Referances

[1] KOSECKA-STROJEK, M., SADOWY, E., GAWRYSZEWSKA, I., KLEPACKA, J., TOMASIK, T., MICHALIK, M., HRYNIEWICZ, W. & MIEDZOBRODZKI, J. 2020. Emergence of linezolid-resistant *Staphylococcus epidermidis* in the tertiary children's hospital in Cracow, Poland. *European Journal of Clinical Microbiology & Infectious Diseases,* 39**,** 1717-1725. https://doi.org/10.1007/s10096-020-03893-w.

[2] FOSTER, T. J. 2020. Surface proteins of Staphylococcus epidermidis. *Frontiers in microbiology,* 11**,** 1829. https://doi.org/10.3389/fmicb.2020.01829.

[3] TANG, B., GONG, T., CUI, Y., WANG, L., HE, C., LU, M., CHEN, J., JING, M., ZHANG, A. & LI, Y. 2020. Characteristics of oral methicillin-resistant Staphylococcus epidermidis isolated from dental plaque. *International Journal of Oral Science,* 12**,** 15. https://doi.org/10.1038/s41368-020-0079-5.

[4] YARAWSKY, A. E., JOHNS, S. L., SCHUCK, P. & HERR, A. B. 2020. The biofilm adhesion protein Aap from Staphylococcus epidermidis forms zinc-dependent amyloid fibers. *Journal of Biological Chemistry,* 295**,** 4411-4427. https://doi.org/10.1074/jbc.RA119.010874.

[5] SOUCHE, A., KOLENDA, C., TEOLI, J., SCHUCH, R., FERRY, T., LAURENT, F. & JOSSE, J. 2022. Activity of exebacase (CF-301) against biofilms formed by Staphylococcus epidermidis strains isolated from prosthetic joint infections. *Antimicrobial Agents and Chemotherapy,* 66**,** e00588-22. https://doi.org/10.1128/aac.00588-22**.**

[6] SICILIANO, V., PASSEROTTO, R. A., CHIUCHIARELLI, M., LEANZA, G. M. & OJETTI, V. 2023. Difficult-to-Treat Pathogens: A Review on the Management of Multidrug-Resistant Staphylococcus epidermidis. *Life,* 13**,** 1126. https://doi.org/10.3390/life13051126

[7] GOULART, D. B. 2023. Pathogenicity and Antimicrobial Resistance in Coagulase-Negative Staphylococci. *Journal of Biosciences and Medicines,* 11**,** 9-29. DOI: 10.4236/jbm.2023.115002.

[8] RANJBAR OMID, M., JAMALI, H., KAFILZADEH, F., BORJIAN, A. & ARZANLOU, M. 2023. Occurrence of Staphylococcus spp. in the wastewaters from Iran: Diversity, antimicrobial resistance, and virulence potential. *Journal of Water and Health,* 21**,** 178-191. https://doi.org/10.2166/wh.2023.199.

[9] MICHALIK, M., SAMET, A., PODBIELSKA-KUBERA, A., SAVINI, V., MIĘDZOBRODZKI, J. & KOSECKA-STROJEK, M. 2020. Coagulase-negative staphylococci (CoNS) as a significant etiological factor of laryngological infections: A review. *Annals of Clinical Microbiology and Antimicrobials,* 19**,** 1-10. https://doi.org/10.1186/s12941-020-00367-x.

[10] FRANCA, A., GAIO, V., LOPES, N. & MELO, L. D. 2021. Virulence factors in coagulase-negative staphylococci. *Pathogens,* 10**,** 170. https://doi.org/10.3390/pathogens10020170.

[11] MYLES, I. A., MOORE, I. N., CASTILLO, C. R. & DATTA, S. K. 2019. Differing virulence of healthy skin commensals in mouse models of infection. *Frontiers in Cellular and Infection Microbiology,* 8**,** 451. https://doi.org/10.3389/fcimb.2018.00451.

[12] YU, X., CHEN, T., HUANG, N., JIN, Y. & YANG, L. 2022. Skin Commensal Bacteria Modulates the Immune Balance of Mice to Alleviate Atopic Dermatitis-Induced Damage. *Evidence-based Complementary & Alternative Medicine (eCAM)*. https://doi.org/10.1155/2022/4731675.

[13] RHEEM SAAD, N. A. A., HUSSEIN, R. H. & AL-SHAKIR, N. M. 2022. Antibiogram Pattern of Uropathogenic Escherichia Coli in Baghdad Province, Iraq. *Journal of Techniques,* 4**,** 134-138.

[14] Aubaid, S.H., E.S. Falih, and S. khalid Ibrahim, *Biofilm Formation of Staphylococcus Aureus in Multiple Sclerosis Patients and its Essential Role in the Pathogenicity of the Disease.* Journal of Techniques, 2022. **4**(3): p. 14-18.

[15] Yaseen, A., et al., *Phenotypic Resistance of (MRSA) Clinical Isolates to Some Macrolide Antibiotic Groups.* Journal of Techniques, 2023. **5**(4): p. 186-191.

[16] MISHRA, A. K., SAHU, K., LAL, A. & MENON, V. 2020. Aortic valve abscess: Staphylococcus epidermidis and infective endocarditis. *QJM: An International Journal of Medicine,* 113**,** 211-212. https://doi.org/10.1093/qjmed/hcz151

[17] SOLANKI, K., PRAJAPATI, B., SINGH, R., PATEL, A., PATEL, R. & KATIRA, B. 2022. Detection of mecA gene-associated methicillin-resistance coagulase-negative staphylococci (MRCoNS) from bovine mastitis in Gujarat. *Indian Journal of Animal Sciences,* 92**,** 935-939. https://doi.org/10.56093/ijans.v92i8.105824

[18] MUTER, B. S. & DEGAIM, Z. D. 2023. Association of S. epidermidis infection and TNF-α gene polymorphism of acne patients in Thi-Qar province/Iraq. *Journal of Population Therapeutics and Clinical Pharmacology,* 30**,** 104-111. https://doi.org/10.47750/jptcp.2023.30.04.010

[19] MICHALIK, M., NOWAKIEWICZ, A., TROŚCIAŃCZYK, A., KOWALSKI, C. & PODBIELSKA-KUBERA, A. 2022. Multidrug resistant coagulase-negative Staphylococcus spp. isolated from cases of chronic rhinosinusitis in humans. Study from Poland. *Acta Microbiologica et Immunologica Hungarica,* 69**,** 68-76. https://doi.org/10.1556/030.2021.01580.

[20] CHEN, J., ZHANG, J., YANG, Z., NIU, Y., CAI, Z., WANG, J., YIN, L., LIN, Y. & LU, X. 2022. Characterization of indigenous coagulase-negative staphylococci isolated from Chinese spontaneously fermented meat products.*Microbiological Research,* 263**,** 127160. https://doi.org/10.1016/j.micres.2022.127160.

[21] KHUSRO, A., AARTI, C., SALEM, A. Z. & BARBABOSA-PILEGO, A. 2020. Techno-functional traits and safety aspects of coagulase-negative Staphylococcus saprophyticus isolated from traditional fermented food. *Food Biotechnology,* 34**,** 77-99. https://doi.org/10.1080/08905436.2019.1711114

[22] SHRESTHA, L. B., BHATTARAI, N. R., RAI, K. & KHANAL, B. 2020. Antibiotic resistance and mecA gene characterization of coagulase-negative staphylococci isolated from clinical samples in Nepal. *Infection and Drug Resistance*, 3163-3169. https://doi.org/10.2147/IDR.S274163

[23] FAMOJURO, O. B., ADESANYA, I. O., AJEWOLE, J. O. & FAMOJURO, T. I. 2023. Genotypic characterization of extended spectrum beta-lactamase in gram negative bacterial contaminants of some door handles in Olabisi Onabanjo University Teaching Hospital, Sagamu, Ogun state. *African Health Sciences,* 23**,** 208-18. https://doi.org/10.4314/ahs.v23i2.23.

[24] ASHAGRIE, D., GENET, C. & ABERA, B. 2021. Vancomycin-resistant enterococci and coagulase-negative staphylococci prevalence among patients attending at Felege Hiwot Comprehensive Specialized Hospital, Bahir Dar, Ethiopia. *Plos one,* 16**,** e0249823. https://doi.org/10.1371/journal.pone.0249823.

[25] CHABI, R. & MOMTAZ, H. 2019. Virulence factors and antibiotic resistance properties of the Staphylococcus epidermidis strains isolated from hospital infections in Ahvaz, Iran. *Tropical medicine and health,* 47**,** 1-9. https://doi.org/10.1186/s41182-019-0180-7.

